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Synthesis and Solution Properties of Hydrophobically Modified Polysaccharides

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Abstract

Hydrophobically modified polymers are amphiphilic macromolecules mainly constituted of a hydrophilic backbone and hydrophobic side groups. In aqueous solutions these polymers undergo inter- or intra-molecular hydrophobic association, which results in unusual properties useful for a number of practical applications. The areas of application of these polymers include associative thickeners for enhanced oil recovery, pharmaceuticals, personal care formulations, coatings, adhesives, surfactants, emulsifiers, *etc.* This review presents the analysis of a literature data on preparation of hydrophobically modified polysaccharides (HMP) and their properties in aqueous solutions. Some of the synthetic methods used for hydrophobic modification of non-ionic (cellulose ethers, starch, dextran, pullulan, *etc.*), anionic (carboxymethylcellulose, hyaluronic acid, pectic acid, alginic acid, heparin) and cationic polysaccharides (chitosan) are presented. The methodology used for the investigation of solution properties of hydrophobically modified polysaccharides is discussed. Special attention is paid to aggregate and micelle formation in solutions of hydrophobically modified polysaccharides, solubilization of hydrophobic compounds, their rheological properties and surface activity. The effects of polymer architecture (level of hydrophobic substitution, nature of hydrophobic groups, molecular weight of a hydrophilic backbone, *etc.*), concentration, temperature, presence of inorganic salts and organic solvents on solution properties of hydrophobically modified polysaccharides are discussed. Some applications of hydrophobically modified polysaccharides are briefly highlighted.

Introduction

Hydrophobically modified polymers are neutral or charged hydrophilic macromolecules bearing low amounts of long chain hydrophobic groups. These polymers have attracted significant attention of researchers during the past several decades due to their unique hydrodynamic properties, surface activity and ability for self-organisation into micelles, nanoparticles and gels. A number of reviews have been published on preparation, properties and application of synthetic hydrophobically modified polymers [1-10]. The intensive research undertaken in the recent years concerning the preparation and properties of hydrophobically modified polymers based on polysaccharides has not been systematised properly except for the review of Zhang [11], devoted partially to hydrophobically modified cellulosic polymers.

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Cellulose is the most abundant polysaccharide on earth, which in its native form is insoluble in water due to high crystallinity and numerous intramolecular hydrogen bonds (Scheme 1). However, it can be converted into water-soluble polymers by chemical modification, which involves positioning a sufficient number of hydrophilic or slightly hydrophobic functional groups along the cellulose backbone to prevent the formation of crystalline areas. The range of commercially produced water-soluble cellulose derivatives includes non-ionic methylcellulose (MC), hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC), hydroxypropylmethylcellulose (HPMC) and anionic carboxymethylcellulose (CMC). They have found very wide applications in industries concerned with oilfield treatments, medical products, protective colloids, coatings, surfactants, hair conditioners, antistatic agents, dispersion agents, adhesives, textiles, *etc.*

Chitin is the most abundant natural amino-polysaccharide and is estimated to be produced annually

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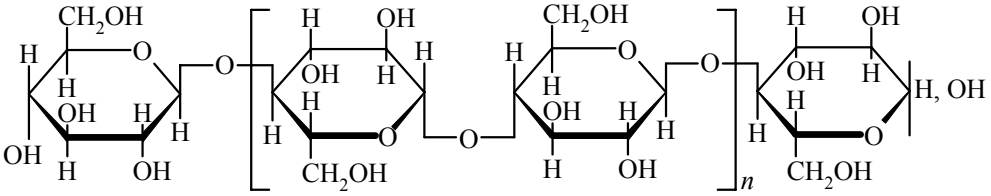
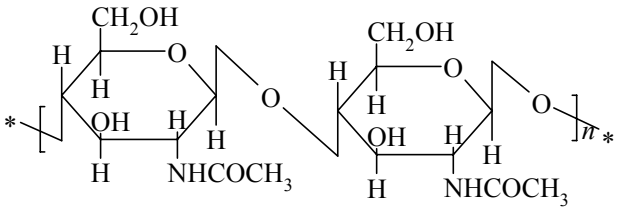


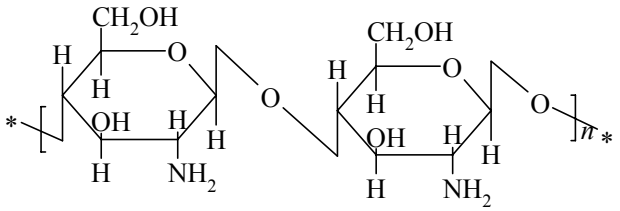
Scheme 1

almost as much as cellulose (Scheme 2). It has been found in a wade range of natural sources (crustaceans, fungi, insects, annelids, molluscs, coelenterate, *etc.* [12]. It is a highly insoluble material resembling cellulose in its solubility and low chemical reactivity but it can be converted by N-deacetylation into chitosan, which is soluble in water at weakly acidic pH conditions (Scheme 3).



Chitin

Scheme 2

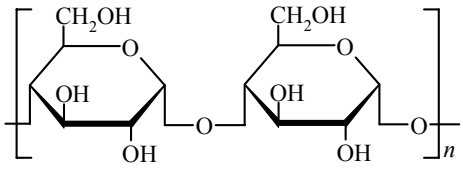


Chitosan

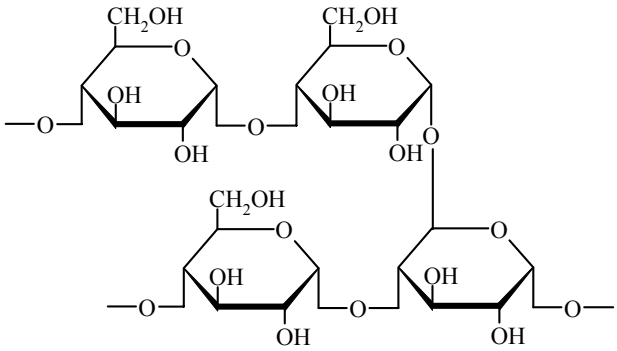
Scheme 3

Starch is also one of the most abundant biopolymers on earth and is present in living plants as energy storage material. Native starches are mixtures of two polyglycans, amylose and amylopectin [13,14]. Amylose is nearly unbranched and its molecular weight is about 10^5-10^6 Da, while amylopectin is a highly branched polymer with molecular weight 10^6-10^7 Da (Scheme 4).

Amylopectin forms crystalline domains in the starch granules of living plants, while linear amylose is believed to be present in the amorphous areas between the crystalline domains. On heating in water, the semicrystalline structure of the granules is broken up, and amylose and amylopectin enter into



Amylose



Amylopectine

Scheme 4

solution. However, in aqueous solution, starch molecules have a strong tendency to associate, causing high viscosity.

Among the other water-soluble polysaccharides it is also important to mention non-ionic dextran, pullulan, inulin and anionic pectic acid, hyaluronic acid and alginic acid.

In the present review the literature data on preparation of hydrophobically modified polysaccharides, their properties in aqueous solutions and some of the potential applications are discussed.

Preparation of Hydrophobically Modified Polysaccharides

The preparation of HMP involves a covalent attachment (conjugation) of hydrophobic groups (alkyl, aromatic or fluorocarbon groups) to the functional groups of a polysaccharide. Depending on the hydrophobicity of these groups and the degree of modification HMP can be either insoluble or soluble in water. For preparation of water-soluble products the degree of its modification should not alter by a few

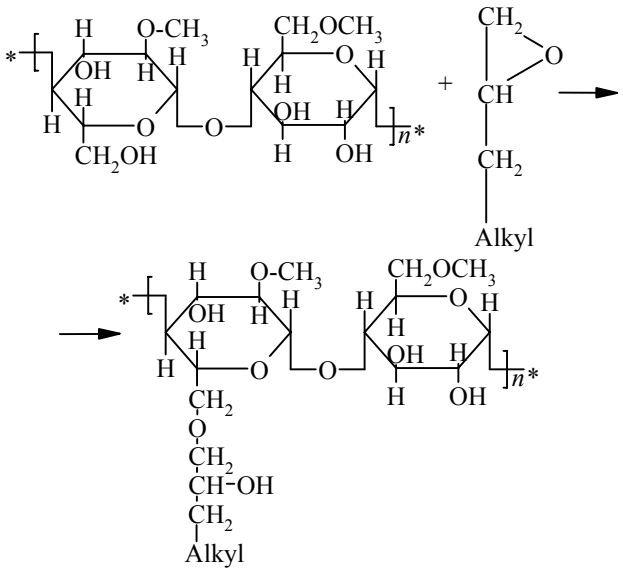
percents (up to about 5% for non-ionic and 10% for ionic macromolecules).

Different modification approaches can be used depending on the structure of water-soluble polysaccharides and the reactivity of their functional groups. We will consider some examples on modification of non-ionic, anionic and cationic polysaccharides.

Modification of non-ionic polysaccharides

The most important water-soluble non-ionic polysaccharides are hydroxyethylcellulose, methylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, starch, dextran, inulin and pullulan. All of them have numerous hydroxyl groups that can be used as a target for hydrophobic modification.

Landoll [15] was one of the first authors to report the preparation of hydrophobically modified polysaccharides by reaction of non-ionic cellulose ethers (HEC and MC) with 1,2-epoxydecane, 1,2-epoxydodecane, 1,2-epoxytetradecane as well as a mixture of 1,2-epoxyeicosane, 1,2-epoxydocosane and 1,2-epoxytetracosane by the Scheme 5:



Scheme 5

In the course of modification the cellulose ether was mixed with degassed isopropyl alcohol and a degassed solution of NaOH in water was added. The mixture was stirred overnight to ensure complete swelling of the polymer and then alkyl epoxide dissolved in isopropyl alcohol was added in excess and stirred for 3 to 6 hrs at 80°C to give various levels of modification. However the reaction was not very efficient and yielded products with a degree of modification ranging within 0.13-4.75 wt.%.

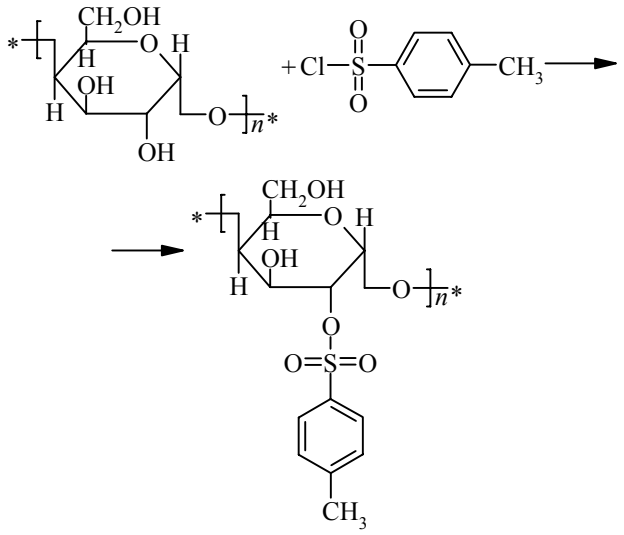
A similar approach has been applied by Wesslen and Wesslen [14] in derivatization of starch using alkyl epoxides with C_6 and C_{12}. In this work starch was dissolved in dimethylsulfoxide and mixed with a solution of NaH in dimethylsulfoxide in order to activate the hydroxyl groups of the polymer. Then the solution was stirred for 24 hrs under nitrogen atmosphere before alkyl epoxide was added. The alkylation reaction was allowed to proceed under stirring at room temperature and resulted in hydrophobically modified derivatives with the degree of molar substitution (MS) of up to 1.8. However in order to reach MS values higher than 1.5, the reaction had to be run for 150-300 hrs.

Heinze and co-workers [16] have suggested an effective homogenous method for hydrophobic modification of starch by reaction with p-toluenesulfonyl chloride. Starch was dissolved in a mixture of N,N-dimethylacetamide (DMA) and lithium chloride. Then this solution was mixed with triethylamine and additional DMA. After cooling it to 8°C, a solution of p-toluenesulfonyl chloride in DMA was added and the homogeneous reaction mixture was stirred for 24 hrs at 8°C and then was precipitated into a water-ethanol mixture, dissolved in acetone and reprecipitated into distilled water. It was shown that the degree of substitution can be controlled from 0.4 to 2.0 by adjusting the molar ratio of the reagents. It was also demonstrated that the remaining OH-groups of tosyl starch are reactive and can be additionally modified by acetylation reactions (Scheme 6).

Vaidya and Kumar [17] have demonstrated the methodology for preparation of water-soluble starch grafted with quaternary amino polydimethylsiloxane (PDMS) by reacting 3-chloro-2-hydroxypropyl, methyl, n-propyl quaternary amino PDMS with granular as well as degraded starches.

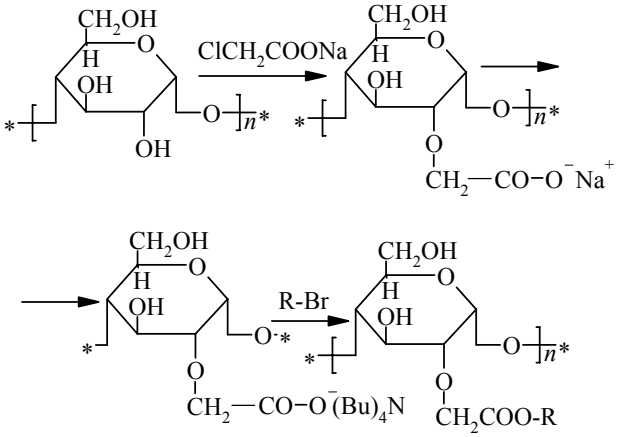
Duval-Terrie and co-workers [18] have prepared hydrophobically modified pullulan by reacting hydroxyl groups of the polysaccharide with sodium chloracetate in the presence of NaOH in water-isopropanol mixture at 70°C with subsequent transformation of the sodium salt of carboxymethylpullulan into its acidic form and then into tetrabutyl ammonium salt and reaction with alkyl bromide (Scheme 7).

The hydrophobic modification of dextran has been performed by Fournier and co-workers [19] in a two-step procedure. Firstly, dextran was allowed to react



Scheme 6

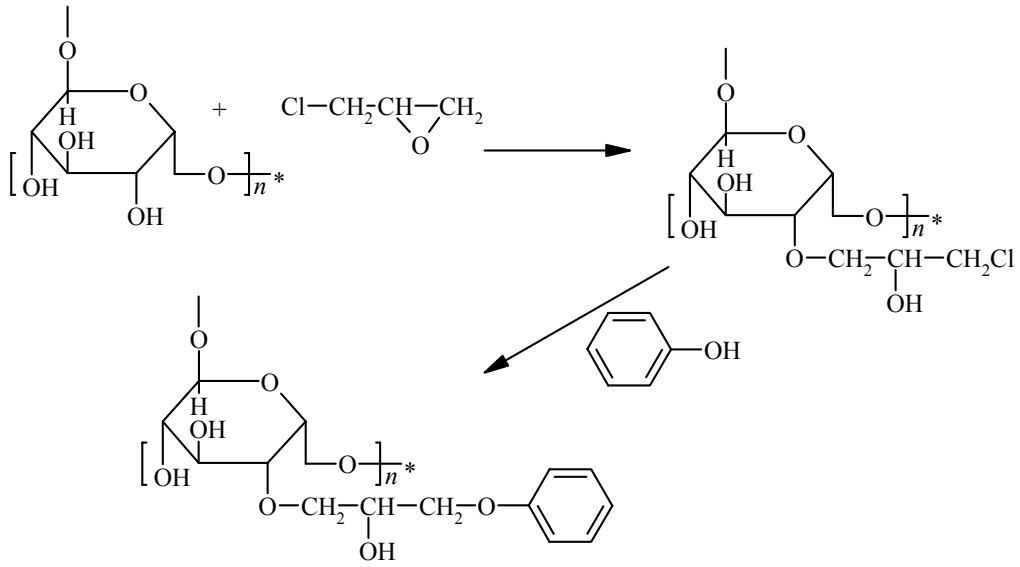
with epichlorhydrin at pH 1 at 80°C for 5 hrs, in the presence of $Zn(BF_4)_2$ in water. Then the product was precipitated once with acetone and 3 times with ethanol. The resulting dextran derivative was treated with an aqueous solution of phenol at pH 11 and ambient temperature for 24 hrs (Scheme 8).



Scheme 7

The crude product was precipitated twice with ethanol and treated with 1 M NaOH for 48 hrs in order to remove all unreacted chlorinated groups and finally it was dialized against water and freeze-dried. In the resulting polymer about 2% of the total saccharide units were substituted.

An original approach on hydrophobic modification of dextran has been reported by Xu and co-workers [20]. First, they have activated dextran by oxidati-



Scheme 8

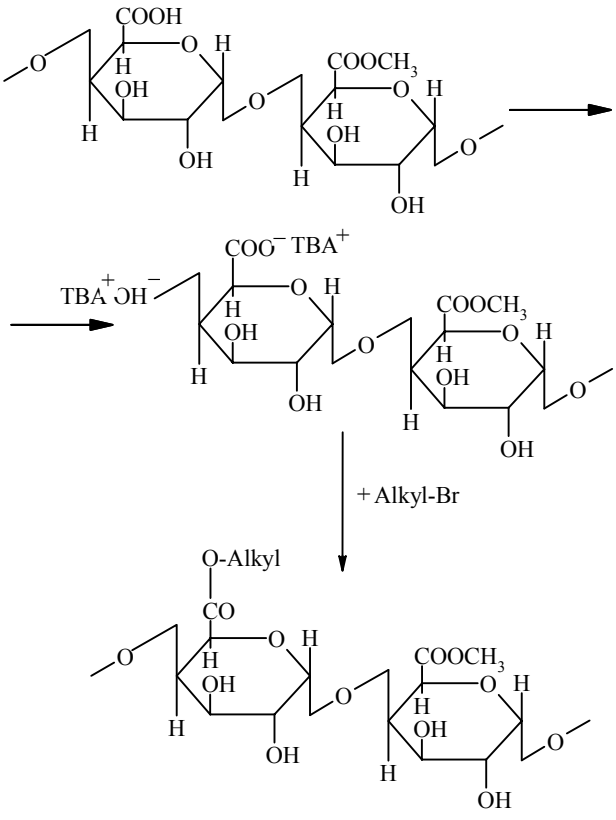
on with sodium periodate resulting in poly(aldehyde dextran) where the aldehyde groups content was 29%. Poly(aldehyde dextran) dissolved in water was then mixed with cholic acid hydrazide dissolved in dimethylformamide and the pH of solution was adjusted to pH 4.0 and stirred for 24 hrs at room temperature. Then the reaction mixture was poured into methanol and the precipitate was isolated by filtration. The resulting dextran derivative was purified by washing with methanol and vacuum dried at room temperature.

Modification of anionic polysaccharides

The most important anionic polysaccharides are carboxymethylcellulose, pectin (pectic acid), hyaluronic acid, alginic acid and heparin. The anionic nature of these polysaccharides is due to the presence of carboxylic groups that can be used for further modification.

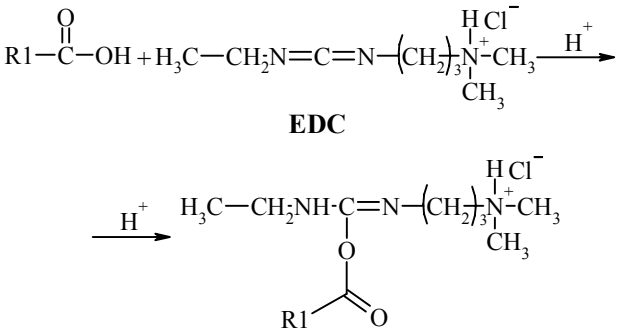
Miralles-Houzelle and co-workers [21] have synthesized hydrophobically modified pectin by covalent attachment of long alkyl chains. A 2% w/v solution of pectin (pH 3.2) was neutralized by tetrabutylammonium hydroxide (TBA^+OH^-) up to pH 7.0 and after freeze-drying, the resulting pectin TBA salt was dissolved in DMSO and a long chain (C_{12}, C_{16} and C_{18}) alkyl bromide was introduced and left to react for 24 hrs, under stirring at room temperature (Scheme 9).

Then the reaction mixture was dialized for 7 days against water containing sodium azide as a bactericide and afterwards it was freeze-dryed and left in contact with sodium chloride solution in 70% aqueous ethanol to exchange the residual TBA^+ by Na^+. A similar procedure has been applied by Babak and co-workers [22] for synthesis of hydrophobically modified alginic acid.



Scheme 9

The application of coupling reagents, which facilitate the conjugation, has become a very popular method for preparation of hydrophobically modified polysaccharides. Carbodiimides is one of the important classes of coupling reagents [23] due to their ability to catalyze the formation of amide bonds between carboxylic acids or phosphates and amines by activating carboxyl or phosphate groups to form an O-urea derivative:

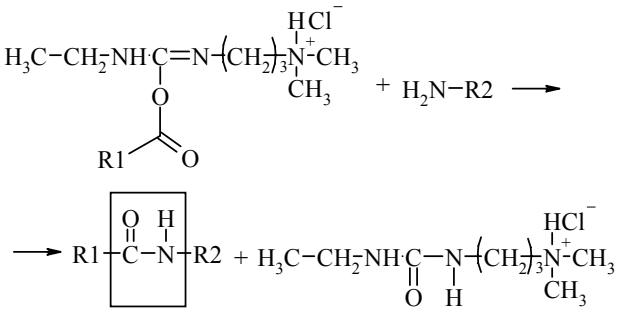


EDC

Unstable amine-reactive intermediate

Scheme 10

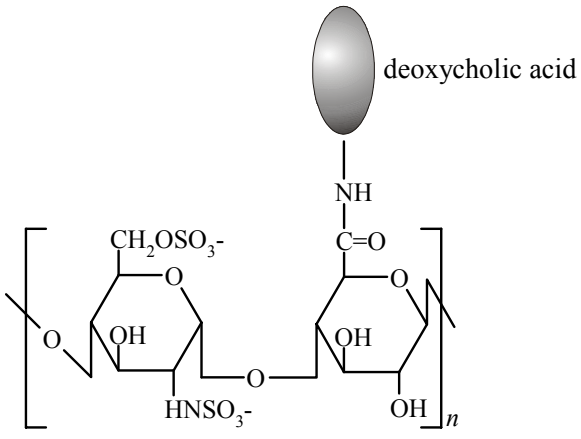
This derivative reacts readily with nucleophiles:



Scheme 11

Carbodiimides can be water-soluble like 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) or organo-soluble like N,N'-dicyclohexylcarbodiimide. Charpentier and co-workers [24] have reported about the synthesis of hydrophobically modified carboxymethylcellulose. The acid form of CMC was dialized against 0.1 N HCl and then against water until neutral pH. The solution of polymer was dried as a film and then it was dissolved in DMSO. One equivalent of N,N'-dicyclohexylcarbodiimide was added under stirring and 30 min later followed by one equivalent of hexadecylamine in chloroform mixed with dimethylaminopyridine as a catalyst. The reaction mixture was stirred for 1 day.

Lee and co-workers [25] have prepared hydrophobic heparin derivatives by its conjugation with deoxycholic acid, cholesterol, lauric acid and palmitic acid. For this purpose deoxycholic acid (DOCA) was mixed with excess of N,N'-dicyclohexylcarbodiimide (DCC) and N-hydroxylsuccinimide (NHS) in DMF. The mixture was reacted for 5 hrs at room temperature under vacuum and then precipitated dicyclohexylurea was removed and washed by distilled water to remove unreacted DCC and HOSU. The activated DOCA was precipitated and liophilized. Then it was reacted with heparin in water/DMF (1:1) for 4 hrs at room temperature. The excess of activated DOCA was removed by precipitating in water and the remaining solution was liophilized to prepare heparin-DOCA conjugate as a white powder. In the case of heparin-cholesterol conjugate, most of the experimental procedures were the same with those of heparin-DOCA conjugate except the carboxylation step. Cholesterol has only a hydroxyl group, and it should be substituted by a carboxyl group via the reaction with chloroacetic acid to couple with the amine group of heparin. Lauric acid and palmitic acid were also coupled with heparin in a similar manner. A slightly different synthetic strategy for conjugation of DOCA with heparin was also reported by the same group [26]. First they introduced a primary amino-group to DOCA using ethylenediamine, DCC and NHS. Then coupled this DOCA derivative with heparin using EDC as a coupling agent. The resulting product had the following structure:



Scheme 12

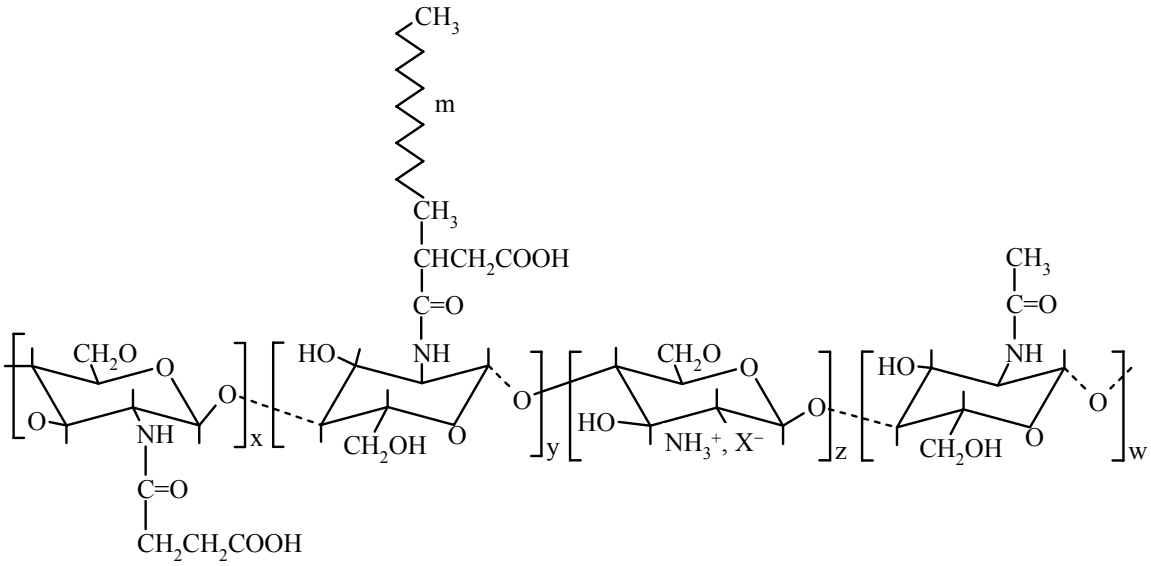
Modification of cationic polysaccharides

One of the most important representatives of cationic polysaccharides is chitosan, which primary amino-groups are quite reactive and can be used for covalent attachment of hydrophobic groups. However an insolubility of chitosan in most organic solvents and aqueous solutions at higher pH values limits the possibilities for its modifications. A lot of attempts have been reported to functionalize chitosan including its hydrophobic modification. Comprehensive information about controlled functionalization of chitin and chitosan can be found in the review of Kurita [27]. Here we will discuss some of the methods reported for hydrophobic modification of chitosan.

Babak and co-workers [28] prepared the hydrophobically modified chitosan by treatment of chitosan with (2-dodecen-1-yl-succinic anhydride in water/methanol (1:1, v/v) (DDC) solution containing 1% (v/v) acetic acid. The degree of hydrophobic substitution after this treatment was found to be 5 mol.%. In order to enhance the solubility of the product the authors treated it with succinic anhydride to obtain the DDC-N-(2-carboxymethyl) chitosan, which displays an enhanced solubility in aqueous media at neutral and basic pH values (Scheme 13).

Liu and co-workers [29] have prepared a hydrophobically modified chitosan by reaction with dodecyl bromide. For this purpose chitosan was suspended in isopropanol followed by addition of 5 N NaOH. The mixture was stirred at 6°C for 30 min and then dodecyl bromide was added. After 4 hrs the reaction was terminated by addition of hydrochloric acid. The precipitate obtained was thoroughly washed with methanol and dried under vacuum at 50°C to obtain dodecylated chitosan powder. The degree of substitution of this derivative determined by potentiometric titration was 22%. Similar synthetic strategy was reported by Wakita and Hashimoto [30] for preparation of chitosan modified by conjugation with 1-bromooctadecane.

The solubility problems associated with chitosan can be resolved by using hydrophilic chitosan derivatives such as carboxymethyl chitosan (CMCHI) or glycol chitosan (GCHI), which are soluble in water in a broad range of pH. These derivatives can be successfully used for further modification. Sui and co-workers [31] have prepared a new kind of chitosan derivative by reacting carboxymethyl chitosan with dodecyl glycidol ether (DGE) in KOH-isopropanol solution. The required amounts of DGE were added drop wise to the solution of CMCHI in KOH-isopropanol at 50°C and stirred for several hours. Then the product was filtered, washed with acetone, redis-

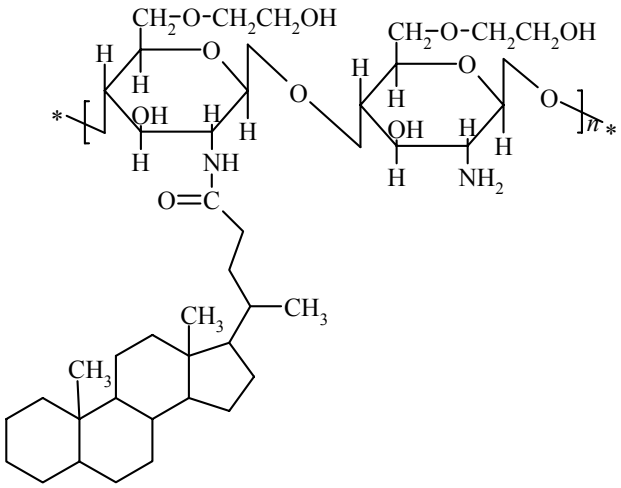


Scheme 13

solved in water and dialized against distilled water. The degrees of hydrophobic modification determined by elemental analysis were found to be 4.1, 9.5 and 11% depending on the initial DGE content in the reaction mixture.

Kwon and co-workers [32] have reported about the modification of glycol chitosan by covalent conjugation with 5β-cholanic acid. GCHI was dissolved in distilled water followed by dilution with methanol. Different amounts of 5β-cholanic acid were added and its carboxylic group was activated by addition of 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and hydroxysuccinimide, which allowed the formation of amide linkage with primary amino-groups of GCHI. The resulting solution was stirred for 24 hrs at room temperature, dialized against water/methanol mixture and freeze-dried (Scheme 14). The degree of hydrophobic substitution determined by colloidal titration with poly(vinyl sulphate) was found to be 12 5β-cholanic acid groups per 100 sugar residues of GCHI.

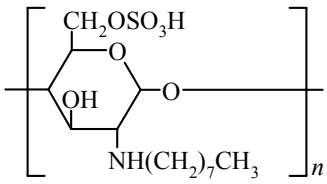
Uchegbu and co-workers [33] prepared a new HMP by conjugation of glycol chitosan with palmitic acid N-hydroxysuccinimide with subsequent quaternisation of remaining primary amino-groups using methyl iodide. GCHI and sodium bicarbonate were dissolved in water/ethanol mixture and a solution of palmitic acid N-hydroxysuccinimide in ethanol was added drop wise with continuous stirring for 1 hr. The mixture was stirred for 72 hrs and the product was isolated by evaporating off ethanol and extracting the remaining aqueous phase with diethyl ether.



Scheme 14

Then the aqueous mixture was dialized against water and freeze-dried. In order to obtain a polymer soluble in water the palmytoylated chitosan derivative was quaternised by reacting with methyl iodide with subsequent extraction, dialysis and ion exchanger treatment. The levels of palmitoylation and quaternisation of GCHI determined by ^{1}H NMR spectroscopy were found to be 5.9 mol.% and 4.0 mol.%, respectively. Similar modification by reacting GCHI with hexadecyl bromide with subsequent quaternisation of the product with methyl iodide was reported by the same group [34,35].

A series of novel chitosan derivatives carrying long alkyl groups (n = 8, 10, 12) as hydrophobic moieties and sulfated groups as hydrophilic moieties were synthesized by Zhang and co-workers [36]:



Scheme 15

Chitosan was suspended in methanol at room temperature, then octaldehyde, decanal or lauryl aldehyde were added and the mixture was stirred for 24 hrs, KBH_4 dissolved in water was slowly added and the reaction mixture was left for another 24 hrs stirring. Then the reaction mixture was neutralized with 2 M HCl and the product was precipitated with methanol and dried under vacuum at 60°C. For further modification the product was suspended in DMF under nitrogen atmosphere and mixed with solution of chlorosulphonic acid in DMF. The mixture was reacted at 10°C under nitrogen atmosphere for 24 hrs and then it was neutralized with 20% NaOH to pH 7 and the filtered solution was dialyzed against water and freeze-dried.

Ramos and co-workers [37] reported about hydrophobic modification of chitosan carrying phosphonic groups – CH_2–PO_3H_2. First they synthesized N-methylene phosphonic chitosan by the reaction of chitosan with phosphorous acid in the presence of formaldehyde. Then this derivative was reacted with lauryl aldehyde in the presence of sodium borohydride. The mixture was stirred at room temperature overnight and then the product was precipitated with methanol. The degrees of phosphomethylation and laurylation were found to be 1.56 and 0.33, respectively.

Properties of hydrophobically modified polysaccharides in aqueous solutions

Water-soluble hydrophobically modified polysaccharides display a number of unique properties such as the ability to form intra- and intermolecular micelles and aggregates in aqueous solutions, unusual rheological properties, surface activity, emulsifying properties, *etc.* These properties will be briefly considered below.

Aggregate and micelle formation in dilute solutions and solubilization of hydrophobic compounds

HMP in aqueous solutions form "polymeric micelles" or micelle-like aggregates of higher hierarchy via intra- and/or intermolecular association between hydrophobic moieties (Fig. 1). Polymeric micelles consist of a hydrophobic core stabilised by a corona of hydrophilic polymeric chains exposed to aqueous environment. The formation of self-assemblies of HMP is generally considered to resemble that of low molecular weight amphiphiles.

The micelle formation and aggregation of HMP in aqueous solutions can be studied by fluorescence techniques using probes such as pyrene and 1,8-anilinonaphtalene sulfonic acid sodium salt (ANS). The ability of pyrene to migrate into a more hydrophobic environment and change the intensity ratio of the first (373 nm) to the third (383 nm) vibronic peaks I_{373}/I_{383} (or vice versa I_{383}/I_{373}) in its emission spectra (Fig. 2a) is well documented and was employed many times in the studies of solution properties of hydrophobically modified polymers [2,38-41]. The I_{373}/I_{383} value for pyrene solubilised in distilled water is around 1.8-2.0 but in more hydrophobic environment this ratio decreases. Critical aggregation concentration (CAC), which is the threshold concent-

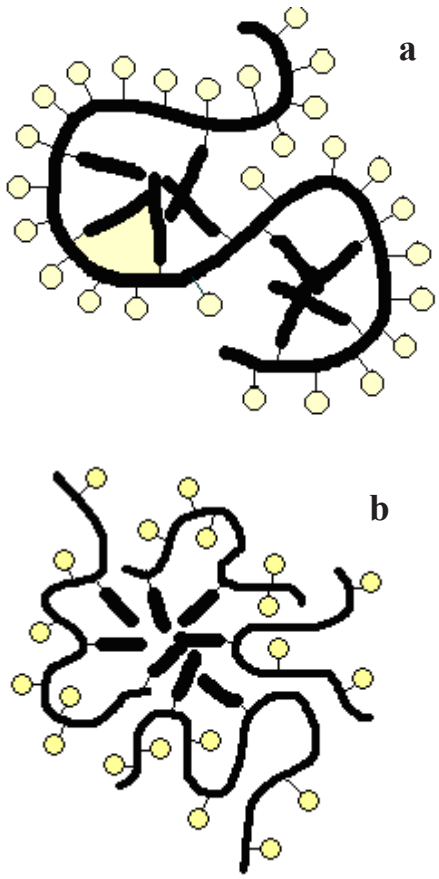


Fig.1. Scheme of intramolecular (a) and intermolecular (b) HMP association.

ration of aggregate formation by intermolecular association, can be determined by analysis of the changes in I_{373}/I_{383} of pyrene in the presence of HMP. A typical dependence of I_{373}/I_{383} on HMP logarithmic concentration is plotted in Fig. 2b. The CAC is defined as the intercept of the tangents to the curve before and after the inflection point. As a rule the CAC values detected for HMP are several orders of magnitude smaller than those found for low molecular weight surfactants. Due to this reason polymeric micelles are more stable to dilution.

Organic dyes such as methyl orange and Coomassie Brilliant Blue (CBB) undergo solvatochromic shifts upon changes in polarity of microenvironment and have also been widely used to explore the hydrophobic domains in polymers [18,33,34,42,43]. The λ_{max} of methyl orange undergoes a hypsochromic shift from 465 nm to smaller wavelength upon exposure to a hydrophobic environment. The λ_{max} of CBB is also shifted from 584 nm in polar media to higher wavelength in apolar media.

Vieira and co-workers [41] have analyzed the dependence of CAC on the degree of hydrophobic substitution in solutions of hydrophobically modified dextran using fluorescence technique with pyrene and ANS probes. They found that the CAC values determined by pyrene and ANS are in good agreement and the logarithms of CAC values decrease linearly with the degree of substitution. An increase in the dextran derivative molecular weight from 11 to 40 kDa decreases the CAC by about two orders of magnitude.

Khutoryanskiy and co-workers [34] analyzed the aggregation in aqueous solutions of hydrophobically modified glycol chitosan as a function of amphipilic polymer molecular weight and hydrophilic/hydrophobic groups ratio using methyl orange as a polarity probe. They found that the CAC is a complex function of molecular weight with more hydrophobic aggregates found in polymers of high (28-261 kDa) and low molecular weight (< 15 kDa). The medium molecular weight polymers (19-22 kDa) form more hydrophilic and looser aggregates. A linear relationship between the CAC and the hydrophilic/hydrophobic ratio was found within each molecular weight class.

The ability of HMP aggregates to solubilize hydrophobic molecules opens an opportunity to use this property in pharmaceutics for formulation of poorly water-soluble drugs. Francis and co-workers [44,45] have prepared hydrophobically modified hydroxypropylcellulose and dextran by grafting polyoxyethylene alkyl ethers and studied the solibilizing potential of polymeric micelles on their basis towards cyclosporine A, a poorly water soluble immunosuppressant. A significant enhancement in cyclosporine A aqueous solubility in solutions of hydrophobically modified derivatives compared to the parent HPC and dextran has been demonstrated.

Miwa and co-workers [46] have examined the solubility of paclitaxel, an anticancer drug, in micelles of N-lauryl-carboxymethylchitosan (LCC) in aqueous solutions. It was found that LCC solubilized paclitaxel by forming micelles with particle sizes less than 100 nm. The concentration of paclitaxel in micellar solutions was found to be up to 2.37 mg/ml compared to 0.001 mg/ml, which is the solubility of the drug in distilled water.

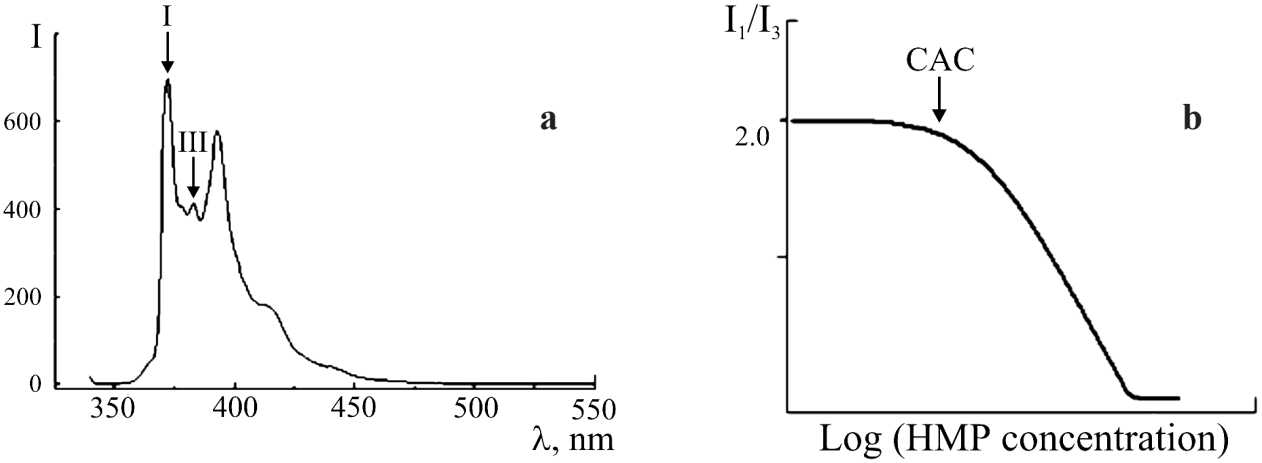


Fig. 2. Fluorescence spectra of pyrene (a) and typical dependence of I_{373}/I_{383} pyrene ratio on HMP logarithmic concentration (b).

Zhang and co-workers [36] have studied the solubilization of paclitaxel in micelles formed by chitosan derivatives carrying long chain alkyl and sulfate groups. The maximal solubilization capacity of these derivatives towards paclitaxel was found to be 2.01 mg/ml. They also found that the drug concentration in micelles is related to the type of alkyl groups and molecular weight of chitosan. The higher the molecular weight of chitosan the greater the formed micelle size was observed, which favoured the solubilization of paclitaxel.

Uchegbu and co-workers [47] have demonstrated a potential of hydrophobically modified glycol chitosan derivatives for solubilization of a number of poorly water soluble drugs (paclitaxel, etoposide, prednisolone and propofol). It was also found that the manipulation in degree of hydrophobic modification and molecular weight of the amphiphiles may lead to the nanostructures of different dimensions.

In addition to solubilization of poorly water soluble drugs the possibilities for pharmaceutical application of hydrophobically modified polysaccharides include polymers for non-viral gene delivery [29,35, 48], topical and mucoadhesive dosage forms [49-53], polymer-drug conjugates [54,55], colloidal drug carriers such as nanoparticles, liposomes and vesicles [30,56-59], *etc.*

Rheological properties

The unusual rheological properties of hydrophobically modified synthetic polymers and polysaccharides in aqueous solutions have been a subject of numerous investigations. These properties are attributed to the association between long-chain hydrophobic moieties, which can be of intra- and intermolecular nature. Because of this unusual behaviour, hydrophobically modified polymers are also called "associative thickeners" and they may act as rheology modifiers and can be used in various industrial applications.

The rheological behaviour of HMP in aqueous solutions depends significantly on the polymer concentration [15,60]. At low polymer concentration macromolecules of HMP behave as individual species and intramolecular interactions are almost exclusively favoured. It usually results in more compact conformations and intrinsic viscosities compared to unmodified parent polymers (Fig. 3). In more concentrated solutions macromolecular coils are forced to be closer together and eventually overlap leading to intermolecular hydrophobic association and significant increase in solution viscosity. It should be noted that some unmodified polymers, for example, chitosan, also can show a sharp increase in viscosity, but at much higher concentrations compared to hydrophobically modified analogues [61].

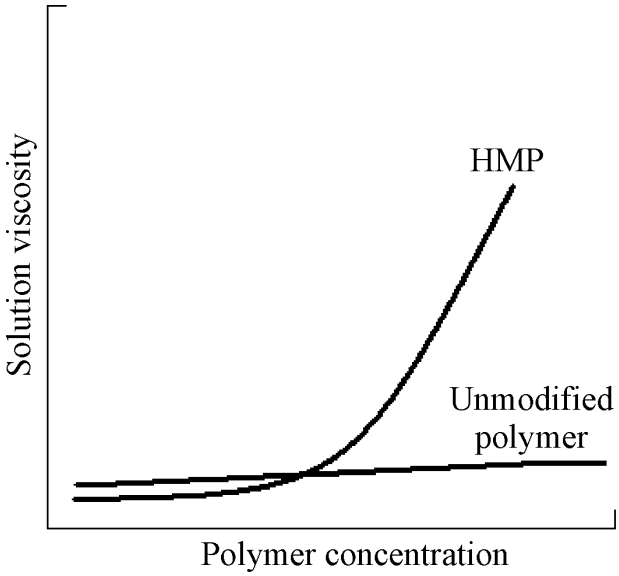


Fig. 3. Solution viscosity of HMP and unmodified polymer as a function of their concentration.

Landoll [15] demonstrated that the solution viscosity of hydrophobically modified hydroxyethylcellulose at constant molecular weight and polymer concentration varies with the length of alkyl chain and degree of hydrophobic modification. As the degree of modification increased solution viscosity first increases and then decreases rapidly, finally yielding an insoluble polymer. The degree of hydrophobic modification, from which an abrupt increase in solution viscosity is observed, was found to be in linear relationship with the length of n-alkyl group within one molecular weight class. Similar results on viscometric behaviour of hydrophobically modified carboxymethylpullulane were reported by Bataille and co-workers [62]. They found that the viscosity of the HMP solution increases gradually upon increase in the hydrophobic groups' content, then a steep change in viscosity is observed when the degree of modification exceeds 5%. It was also noted that carboxymethylpullulan derivatives are soluble in water up to 6.8% of C_{16} alkyl chains, whereas less than 2% are sufficient to confer water-insolubility to neutral hydrophobized cellulose ethers as reported by Landoll [15].

Sinquin and co-workers [60] have studied the effect of shear rate and time on the rheological behaviour of hydrophobically modified propyleneglycol alginate derivatives. It was shown that the hydrophobic associations are reversible and the intermolecular junction network can be quickly disrupted at relatively high shear stress, whereas in the quasi-rest state the network can be restored but rather slowly.

An addition of an organic solvent, which is able to disrupt hydrophobic association, can modify the rheological properties of HMP significantly. Gelman and co-workers [63] have reported that an addition of 50% v/v methanol in water was able to eliminate the association of hydrophobically modified HEC. In these conditions HEC and its hydrophobically modified derivative have similar viscosities in the concentration range 1-20 g/l. Bataille and co-workers [62] have used water/ethanol mixtures with ethanol content from 0 to 40% v/v to evaluate the effect of organic solvent on the viscosity of carboxymethylpullulan and its hydrophobically modified derivative. A slow decrease in viscosity was observed for solution of carboxymethylpullulan upon addition of ethanol. This is opposed to the viscosity of its hydrophobically modified derivative, which decreased.

Desbrieres and co-workers [64,65] have studied the effect of the hydrophobic chain and the degree of substitution, polymer concentration, temperature and ionic content of solution on the rheological properties of hydrophobically modified chitosan. They observed that an increase in temperature up to 60°C induced the gelation in solutions of chitosans substituted with C_8 or C_{12} alkyl chains and for substitution degrees larger than 0.1 and 0.05, respectively. An addition of NaCl to solutions of unmodified chitosan and its derivative with C_8 alkyl chains leads to reduction in relative viscosity, which is attributed to the screening of the charge repulsion between ionized amino-groups. The viscosity of chitosan with C_{10} is different from unmodified chitosan and its derivative with C_8 alkyl chains. It increases upon addition of NaCl, which is due to strengthening of hydrophobic association.

Rheological properties of HMP can also be significantly affected by the presence of small or large molecules, which are able to interact with either functional groups of parent polysaccharides or with hydrophobic moieties. For example, all HMP have unreacted hydroxyl groups, which can participate in complexation with carboxylic acid containing polymers via hydrogen bonding resulting in significant modification of their properties. The complexation reactions between non-ionic water soluble polysaccharides and poly(carboxylic acids) were considered in our recent review [66]. It is expected that HMP can form stronger complexes with poly(carboxylic acids) compared to unmodified polysaccharides.

Another possibility arises when polysaccharides have some ionic groups (cationic or anionic) and can form polyelectrolyte complexes with oppositely charged polymers and surfactants. Shchipunov and co-workers [67], for example, studied the polyelectrolyte complexation in aqueous mixtures of naturally occurring sulfated fucoidans and hydroxyethyl cellulose derivatives containing cationic and hydrophobic substituents. It was shown that the formation of polyelectrolyte complexes is determined by the type and arrangement of substituents and the degree of charging, as well as the composition of polysaccharide molecules. In the case of fucoidans, xylose residues that hamper association with cationic derivatives of hydroxyethyl cellulose were found to exert a strong effect on the process under consideration. As for hydroxyethyl cellulose derivatives, hydrophobic substituents and their location in a molecule play an important role. If a hydrocarbon chain is directly attached to a cationic group, the generation of polyelectrolyte complexes is hampered owing to the loosening of electrostatic interactions as a result of shielding of a charged site.

The effect of surfactants having the same charge as HMP has also been reported in several studies and been attributed to the hydrophobic association. Nystrom and co-workers [68] have examined the effects of polymer concentration, temperature, pH, and surfactant addition on the rheological properties of hydrophobically modified chitosans. They found that the viscosity of the HMP rises with increasing polymer concentration, but the viscosity enhancement is stronger as the hydrophobicity of the polymer increases. In the presence of the cationic surfactant cetyltrimethylammonium bromide the unmodified chitosan and its hydrophobically modified derivatives show different dependence of rheological properties on pH. In the case of hydrophobically modified chitosan the rheological parameters of solution (storage modulus G', loss modulus G" and complex viscosity) pass through minima at pH 4, whereas the parent polymer does not show this dependence.

Sivadasan and Somasundaran [69] have studied the interactions of hydrophobically modified hydroxyethylcellulose (HMHEC) with sodium dodecyl sul-

phate and dodecyloxyheptaethoxyethyl alcohol in dilute aqueous solutions. It was found that the presence of hydrophobic groups in HMHEC favours its stronger association with the surfactants compared to unmodified HEC. The reduced viscosity of HMHEC increases gradually with dodecyloxyheptaethoxyethyl alcohol concentration and then decreases in the vicinity of its critical micelle concentration. Minimum viscosity is observed at a surfactant concentration of $3.26 \cdot 10^{-4}$ mol/l. Above this concentration the viscosity increases again and approaches the value of pure aqueous solution at the surfactant concentration of $2.04 \cdot 10^{-1}$ mol/l. In contrast to HMHEC the viscosity of HEC is unaltered in the presence of the surfactant. An addition of SDS to the HMHEC solution increased the viscosity initially as in the case of the non-ionic surfactant; at concentrations above $5 \cdot 10^{-3}$ mol/l SDS the polymer was observed to precipitate. However, the polymer redissolved at surfactant concentrations above $5 \cdot 10^{-1}$ mol/l SDS. The reduced viscosities obtained after redissolution were very much lower than that observed for the HMHEC in water. It was also found that the presence of a miscible organic solvent (ethanol) also reduces the association by preferential solvation of the hydrophobic groups.

Gruber and Konish [70] have found that when hydrophobically modified cationic cellulose ether is mixed with amylose (AM) and dissolved together at high temperature and then carefully cooled down this results in increased viscosity compared to that of the polymer acting alone. The mode of viscosity enhancement was attributed to formation of a cross-linked network created when the amylase forms a non-covalent helical clathrate with the hydrophobic groups of HMP. Such an effect is not observed when the HMP is replaced with non-hydrophobic water-soluble cationic cellulose or when the amylose is replaced by amylopectin or α-, β- or γ-cyclodextrin. The resulting non-covalent cross-linked network is pseudoplastic and thermally unstable. Heating of this association product results in complete loss of solution viscosity, which then gradually rebuilds as the solution is recooled. Recently Egermayer and co-workers [71] have used the gels prepared by inclusion complexation between the HMHEC and AM for the study of the surfactants effect on the rheological properties. The competition effects of seven different surfactants (cationic, anionic and non-ionic) when mixed at room temperature with preformed HMHEC-AM gels have been studied. The aqueous mixtures of AM, HMHEC, and surfactant were compared with reference mixtures of AM-HMHEC, AM-surfactant and HMHEC-surfactant, respectively. All the surfactants mixed with HMHEC gave rise to an increase in shear storage modulus compared to pure HMHEC solution. In addition, all added surfactants, except Triton X-100, form inclusion complexes with AM. The mechanical properties of the AM/HMHEC/surfactant mixtures closely resemble those containing only HMHEC and surfactant but are quite distinct from that of the AM-HMHEC gel, demonstrating that all surfactants can compete with the AM-HMHEC complexation.

Surface activity and emulsifying properties

Some hydrophobically modified polysaccharides display a remarkable surface activity at the air-water or the oil-water interfaces. The surface activity of polymers is understood as their ability to decrease the surface tension at the interface. For example, Rosilio and co-workers [72] have studied the interfacial behaviour of carboxymethylcellulose (CMC) and its hydrophobically modified derivatives by surface tension and surface pressure measurements. They found that the unmodified CMC does not display any surface activity. The hydrophobically modified CMC derivatives, on the contrary, exhibited surface activity in aqueous solutions, which depended on the length of hydrophobic groups and degree of polysaccharide modification. The CMC derivative containing 6% hexadecyl-groups (C_{16}-derivative) showed the most pronounced surface tension lowering ability compared to the CMC derivatives having 6% of hexadecyl and 30% butyl groups (C_4C_{16}-derivative) as well as 6% hexadecyl and 30% octyl-groups (C_8C_{16}-derivative). Moreover, it was found that the surface activity of all derivatives is enhanced in 0.5 M NaCl solutions, which is explained by screening of electrostatic repulsion forces between the charged groups and additional aggregation of alkyl chains.

Babak with co-workers [28,73] have studied the kinetics of the adsorption of alkylated chitosan derivatives at the air-water interface as a function of its bulk concentration and the ageing time of the adsorption layers. It has been shown that the dynamic surface tension of this polysoap aqueous solution measured by the axisymmetric rising bubble shape analysis is characterized by several stages with different characteristic relaxation times. During induction and post-induction times the adsorption of macromolecules is the diffusion-controlled process from the bulk of the

solution to the interface. At the final stage the adsorption is characterized by a much lower rate due to the steric hindrance exerted by the yet to be formed adsorption layer on newly adsorbing macromolecules. The same group [22] have studied the surface tension properties of sodium alginate and its hydrophobically modified derivative. They found that the adsorption capacity of sodium alginate at the air-water interface is low due to its hydrophilic nature. The covalent attachment of 12% (mol/mol saccharide unit) of dodecyl chains exerted no substantial effect on its surface properties. In contrast the hydrophobic modification of alginate by electrostatic attachment of 12% of oppositely charged cationic surfactant results in a hundredfold increase in the surface activity of the polysaccharide-surfactant complex.

Rouzes and co-workers [74] reported the surface activity and emulsification properties of dextrans modified by covalent attachment of hydrophobic aromatic rings. The surface activity of these derivatives was studied by surface tension at the air/water interface and interfacial tension at the dodecane/water interface. It was found that the surface or interfacial tension levelled down above a critical polymer concentration, which was attributed to the formation of a dense polymer layer at the liquid/air or liquid/liquid interface. Dodecane-in-water emulsions were prepared using the polymeric surfactants as stabilisers, with oil volume fractions ranging within 5-20%. The dextran hydrophobically modified derivatives were also used as emulsifiers in emulsion polymerisation of styrene. Stable polystyrene particles were obtained with a permanent adsorbed dextran layer at their interface.

Kawaguchi and co-workers [75,76] have reported that palmitoylated derivatives of sodium hyaluronate displayed an emulsification activity with respect to soybean oil-water emulsions. It was found that the derivatives with lower molecular weight have better emulsification properties. The same group [77] reported about lowering of the interfacial tension of n-octane to water in the presence of hyaluronates partially modified with various acyl chains.

Tadros *et al.* [78] prepared oil-in-water emulsions stabilized by hydrophobically modified inulin using Isopar M and silicone oil EU 344 (cyclomethicone) as oil components. They found that emulsions stabilized by the inulin derivative INUTEC®SP1 alone had large droplets, but this could be significantly reduced by addition of a cosurfactant Span 20 to the oil phase. The authors demonstrated the superiority of INUTEC®SP1 as an emulsion stabiliser when compared with surfactants based on polyethylene glycol.

Concluding Remarks

Different synthetic strategies can be used for preparation of hydrophobically modified polysaccharides. In this review we considered only few examples on hydrophobic modification of non-ionic, anionic and cationic polysaccharides and pointed out some of the features arising from carbohydrate nature of these polymers. Intensive research has been carried out on the synthesis and characterization of HMP during the past 10 years, however a lot of questions remain unanswered and problems unresolved. The formation, structure and stability of aggregates formed by HMP depend on many factors and the establishment of the structure-property relationship in these systems is of significant importance. Technical difficulties such as low modification efficiency, poor reproducibility, and batch-to-batch variability may also be experienced in synthesis of HMP.

The areas of HMP application include associative thickeners for enhanced oil recovery, pharmaceuticals, personal care formulations, coatings, adhesives, surfactants, emulsifiers, *etc.* Some of these applications have been briefly described in this review.

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