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UNIVERSITÉ DE GRENOBLE

THÈSE

Pour obtenir le grade de

DOCTEUR DE L'UNIVERSITÉ DE GRENOBLE

Spécialité : **Sciences des polymères**

Arrêté ministériel : 7 août 2006

Présentée par

Xia MIAO

Thèse dirigée par **Rachel Auzély-Velty** et **Bo JIANG**

préparée au sein du **Centre de Recherches sur les Macromolécules Végétales (CERMAV-CNRS)**

dans **l'École Doctorale de Chimie et Science Vivante**

Hydrophobically modified derivatives of polysaccharides

Thèse soutenue publiquement le **4 Octobre 2011**,

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General introduction (*En*)

In the family of water-soluble associating polymers, amphiphilic systems composed of polar and non-polar units, which are soluble and non-soluble in water, respectively, exhibit original rheological properties in aqueous solution. These properties lead to numerous industrial applications in particular in pharmacy, food, cosmetic, paints, oil recovery, where peculiar properties such as thickening, gelling or viscoelastic properties that can be modulated with temperature or the presence of additives (salt, surfactant), are desired. The viscosity character of the aqueous solutions of such polymers is mainly due to inter-chain associations between hydrophobic groups leading to physical cross-linkages between polymer chains.

In the general context of using water-soluble polymers for various applications ranging from oil recovery to drug delivery, we focused on the synthesis of hydrophobically modified neutral or charged polysaccharides. These glucidic polymers, derived from living organisms - in other words plants, animals or bacteria, are candidates of choice for the development of a ″green chemistry″. Besides being renewable, they are generally biocompatible and biodegradable and, can also exhibit a biological activity which can be advantageously used for biomedical applications. This is typically the case of hyaluronic acid, a negatively charged linear polysaccharide which is ubiquitous in the body.

The main goal of this thesis was thus to synthesize new amphiphilic systems based on neutral polysaccharides (guar, hydroxyethyl guar) or charged polysaccharides (hyaluronic acid), based on the grafting of hydrophobic groups along the polysaccharidic chain in easy handling conditions. The synthesis strategy we proposed relies on etherification reactions in alkaline media of the polysaccharides from commercial and non-commercial hydrophobic glycidyl ethers (epoxides).

This work was performed in the team ″Structure and Modification of Polysaccharides″ in the Centre de Recherches sur les Macromolécules Végétales (CERMAV-CNRS, Grenoble) and the “Function Polymer Lab” (Sichuan University), in the framework of a co-supervision convention between the Joseph Fourier University of Grenoble and the University of Sichuan.

The first chapter of this manuscript consists in a bibliographic review on hydrophobically modified neutral or charged polysaccharides and, the study of their thickening properties in aqueous media.

The chapter 2 is devoted to the grafting of various hydrophobic groups on guar, hydroxyethyl guar as well as hydroxyethyl cellulose, used in order to compare the efficiency of reactions involved as a function of the polysaccharide backbone. The reactions, based on the opening of hydrophobic epoxides in alkaline media by the alcoholate functions of polysaccharides, are conducted by varying some experimental parameters (solvent, alkylating (arylating) agent/polysaccharide molar ratio...) in order to modulate the hydrophobic/hydrophilic balance of the final products. The analysis of their behavior in aqueous solution by viscosity measurements is also presented.

Finally, chapter 3 presents the synthesis and characterization of new amphiphilic derivatives of hyaluronic acid resulting from the grafting of Brij®56 groups. Original properties in aqueous media observed from these conjugates, which contrast with previous results obtained from alkylated derivatives of hyaluronic acid in the team ″Structure and Modification of Polysaccharides″, are discussed.

Introduction générale (*Fr*)

Dans la famille des polymères associatifs hydrosolubles, les systèmes amphiphiles composés d'unités polaires et apolaires, respectivement solubles et insolubles dans l'eau, présentent en milieu aqueux des propriétés rhéologiques originales. Ces propriétés sont à l'origine de nombreuses applications industrielles notamment en pharmacie, agro-alimentaire, cosmétique, peinture et pétrole, où des propriétés particulières comme un effet épaississant, gélifiant ou viscoélastique modulables en fonction de la température et de la présence d'additifs (sels ou tensioactifs) sont recherchées. L'origine des propriétés viscosifiantes des solutions aqueuses est liée principalement aux associations interchaînes des groupements hydrophobes conduisant à une réticulation physique et réversible du milieu.

Dans une problématique générale d'utilisation de polymères hydrosolubles pour diverses applications allant de la récupération assistée des hydrocarbures à la délivrance de principes actifs, notre intérêt s'est porté sur la synthèse de dérivés hydrophobiquement modifiés de polysaccharides neutres ou chargés. Ces polymères glucidiques, d'origine végétale, animale ou bactérienne, sont des candidats de choix pour le développement d'une chimie dite ″verte″. En plus d'être issus de ressources renouvelables, ils sont en général biodégradables et biocompatibles et peuvent également montrer une activité biologique pouvant être avantageusement mise à profit pour des applications biomédicales. C'est le cas notamment de l'acide hyaluronique, polysaccharide chargé négativement, omniprésent dans le corps.

Cette thèse avait ainsi pour principal objectif de synthétiser de nouveaux systèmes amphiphiles à base de polysaccharides neutres (guar, hydroxyéthyl guar) ou chargés (acide hyaluronique), par greffage de groupements hydrophobes le long de la chaîne polysaccharidique dans des conditions simples à mettre en oeuvre. La stratégie de synthèse proposée repose sur des réactions d'éthérification en milieu basique des

polysaccharides à partir d'éthers de glycidyle (époxydes) hydrophobes commerciaux et non commerciaux.

Ce travail a été effectué au sein de l'équipe ″Structure et Modification des Polysaccharides″ du Centre de Recherches sur les Macromolécules Végétales (CERMAV-CNRS, Grenoble) et de l'équipe "Function Polymer Lab" (Université de Sichuan), dans le cadre d'une convention de co-tutelle entre l'Université Joseph Fourier de Grenoble et l'Université de Chengdu.

Le premier chapitre de ce manuscrit consiste en une mise au point bibliographique sur la synthèse de dérivés hydrophobiquement modifiés de polysaccharides neutres ou chargés et l'étude de leur propriétés épaississantes en milieu aqueux.

Le chapitre 2 est consacré au greffage de groupements hydrophobes variés sur le guar, l'hydroxyéthyl guar ainsi que l'hydroxyéthyl cellulose, utilisée afin de comparer l'efficacité des réactions mises en œuvre en fonction du squelette polysaccharidique. Les réactions, reposant toutes sur l'ouverture d'époxydes hydrophobes en milieu basique par les fonctions alcoolate des polysaccharides, sont menées en faisant varier certaines paramètres expérimentaux (solvant, rapport molaire greffon/polysacccharide…) afin de moduler la balance hydrophobe/hydrophile des produits finaux. L'analyse de leur comportement en milieu aqueux par des mesures de viscosité est également présenté.

Enfin, le chapitre 3 présente la synthèse et la caractérisation de nouveaux dérivés amphiphiles de l'acide hyaluronique résultant du greffage de groupements Brij56®. Des propriétés originales en milieu aqueux observées à partir de ces conjugués, contrastant avec des résultats antérieurs obtenus à partir de dérivés alkylés de l'acide hyaluronique au sein de l'équipe ″Structure et Modification des Polysaccharides″, sont discutées.

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CHAPTER 1

Hydrophobically modified polysaccharides

- Bibliography Review

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Résumé (*Fr*)

Ce chapitre, situant le travail dans son contexte bibliographique, s'organise en deux volets. Les différentes approches proposées dans la littérature pour la synthèse de polysaccharides hydrophobiquement modifiés sont tout d'abord présentées. Elles reposent sur des réactions d'étherification, d'estérification ou de couplage amine-acide à partir de réactifs alkylés ou arylés variés. Celles-ci ont été appliquées principalement à des polysaccharides neutres courants (cellulose, amidon, dextranes, guar) ou chargés (acide hyaluronique) afin de leur conférer des propriétés épaississantes. Celles-ci font l'objet de la seconde partie de ce chapitre qui fait le point sur les principales études rhéologiques effectuées à partir de dérivés hydrophobiquement modifiés de la cellulose, de l'amidon, de dextranes, du et de l'acide hyaluronique.

Summary (*En*)

This chapter, situating the work in context bibliography, organized into two main parts. The different approaches proposed in the literature for the synthesis of hydrophobically modified polysaccharide by grafting hydrophobic alkyl chain on the natural polysaccharide are presented. They are based on the use of various functional groups as interlinkage for the preparation of hydrophobically modified polysaccharide. These reaction conditions and the degree of substitution of hydrophobic alkyl chain are categorized and compared by etherification, esterification, arylation or amine-acid coupling reactions. The literature shows a wide variety of polysaccharides used for the synthesis of hydrophobically modified polysaccharide. These polysaccharides come from animals or plants and vary in molecular weight between 25 000 and 1,450,000.

After hydrophobically modified, these amphiphilic polysaccharides that associate in solution are often efficient rheology modifiers. These are the subject of another part of this chapter. As used as thickener, their solution properties have been largely examined on different hydrophobic substituent, on different molar substitution levels, on the effects of operating parameters and so on. Particular attention is paid to cellulose, starch, dextran, guar and hyaluronic acid.

1.1 Introduction

Hydrophobically modified water soluble polymers that associate in solution via physical interactions are often efficient rheology modifiers. These "associating systems" are used as thickening agents in many fields of applications such as paints, cosmetics, foods, oil recovery.[1-4] The hydrophobic associations can be intramolecular and/or intermolecular leading to aggregating structures and/or three-dimensional networks. Thus very viscous solutions, physical gels exhibiting a shear thinning behavior or, micellar aggregates, can be obtained.

Natural polysaccharides have been widely used as the hydrophilic backbone of the hydrophobically modified amphiphilic macromolecule. They can have the properties of synthetic counterparts as well as being intrinsically biodegradable, abundant in nature, renewable, nontoxic, and relatively cheap.[5,6] Their characteristics at the structural level are associated with their hydrogen-bonding ability, side-group reactivity, which can be modified covalently or by ionic bonds.[7]

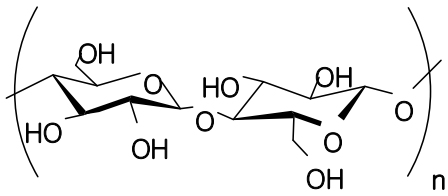
The first part of this chapter describes the strategies for the synthesis of hydrophobically modified natural polysaccharides, including etherification reaction using halogenated or epoxide derivatives, ester formation and alkylation using isocyanate derivatives. The conditions of the currently investigated reactions of these synthetic routes and the grafting efficiency are reviewed. This allows one to clarify the advantage and disadvantage of each synthesis strategy and different reaction parameters.

Next, we focus on the rheological properties of the modified polysaccharides in aqueous solution. Their thickening properties are compared.

1.2 Strategy to synthesis hydrophobically modified polysaccharide

1.2.1 Introduction

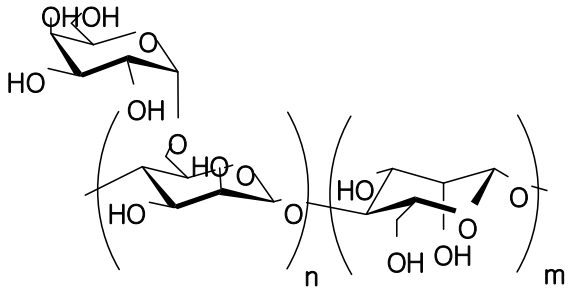
Polysaccharides used for the development of "associating systems" come from various origins.[8] Polysaccharides which are extracted from plant include cellulose, guar, starch and xylan (**Figure 1.1**). **Figure 1.2** shows the chemical structure of polysaccharides extracted from bacterial sources (dextran, xanthan and hyaluronic acid), from animals (chitin and hyaluronic acid) and from fungi (pullulan and chitosan).



β-(1→4)-D-glucose

Cellulose

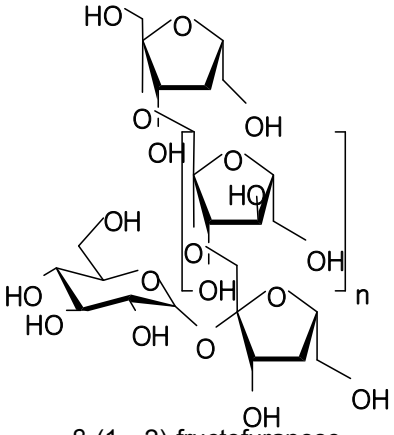
1



β-(1→4)-D- mannose main chain,
D-galactose branches

Guar

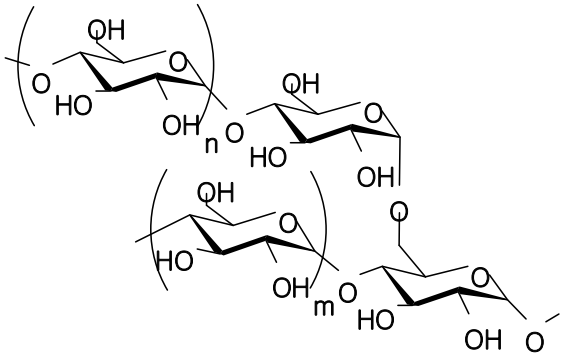
2



β-(1→2)-fructofuranose

Inulin

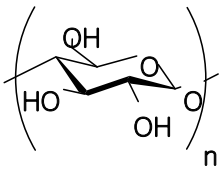
3



α-(1→4)- and α-(1→6)-D-glucose

Amylopectin

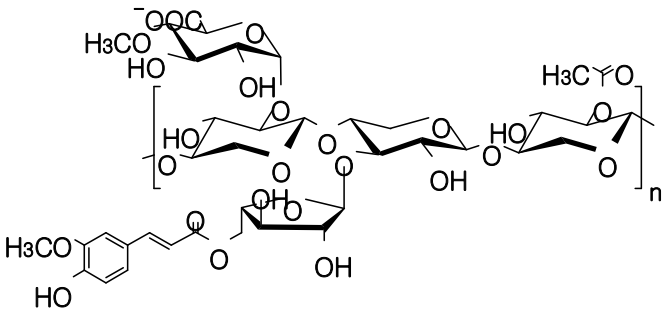
4



α-(1→4)-D-glucose

Amylose

5

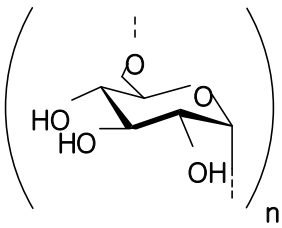


β-(1→4)-D-xylose main chain

Xylan

6

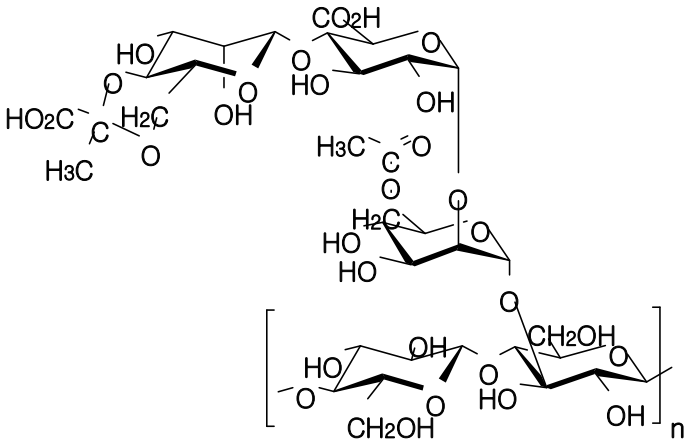
Figure 1.1: Structure of polysaccharides isolated from plant.



α-(1→6)-D- glucose main chain

Dextran

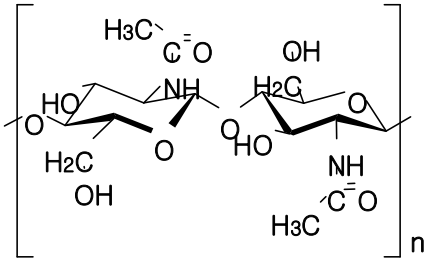
7



β-D-glucose main chain

Xanthan

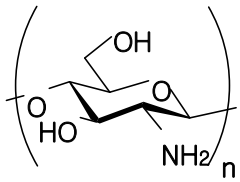
8



β-(1→4)-(N-acetyl)glucosamine

Chitin

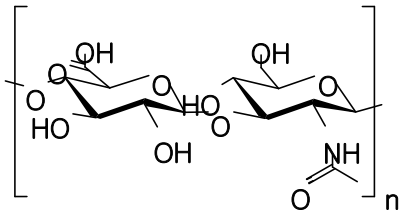
9



β-(1→4)-D-glucosamine

Chitosan

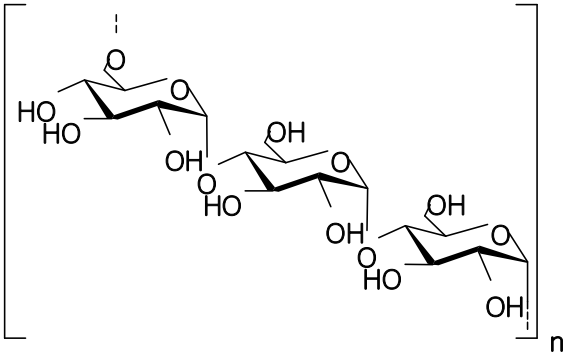
10



β-D-Glucuronic acid,
N-Acetyl-β-D-Glucosamine

Hyaluronic acid

11



α -(1→6) linked maltotriosyl units

Pullulan

12

Figure 1.2: Structure of polysaccharides obtained from bacteria (**7, 8** and **11**), animals (**9** and **11**) and fungi (**10** and **12**).

The solubility of these polysaccharides is different depending on different origins, as listed in **Table 1.1**. Some polysaccharides are insoluble in water (e.g. cellulose and chitin) or not very soluble (e.g. amylose and xylan), therefore, these polysaccharides have to be modified in order to be dissolved in water; moreover, the solubility of the hydrophobically modified polysaccharide with long alkyl chains can be increased by its derivatization with short chain. Most well known derivatives of these polysaccharides are cellulose ethers (methyl-, hydroxyethyl-, hydroxypropyl-, carboxymethyl-cellulose etc.), starch derivatives (hydroxyethyl-, hydroxypropyl-, carboxymethyl-starch, degraded starch, cationic starch, esterified starch etc.) and guar derivatives (hydroxyethyl-, hydroxypropyl-, carboxymethyl-guar, depolymerized guar, cationic guar, etc.).

Table 1.1: Solubility of polysaccharides in dimethylsulfoxide (DMSO), dimethylformamide (DMF) and water. [8,9]

Polysaccharide	**Solubility in different solvent**		
	DMF	**DMSO**	**H_2O**
Cellulose	-	+ (TBAF)	-
Guar	x	+ (TBAOH)	+
Dextran	+ (LiCl)	+ (40 ^oC)	+[a]
Hyaluronic acid	x	+ (NaOH)	+
Starch	-	+ (80 ^oC)	-[b]
Amylose	-	+ (80 ^oC)	+ (70 ^oC)
Amylopectin	-	+ (80 ^oC)	+
Alginate	-	-	+
Chitin	-	-	-
Chitosan	-	-	+

Inulin	+	+	+
Pullulan	+ (80 ^{o}C)	+	+
Xylan	+ (LiCl)	+	- (NaOH)
Xanthan	-	-	+

+. Soluble.

-. Insoluble.

( ). Soluble under the additional condition.

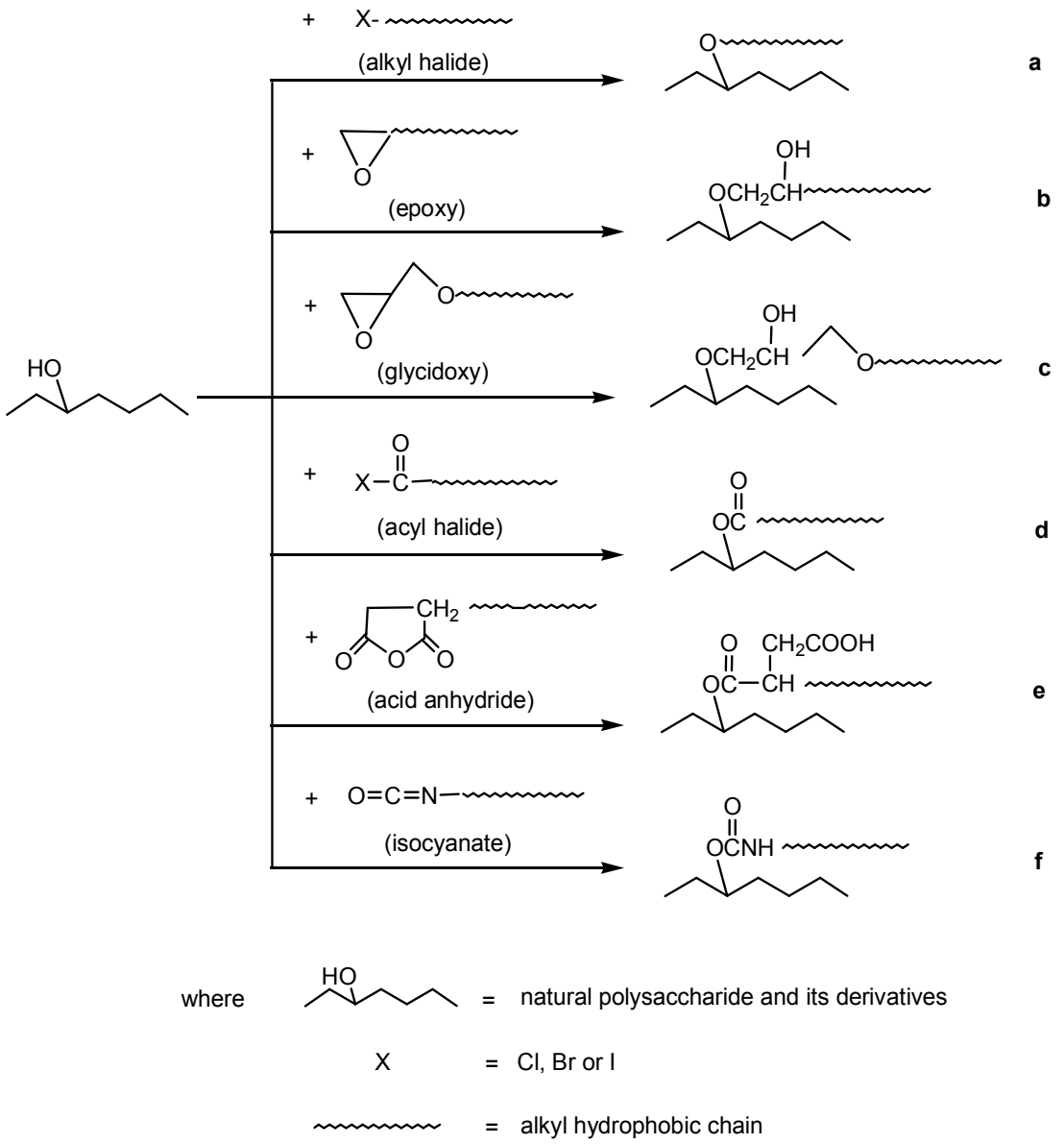
x. No date

a. The crystalline form is insoluble.

b. Amylose is water soluble at 70 ^{o}C.

To handle the incompatibility of the hydrophilic polysaccharide and the hydrophobic reagents, the reactions carried out in both homogeneous media and heterogeneous media were extensively studied. Most of the related literatures of synthesis strategies and procedures are patents.

As shown by **Scheme 1.1**, several typical alkylating agents can be used to prepare hydrophobically modified polysaccharides. These include alkyl halides (such as chlorides or bromides), alkyl or aryl epoxides, acyl halides, anhydrides or isocyanates, which can be reacted with the free hydroxyl groups of the polysaccharide.[6,10] Alkyl amines have also been used to prepare hydrophobically modified derivatives of polysaccharides possessing carboxyl groups along the chain.



Scheme 1.1: Strategy to synthesize hydrophobically modified polysaccharide derivatives by reacting polysaccharides with hydrophobic reagents containing long hydrocarbon chains.[10]

In this chapter, the reaction conditions and results of these strategies will be summarized and represented into four types:

1) Etherification reaction using halogenated derivatives (**Scheme 1.1.a**).
2) Etherification reaction using epoxide derivatives (**Scheme 1.1.b and c**).
3) Ester formation (**Scheme 1.1.d and e**).

4) Alkylation using isocyanate derivatives (**Scheme 1.1.f**).

1.2.2 Etherification using halogenated derivatives

The more common strategy to graft long alkyl chains on polysaccharides is etherification. This reaction provides easy access to a variety of bio-based materials with valuable properties, the etherifying reagents are usually more easily handled than the reagents employed for modification via the ester or urethane linkages and the resulting ether linkage is usually more resistant to further reactions.[11,12]

Etherification of polysaccharides via irreversible nucleophilic substitution using aliphatic halides is shown in **Figure 1.3**. Generally, the polysaccharide is first mixed with strong alkali, e.g., aqueous sodium hydroxide under a nitrogen atmosphere, subsequently, this alkali polysaccharide is reacted with etherifying agent (alkyl halide), to form alkyl ethers.

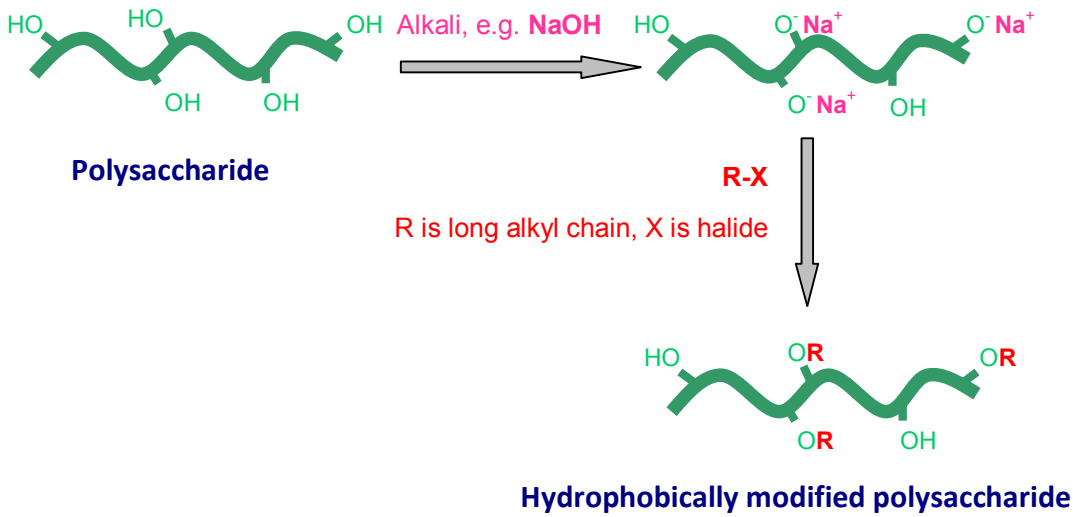


Figure1.3: Etherification reaction of polysaccharides with alkyl halide, represented here as **R-X**, in which R is long alkyl chain, X is halide.

This strategy has been applied on various polysaccharides including cellulose and

derivatives (hydroxyethyl cellulose (HEC), carboxymethyl cellulose (CMC), hydroxypropyl cellulose (HPC)) as summarized in **Table 1.2**, hydroxybutyl guar and, hyaluronic acid. As the alkyl halide agent (**R-X**) listed in **Table 1.2**, **R** is a long alkyl chain containing from 8 to 22 carbon atoms (C_8-C_{22}). Alkyl iodides are excellent alkylating agents, but expensive, therefore the preferred alkylating reagents are alkyl chlorides or bromides.

The reaction conditions are similar to those usually used with short alkyl halides except the solvent. In the case of reactions with short chain (C_1-C_4) alkyl halides, water is used as the solvent [13]. In contrast, reactions with long alkyl halides require the use of an organic solvent such as isopropanol (IPA), *tert*-butyl alcohol (TBA), acetone, *N,N*-dimethylacetamide (DMAc) or dimethylsulfoxide (DMSO) (**Table 1.2**) because of their insolubility in water. These reactions are thus generally performed under heterogeneous conditions as the polysaccharide is suspended in the organic solvent [14]. In some cases, for instance, reactions **No. 2, 5, 7** and **8**, a small amount of water (10~50%, v/v) is added to the organic solvent, allowing the polysaccharide to swell but not to dissolve. This can provide higher reaction efficiency. However, excess of water can result in difficulty of post-treatment of product during purification and recovery.

The pH of the reaction medium must be higher than 11, and is generally adjusted to 13 using strong alkali, such as lithium hydroxide (LiOH), sodium hydroxide (NaOH) or potassium hydroxide (KOH). However, if DMAc is used as a solvent, the addition of an extra base is not required, as can be seen from reactions **No. 3, 4** and **6**. The use of NaH in DMSO appeared to be more efficient than that of NaOH in the same solvent, but the NaH-DMSO system seems to cause more depolymerisation than NaOH-DMSO [15]. In some cases, a phase transfer agent such as tetramethylammonium chloride (TMAC) is used in combination with the base (reaction **No. 2**).

From the examples given in **Table 1.2**, the reactions are generally performed between 70-105 ^{o}C for short time (1-8 hours). The alkylation reactions carried out at low temperature (RT) need longer times (24-92 hours) [14]. As an example, in the case of the reaction of cellulose with octyl bromide at 22 °C, there was no significant substitution after 3 hours, but the degree of substitution (DS) of octyl chain reached 0.11 after 25 hours. With octadecyl bromide, the DS reached 0.10 after 26 hours, and 0.30 after 72 h [16].

The degree of substitution (DS) of polysaccharides with long chain is often lower than 5%, as higher DS lead to problems of water insolubility of the final polysaccharide derivatives. The DS is determined by ^{1}H RMN in $CDCl_3$[16], DMSO-d6[17] or D_2O[18,19].

Table 1.2: Etherification of polysaccharides using alkyl halides.

No.	Polysaccharide substrate	Alkyl halide (R-X, R is long alkyl chain, X is Cl or Br)	Reaction condition: solvent, alkali, temperature, time	DS (%)	Ref.
1	Cellulose	$CH_3(CH_2)_nCH_2Br$, n=6~16	DMSO, NaOH or NaH*, 22 ^{o}C, 15~92 h	11~30*	16
2	CMC (DS=0.55~1.0)	$CH_3(CH_2)_n$ CH_2Br, n=20	50 % (wt) tert-butyl alcohol/ water, LiOH or NaOH (pH=11.4~14), with or without TMAC 90 ^{o}C, 3 h	0.09~2.7	15
3	HEC (MS=1.8)	$CH_3(CH_2)_7CHCH(CH_2)_8Cl$	DMAc, RT, 24 h	1.4	6
4	HEC(MS=2.9~4)	$CH_3(CH_2)$n Br, n=7, 8, 12 or 16 (two or more mixed alkyl halides used at the same)	DMAc, RT, 24 h	0.17~2	20
5	HEC (MS=1.8)	$CH_3(CH_2)_nCH_2Cl$, n=16	88% (wt) IPA/water, NaOH (, pH=13), 80 ^{o}C, 8 h	1	21, 17

6	HPC(MS=4)	$CH_3(CH_2)_nCH_2Cl$, n=16	DMAc, RT, 24 h	1.5	[6]
7	CMHEC (CMMS=0~1.0, HEMS=2.7)	$CH_3(CH_2)_nCH_2Br$, n=14	Water/ *tert*-butanol/ acetone, NaOH(pH=14), 95 ^oC, 2.5 h	0.1~4	[22]
8	Hydroxybutyl guar	$CH_3(CH_2)_nCH_2Br$, n=14	IPA/water KOH(, pH=13.5), 70 ^oC, 1~3 hours	0.5~10	[12, 23]
9	Hyaluronic acid	n-alkanoyl halide (C_4~C_{18})	non-aqueous solvent	--	[24]

1.2.3 Etherification using epoxide derivatives

Epoxide derivatives are the most important intermediates used for the synthesis of hydrophobically modified polysaccharides via ether linkage (**Scheme 1.1.b and c**). Two kinds of epoxide derivatives have been investigated: alkyl epoxide and alkyl glycidyl ether (2-alkyloxymethyloxiranes) (**Figure 1.4**). The "alkyl epoxide" or "alkyl glycidyl ether" are abbreviated to "**R**-epoxide" or "**R**-glycidyl ether", in which **R** could be not only long alkyl chain **(ii)** but also phenyl **(i)**, or poly(ethylene oxide) alkyl chain **(iii)**, or poly(ethylene oxide) phenyl alkyl chain **(iv)**.

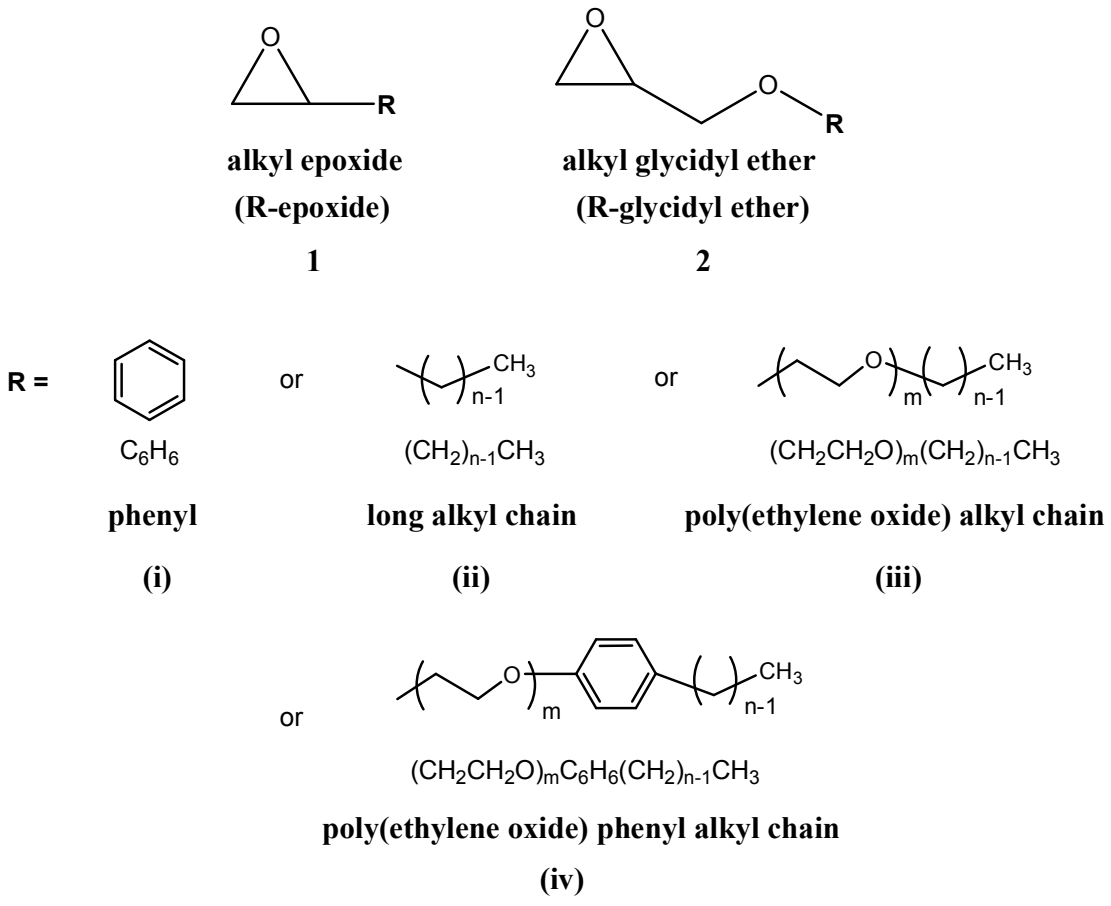


Figure 1.4: Two kinds of epoxide derivatives used to prepare hydrophobically modified polysaccharides: alkyl epoxide (**R**-epoxide) and alkyl glycidyl ether (**R**-glycidyl ether).

The epoxide derivatives **(i)** and **(ii)** is commercially available while **(iii)** and **(iv)** need to be synthesized via the reaction of alcohols with epichlorohydrin. Typically, the hydroxyl group of the alcohol is reacted with NaH in DMF and epichlorohydrin is then added[25].

The ring opening of epoxides requires a catalyst in order to achieve nucleophilic addition. Generally, the polysaccharide is first mixed with alkali base, such as NaOH, KOH, NaH, or tetrabutylammonium hydroxide (TBAOH), under a nitrogen atmosphere, and the etherifying agent (alkyl epoxide or alkyl glycidyl ether) is subsequently added to the reaction medium to form alkyl ethers (**Figure 1.5**).

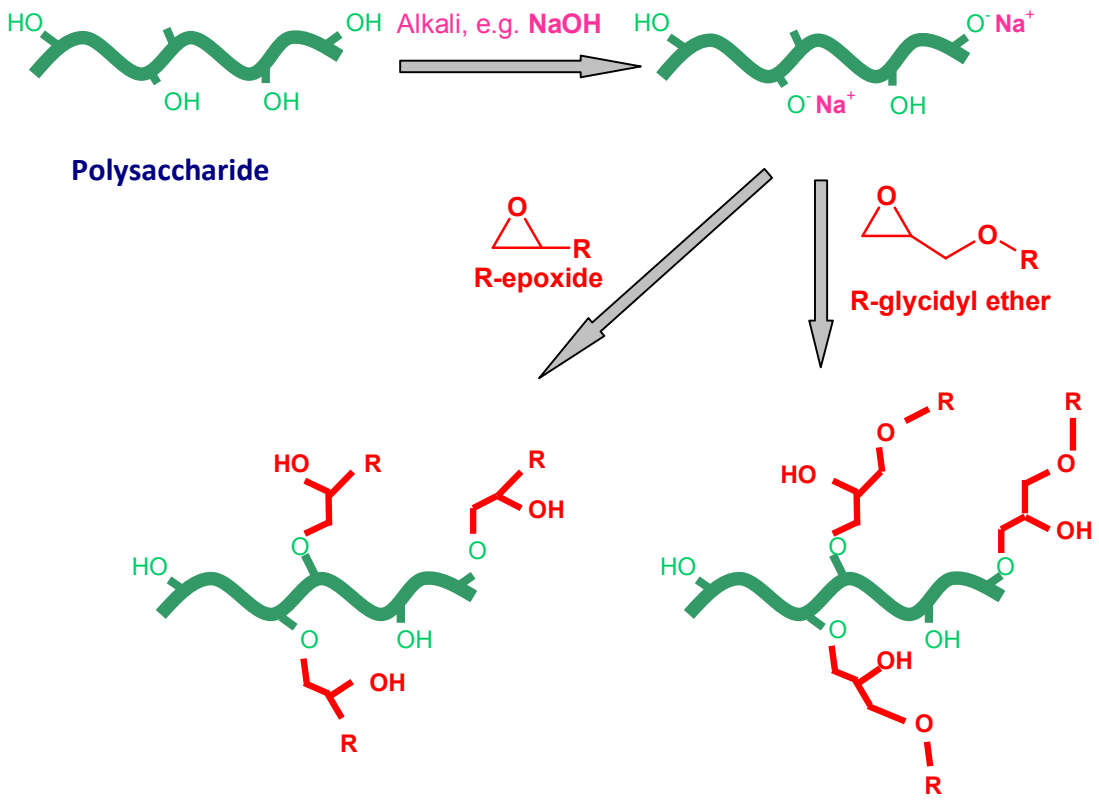


Figure1.5: Etherification reaction of polysaccharides with alkyl epoxide (**R**-epoxide) and alkyl glycidyl ether (**R**-glycidyl ether).

Similar to alkylation reactions with alkyl halides, the ring opening of epoxides by polysaccharides can be performed under homogeneous or heterogeneous conditions. The etherification of polysaccharides in different reaction conditions are summarized in **Table 1.3**. This table was arranged according to the different sorts of polysaccharide substrates used: cellulose and its derivatives (reactions **No. 1-5**), guar and its derivatives (reactions **No. 6**), dextran and its derivatives (reactions **No. 7-9**), starch (reactions **No. 11**) and hyaluronic acid (reactions **No. 12-13**).

Table 1.3: Etherification of polysaccharides using aliphatic or aromatic epoxide derivatives.

No.	Polysacch. substrate	Epoxide derivatives	Reaction condition*: solvent, alkali, temperature, time	DS (%)	Ref.
1	MeC (MS=1.8) or HPC (MS=3.5) or MeHPC (MMS=1.3, HPMS=0.2)	CH_3 $(CH_2)_{n-1}$ epoxide, n=8~24 or CH_3 $(CH_2)_{n-1}$ glycidyl ether, n=8~18	50~90% IPA, NaOH (pH=13 or 14), 75~80 °C, 2~8 h	0.25~2.9	6,17,21,26
2	HEC (MS=1.8~2.5)	$CH_3(CH_2)_{n-1}(O\ CH_2CH_2)_m$ glycidyl ether, n=8~18, m=9~20	50~88% IPA, NaOH (pH=13), 80 °C, 2~8 h	0.32~2	27
3	HEC (MS=3.5~4.5)	CH_3 $(CH_2)_{n-1}$ epoxide, n=16 or $(C_nH_{2n+1})_x(C_6H_6)(OCH_2CH_2)_m$ glycidyl ether, n=9 or 12, x=1 or 2, m=0-100	90% TBA, NaOH (pH=13.5), 75~90 °C, 4.5~12 h	0.25~1	28
4	CMC (DS=0.55~0.9)	CH_3 $(CH_2)_{n-1}$ epoxide, n=18 or CH_3 $(CH_2)_{n-1}$ glycidyl ether, n=12 or $CH_3(CH_2)_n(OC_pH_{2p})_m$ glycidyl ether or epoxide, n=1-30, p=2-4, m=0-20 e.g.: Brij 76^R or Brij 30^R	42~50% TBA, LiOH or NaOH (pH=12.2~14), with or without TMAC, 75~90 °C, 3~4 h	0.014~5.6	15
5	HEC (MS=2.5)	CH_3 $(CH_2)_{n-1}$-epoxide, n=10~24	DMSO, potassium *tert*-butoxide 80 °C, 6 h,	3.5~5.8	6

6	HPG (MS=0.7, 1, 1.2 or 1.3)	CH_3 $(CH_2)_{n-1}$ epoxide, n=12, 14, 18 or 24~28 CH_3 $(CH_2)_{n-1}$ glycidyl ether, n=18 or 22	50~100% IPA, NaOH (pH=13.3~14), or KOH, 60~80 °C, 1.5~6 h	0.04~1	29-32
7	Dextran (T40)	Phenyl glycidyl ether	water, NaOH (pH=14), RT/ 50 °C / 75 °C, 48 h~96 h	6~31	5, 33-36
8	Dextran (T40)	Phenyl glycidyl ether	0.25-3, DMSO TBAOH, RT, 96 h, (dialysis)	17~250	5
9	Dextran (T40)	CH_3 $(CH_2)_{n-1}$ epoxide, n=10	DMSO, TBAOH, 50 °C, 96 h	164	36
10	Hydroxypropyl dextran	CH_3 $(CH_2)_{n-1}$ epoxide n=11~18	DCM BF_3, RT, 20 minute	--	37
11	HA	CH_3 $(CH_2)_{n-1}$ glycidyl ether, n=4, 6, 8, 12 or 14	DMSO/water = 1/1, NaOH (pH=9.0-10.0), 30 °C, overnight	11	38
12	Amylose or starch or degraded starch	CH_3 $(CH_2)_{n-1}$ epoxide, n=6 or 12	DMSO, NaH, RT, 6~286 h	30~230	39
13	Potato starch	CH_3 $(CH_2)_{n-1}(OC_2H_4)_m$ glycidyl ether, n=12, m=10 or 12	80% IPA, NaOH (pH=13), 80 °C, 8 h	0.5 or 0.7	27

* For avoiding oxidative degradation, in all the reactions listed, the oxygen is expediently removed from the reaction mixture by evacuation and flushing with nitrogen.

In the heterogeneous conditions, the etherification is carried out in an alcohol/water mixtures (reactions **No. 1-4, 6** and **13**), in the presence of NaOH or KOH.

In the alcohol/water mixtures, the concentration of the alcohol is from 50% to 100% (v/v), and generally about 90%. Such concentrations are suitable for swelling and suspending the polysaccharide powder and dissolving the long chain alkyl epoxide derivatives and base at the same time. By using this type of solvent, the long chain alkyl epoxide derivatives can be reacted with the polysaccharide so that the alkylation reaction can take place.[6,15,17,21,26,27,30-32,40,41] The alcohol-based reaction media have notable advantages. They are relatively low cost, non-toxic and the modified polysaccharide can be easily isolated by filtration.

Besides using NaOH (or LiOH, pH=12.2~14), phase transfer catalysts (TMAC), were used in the heterogeneous reactions (reaction **No. 4**).

The heterogeneous reactions are performed at temperatures ranging from 55 ^{o}C to 90 ^{o}C for 1.5 ~ 16 h. The preferred conditions in most of the reactions are 80 ^{o}C for about 8 h. After the reaction, the DS of the alkylated polysaccharides are not more than 1%; higher DS generally lead to water-insoluble polysaccharide derivatives. In homogeneous conditions, the polysaccharide is generally solubilized in DMSO (reactions **No. 5, 8, 9** and **12).**

Potassium *tert*-butoxide, tetrabutylammonium hydroxide (TBAOH) or NaH are used as a basic catalyst (reactions as examples **No. 5, 8, 9** and **12**). In DMSO, they have special virtues. For potassium *tert*-butoxide, DMSO interacts with the potassium center, enhancing the basicity of the butoxide. TBAOH is more soluble in organic solvents relative to more conventional inorganic bases. NaH is insoluble in organic solvents, all reactions involving NaH occur at the surface of the solid, and NaH is propitious to keep the anhydrous conditions.

Theoretically, the homogeneous procedure is expected to provide higher reaction efficiency and higher DS than the heterogeneous one. Moreover, it is assumed to favor a random distribution of alkyl groups along the polysaccharide chain as the polysaccharide and alkylating agent are soluble in the same reaction medium. In these conditions, the modified polysaccharide is isolated at the end of the reaction by precipitation or by dialysis and freeze-drying.

The higher efficiency of the homogeneous procedure was confirmed by Durand et. al.[42] In the case of dextran modified by phenyl glycidyl ether (PGE) at room temperature for several hours, at a given amount of PGE, the DS obtained is much higher with the homogeneous procedure than with the heterogeneous one. This can be explained by a partial hydrolysis of the epoxide when using the heterogeneous procedure. Around 70% of the added PGE is grafted onto dextran at the end of the reaction with the homogeneous procedure, while it is only 10% with the heterogeneous procedure.[42]

However, the use of such strong base often causes partial degradation of the polysaccharide, thereby causing the formation of polysaccharide derivatives having molar masses lower than desired. Comparing with the heterogeneous conditions, DMSO is more expensive than the alcohol solvent, the reaction needs anhydrous conditions and the treatment for recovering the polysaccharide is more complicated. These disadvantages limit the industrialization of this strategy.

There are still other types of solvent used individually, for example, a mixture of DMSO and water was used to modify hyaluronic acid (HA) with alkyl glycidyl ethers (reaction **No. 11**). Effect of concentration of DMSO in reaction mixture on product features (for decyl derivative) showed that increasing amount of DMSO from 20 % to 90 % resulted in increase of DS from 17 % to 78 % but slight decrease of Mw.

1.2.4 Esterification using hydrophobic carboxylic acid derivatives or alkyl halides

Esterification reactions have also been used to prepare alkyl derivatives of polysaccharides. These consist in reacting either a neutral polysaccharide with an acyl anhydride (**Figure 1.6 (i)**) or an acyl halide (**Figure 1.6 (ii)**) or, an acid polysaccharide (i.e. hyaluronic acid) with an alkyl halide (**Figure 1.7**). In all cases, the reactions are performed in an organic solvent such as DMSO or DMF. Therefore, in order to make the acid polysaccharide soluble in DMSO or DMF, it is preliminary transformed into the tetrabutylammonium (TBA) salt to shield highly anionic carboxylic acid groups and to disrupt inter/intramolecular hydrogen bonds (**Figure 1.7**). The esterification of polysaccharides in different reaction conditions are summarized in **Tables 1.4**, ranged by different esterifying reagents used.

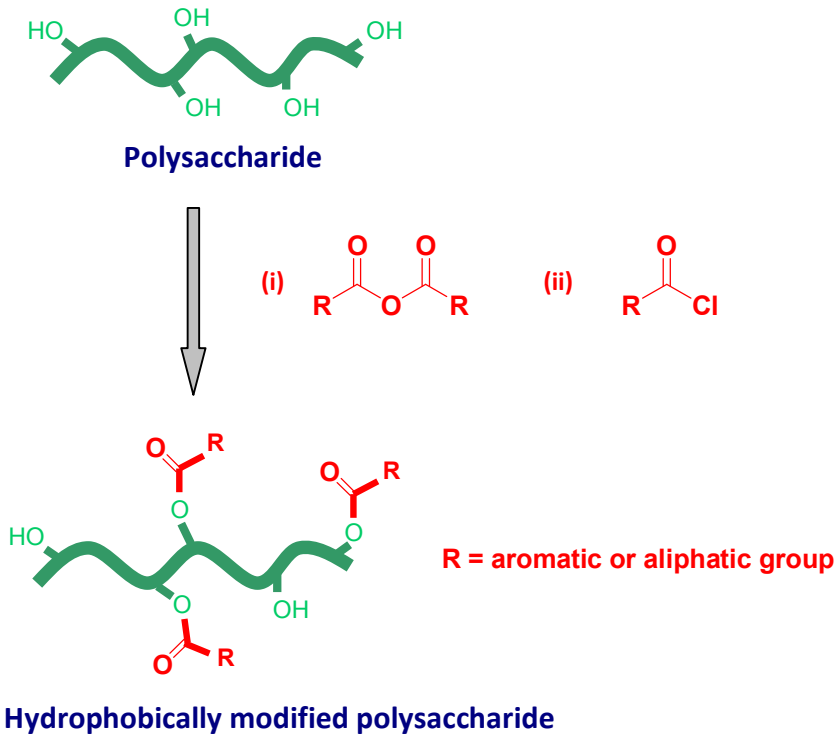


Figure1.6: Esterification of neutral polysaccharides with hydrophobic carboxylic acid derivatives with **(i)** acyl anhydride or **(ii)** acyl halide.

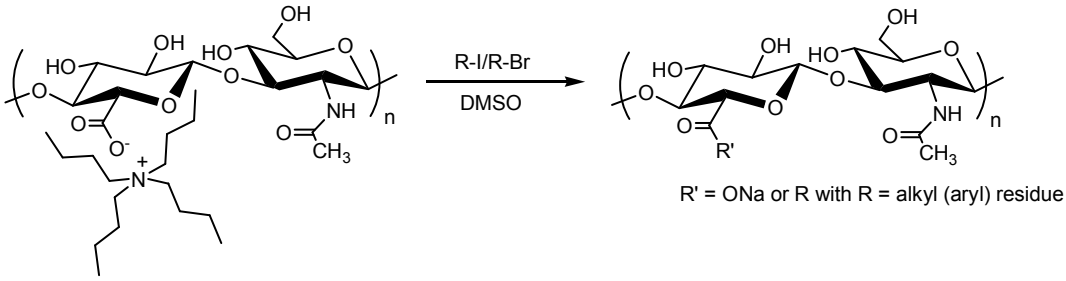


Figure1.7: Esterification of acid polysaccharides (i.e. hyaluronic acid) with alkyl (aryl) halides.

Table 1.4: Esterification of polysaccharides.

No.	Polysaccharide substrate	Esterifying reagent (Figure 1.8 and 1.9)	Reaction condition: Medium, Catalyst, Temperature, Time	DS (%)	Ref.
1	Starch	carboxylic acid anhydride **15-23** or **24**	DMSO, DMAP with or without $NaHCO_3$, 30 °C, 1h	140~260	43
2	Inulin	carboxylic acid anhydride **15-23** or **24***	pyridine, without or with* DMAP, 40 or 60 °C, 24 h	3~6 or 40~230*	44
3	a-cellulose	Soybean fatty acid chloride	DMF/pyridine, 90°C, 24 h	80-270	45
4	Dextran	Carboxylic acid chloride **25-27**	formamide or NMP or DMSO, sodium bicarbonate, 45 °C, 48 h	10~40	46
5	Starch	Stearoyl chloride **28**	Water, NaOH, with or without pyridine/ or TEA or DIEA, RT, 1 h	10-29	47
6	Carboxymethyl pullulan (CMP)	Alkyl bromide (C_8, C_{10} or C_{12}) or/and $C_6H_5(CH_2)_3Br$	Firstly to form a tetrabutylammonium salt of CMP, then react with alkyl bromide, DMSO, 40 °C, overnight	2.8~48	48

7	HA	Alkyl bromide (C_{12} or C_{18})	Firstly to form a tetrabutylammonium salt of HA, then react with alkyl halide, DMSO, RT, 24 h	1-8	[18,19, 49]
8	Cellulose	carboxylic acid: $CH_3(CH_2)_nCH_2COOH$, n=9,11,15,17	Py/TsCl=3, DMAc/LiCl, 50~65 ^oC, 24 h	280~290	[50]
9	Starch (Potato)	Octenylsuccinic anhydride or Dodecenylsuccinic anhydride	Water, NaOH (Ph=8.5), 25 ^oC, 4 h	0.5-2.3	[51]

--. no data.

Esterification with an acid anhydride in the presence of a tertiary base, commonly pyridine (Py) [52], has been applied to prepare alkyl derivatives of neutral polysaccharides (reaction **No. 1** and **2**). As can be seen from reaction **No. 2**, pyridine has been used as the slurry medium. Long-chain fatty acid cellulose esters from soybean fatty acids can be obtained applying the DMF/Py mixture (reaction **No. 3**). It should be noticed that the utilization of an excess of base diminishes the DS values [53]. Another tool to increase the reactivity of this acylation system is the addition of 4-dimethylaminopyridine (DMAP), a useful nucleophilic catalyst [54] for highly functionalizing polysaccharide [55].

For the introduction of more complex carboxylic acid moieties, i.e. fatty acid or aromatic moieties, anhydrides are not reactive enough. In these cases, acid chlorides in combination with a base are applied (reactions **No. 3** and **4**). The esterification of polysaccharide with acyl chlorides is combined with less side reactions if conducted in DMF, *N*-Methy1-2-pyrrolidone (NMP) or *N, N*-dimethylacetamide (DMAc) instead of in DMSO. LiCl is added when cellulose is used (reaction **No. 8**) in order to make the homogeneous esterification into an efficient synthesis which allows excellent

control of the DS in the range from 1 to 3 [56-58]. Although DMAc/LiCl is among the best studied solvents because it dissolves a wide variety of polysaccharides including cellulose and does not cause degradation, it is not known how DMAc/LiCl dissolves polysaccharides.

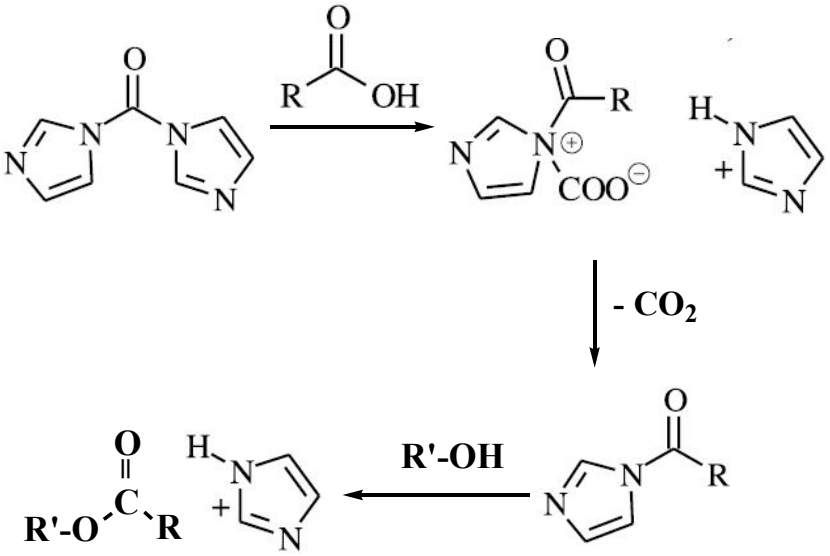
Despite the fact that water is commonly not an appropriate medium for esterification reactions, a number of polysaccharide esters may be obtained in this solvent, owing as it provides an economical and easy method. Acylation in aqueous media with aromatic acid chlorides (reaction **No. 5**), e.g. benzoyl chloride [8,59] or acyl imidazolides, can be carried out well [60]. Esters of other aliphatic acids are prepared in a similar manner using the appropriate anhydride [61] (reaction **No. 9**).

To reach higher degree of hydrophobic modification than via direct esterification and avoid water-insoluble derivatives, the polysaccharide is converted into the acid form firstly, subsequently into the tetrabutylammonium salt, and finally this salt is converted homogeneously in DMSO with long-chain alkyl bromides (reaction **No. 6** and **7**) [18,19,49].

The methods described above are well-established, reproducible tools for the synthesis of defined polysaccharide esters of pronounced commercial importance. In 2006, Thomas Heinze et. al. have given a review of these new esterifications of polysaccharides [62]. Still, these synthesis methods are limited for the preparation of common aliphatic and aromatic carboxylic acid esters. To achieve acylation, a broad variety of new synthesis paths are under investigation.

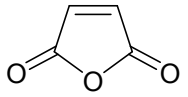
The application of a sulphonic acid chloride, especially 4-toluenesulfonyl chloride (TsCl), for the in situ activation of carboxylic acids is an easy procedure, valuable for the preparation of long-chain aliphatic and alicyclic esters of polysaccharides in DMAc/LiCl [63,64], as shown in reaction **No. 8** in **Table 1.4**. Application of *N,N'*-dicyclohexylcarbodiimide (DCC), in the presence of DMAP, for the in situ activation of carboxylic acids is an easy procedure. Functionalisation with bulky

hydrophobic carboxylic acids (such as bile acid, heptadecafluoroundecanoic acid, adenine-9-butyric acid or thymine-1-yl butyric acid)/DCC was studied for the synthesis of amphiphilic dextran [65]. In contrast to DCC or TsCl as reagents for in situ activation, the *N*, *N*-carbonyldiimidazole (CDI) is associated with no significant side reactions [63], even when DMSO is used as solvent, if the CDI is completely transformed to the imidazolide in the first step (**Scheme 1.2**).

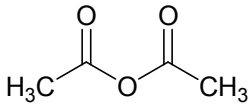


Scheme 1.2: Reaction pathway leading exclusively to esterification if the polysaccharide is treated with CDI in the first step (adapted from [66]).

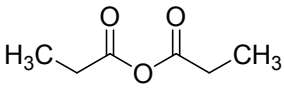
In summary, new state-of-the-art esterifications can yield a broad spectrum of polysaccharide derivatives, but are currently only used under lab-scale conditions.[8,61]



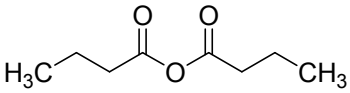
Maleic anhydride
15



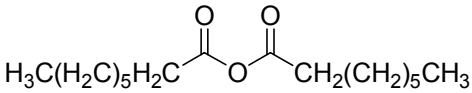
Acetic anhydride
16



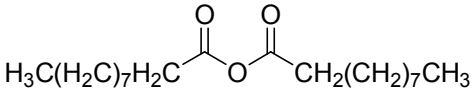
Propionic anhydride
17



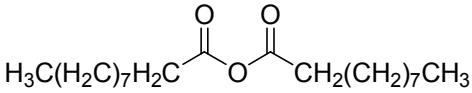
Butyric anhydride
18



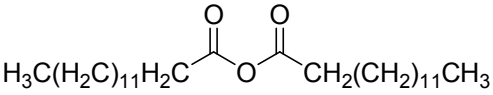
Caprylic anhydride
19



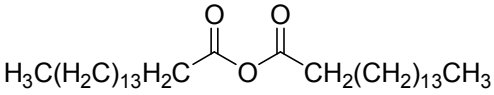
Capric anhydride
20



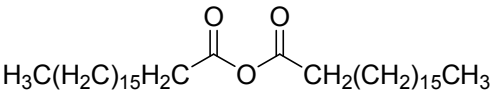
Lauric anhydride
21



Myristic anhydride
22

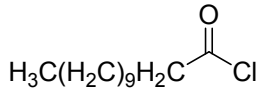


Palmitic anhydride
23

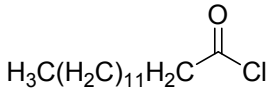


Stearic anhydride
24

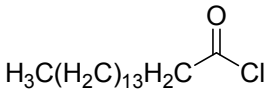
Figure1.8: Esterifying reagent: (i) Carboxylic acid anhydride.



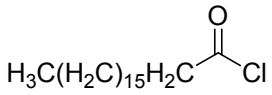
Lauric chloride
25



Myristic chloride
26



Palmitic chloride
27



Stearoyl chloride
28

Figure1.9: Esterifying reagent: (ii) Carboxylic acid chloride.

1.2.5 Alkylation (arylation) using isocyanate derivatives

Polysaccharide has been also alkylated using aromatic or aliphatic isocyanates [46,47] as shown in **Figure 1.10**.

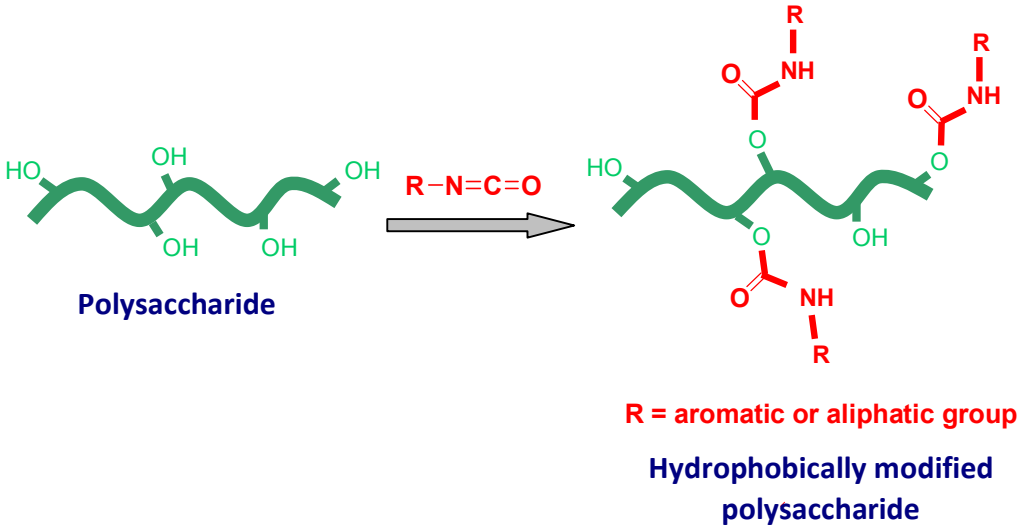


Figure1.10. Carbamoylation reaction of polysaccharide.

As isocyanate groups will also react spontaneously with water, the modifications of polysaccharide by carbamoylation are usually performed in organic solvents.

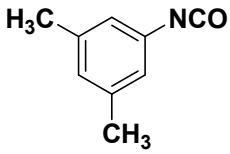
Examples of hydrophobization reactions of polysaccharides using isocyanate in organic solvents are given in **Table 1.5**. Additionally, the structures of some isocyanate reagents are displayed in **Figure 1.11**.

Table 1.5: Synthesis of hydrophobically modified polysaccharides using isocyanate derivatives.

No.	Polysaccharide substrate	isocyanate reagent	Reaction condition*: Ratio of Isocyanate / Polysaccharide, Medium, Catalyst, Temperature, Time	DS (%)	Ref.

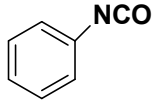
1	Cellulose	3,5-Dimethylphenyl isocyanate: **29**	3.4mol/1mol, pyridine, 90~100 ^{o}C, 12 h	340	[68]
2	Cellulose, Amylase, Xylan, Chitosan, Dextran, Inulin	Phenyl isocyanate: **30**	10~30 eq/1 eq, pyridine, 80~110 $^{\circ}$C, --	>85	[69]
3	Inulin	Octylisocyanate: **31**	0.6, DMF, 70 $^{\circ}$C, 24 h	56	[70]
4	Inulin	Alkyl isocyanate ($C_{4\sim18}$)	different dosage, *N*-methylpyrrolidone or DMF, 80 ^{o}C, 24 h	DS = matching of equivalents of isocyanate added±10%	[71]
5	Starch (containing 28% of amylose)	Phenyl isocyanate: **30**	0.6 g/ 0.25g, methylene chloride or xylene, DBTL, at RT for 24 h or at 80~90 ^{o}C for 75 minutes	--	[72]
6	Pullulan	N-(6-isocyanotohexyl) cholesteryl carbamate: **32**	0.013, DMSO, pyridine, 90 $^{\circ}$C, 3 h	1.1	[73]
7	Hyaluronic acid	Alkyl isocyanate: **31, 33**	1 or 0.5 or 0.1, DMSO, DBTL, 65 ^{o}C, 8 h	100 or 50 or 10	[74]

* All the reactions were carried out under a nitrogen atmosphere.



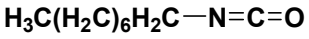
3,5-Dimethylphenyl isocyanate

29



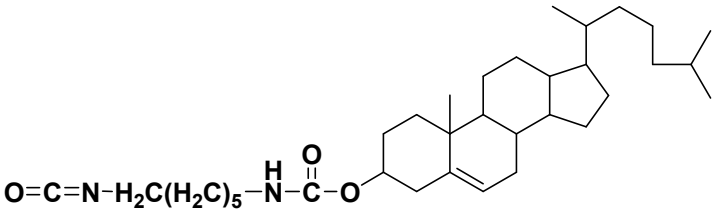
Phenyl isocyanate

30



Octylisocyanate

31



N-(6-Isocyanatohexyl) cholesteryl carbamate

32

Dodecyl isocyanate

33

Figure1.11: Isocyanate reagent.

In most protocols, DMSO was used as the derivatization solvent, whatever the organic solvent used, anhydrous conditions are required.

There are two broad categories of polyurethane catalysts, amine compounds and organometallic complexes. The latter ones are very highly selective towards the polysaccharide/isocyanate reaction. As shown in **Table 1.5**, in reaction **No. 5** and **7**, isocyanate reagent were used as received in conjunction with commercial dibutyltin dilaurate (DBTL) as catalyst.

The main disadvantage, however, is polysaccharide degradation upon carbamoylation. Furthermore, despite the advances made in the art of alkylation using isocyanate derivatives, many of the approaches discussed above involve the use of relatively expensive materials which can be complicated to utilize in a commercial size facility.

From all four kinds of strategies to hydrophobically modify polysaccharide introduced above, it could be found that most of the literatures are investigated on cellulose. Most of the detailed synthesis methods are in patent, especially for guar, most of the

literatures referring to guar are patents.

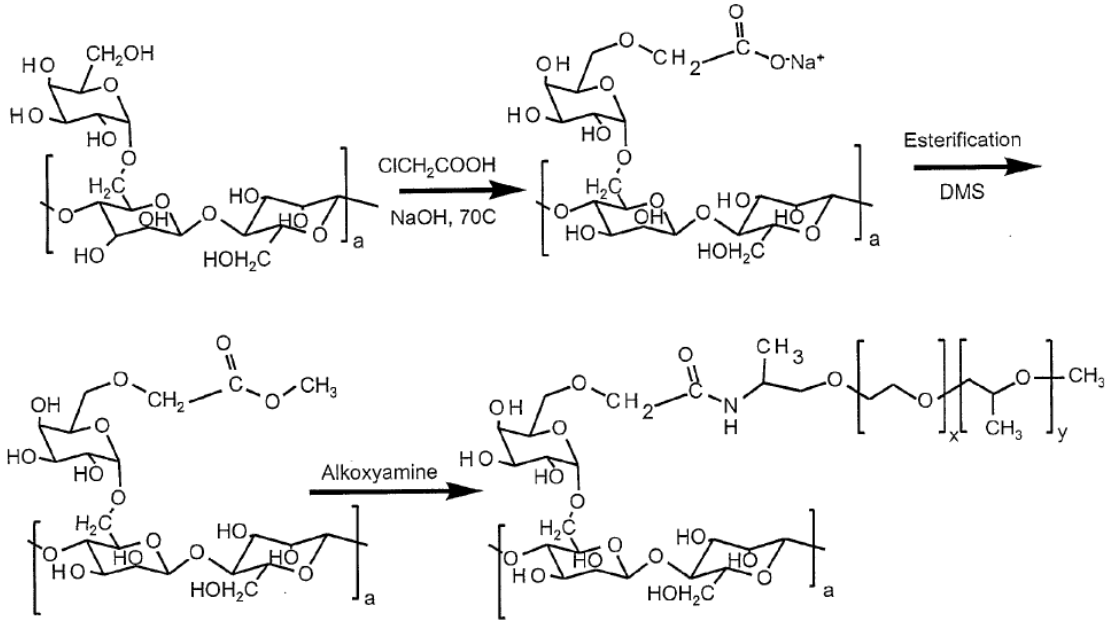
1.2.6 Hydrophobization by amine-acid coupling reactions

Amine-acid coupling reactions using alkylamines or alkyl amino acids have been used as an alternative strategy for the hydrophobization of acidic polysaccharide (**Table 1.6).**

Table 1.6: Hydrophobically modification of polysaccharides using amine derivatives.

No.	PS (Polysaccharide substrate)	Halogenated derivatives	Reaction condition*: Medium, Catalyst, Temperature, Time	DS (%)	Ref.
1	Carboxymethyl guar or Carboxymethyl hydroxypropyl guar	Polyoxyalkyleneamines	DMSO, 90~95 ^{o}C, 24~48 h	18~41	75
2	High-methoxyl citrus pectin (DM=78)	Long alkyl chain amine (C12, C16 or C18)	DMF, RT, 90 minutes	11	76
3	Carboxymethyl pullulans acid (DS=0.76 to 0.84)	Hexadecylamine	DMSO, DCC, RT, 48 h	1.3 to 6.8	77
4	Sodium dextran succinate (DS=1.04)	Dodecylamine	DMF, NMM and EtOCOCl, or TEA and PTSA, 10 ^{o}C, 3 h	10	78 79
5	HA tetrabutylammonium salt	Hexadecylamine	DMSO, methanesulfonic acid and CDI, 42 ^{o}C, 16 h	2.4	80

It consisted of the nucleophilic displacement of some of the polysaccharide's methyl esters by a given long chain alkylamine without coupling reagent (reaction **No. 1** and **2**). Synthesis of guar polyoxyalkylamine derivatives (reaction **No. 1**) is depicted and the structures of several polyoxyalkyleneamines are given below (**Scheme 1.3**) [75].



Polyoxyalkyleneamines Name	Structure	Ratio PO/EO (y/x)	Approximate Mol. Wt.
XTJ-505 (M-600)	CH_3—[OCH_2—CH^2]$_x$—[OCH_2—$CH(CH^3)$]$_y$—NH_2	9/1	600
Jeffamine M-715	CH_3—[OCH_2—CH^2]$_x$—[OCH_2—$CH(CH^3)$]$_y$—NH_2	2/11	715
XTJ-506 (M-1000)	CH_3—[OCH_2—CH^2]$_x$—[OCH_2—$CH(CH^3)$]$_y$—NH_2	3/19	1000
Surfonamine L-300	CH_3—[OCH_2—CH^2]$_x$—[OCH_2—$CH(CH^3)$]$_y$—NH_2	8/58	3000
Surfonamine MNPA-1000 (B100)	C_9H_{19}—C_6H_4—O—[$CH_2CH(CH_3)$]$_{12.5}$—$OCH_2CH(CH_3)$—NH_2	—	1004
Surfonamine ML-300 (B30)	$CH_3(CH_2)_{12}OCH_2CH(CH_3)$—$OCH_2CH(CH_3)$—$NH_2$	—	325

Scheme 1.3: Synthesis of guar gum polyoxyalkylamine derivatives. Guar was first reacted with sodium chloroacetate in presence of NaOH to obtain sodium carboxymethyl guar (NaCMG). NaCMG then reacted with dimethyl sulfate (DMS) to obtain methyl carboxymethyl guar (MCMG). At last, polyoxyalkyleneamine (see the table) was added to react for 24-48 hours at 90-95° C to obtain [75].

N,N'-Dicyclohexylcarbodiimide (DCC), 1,1'-Carbonyldiimidazole (CDI) compound with methanesulfonic acid, N-methylmorpholine (NMM) compound with isobutylchloroformate (EtOCOCl), triethylamine (TEA) compound with p-toluenesulfonic acid (PTSA) are used as catalyst for the coupling of amino acids for peptide synthesis (reaction **No. 3, 4** and **5**).

Hydrophobically modified polysaccharide by amine derivatives could reach a rather high hydrophobic modification ratio, and the product possess some outstanding rheology behaves. However, these reactions need multiple steps and additional purification measures since the selectivity can be problematic [81-84]. Moreover, these modification steps were slightly degrading towards the polysaccharidic backbone.

In order to avoid the problems with solvent removal, our group proposed an alkylation procedure for HA having the advantage of producing selectively modified HA in aqueous solution under mild conditions. This was based on the selective functionalization of HA by reactive dihydrazide groups [85,86] followed by the coupling with an aldehydic alkyl chain using reductive amination conditions (**Fig. 1.12**).

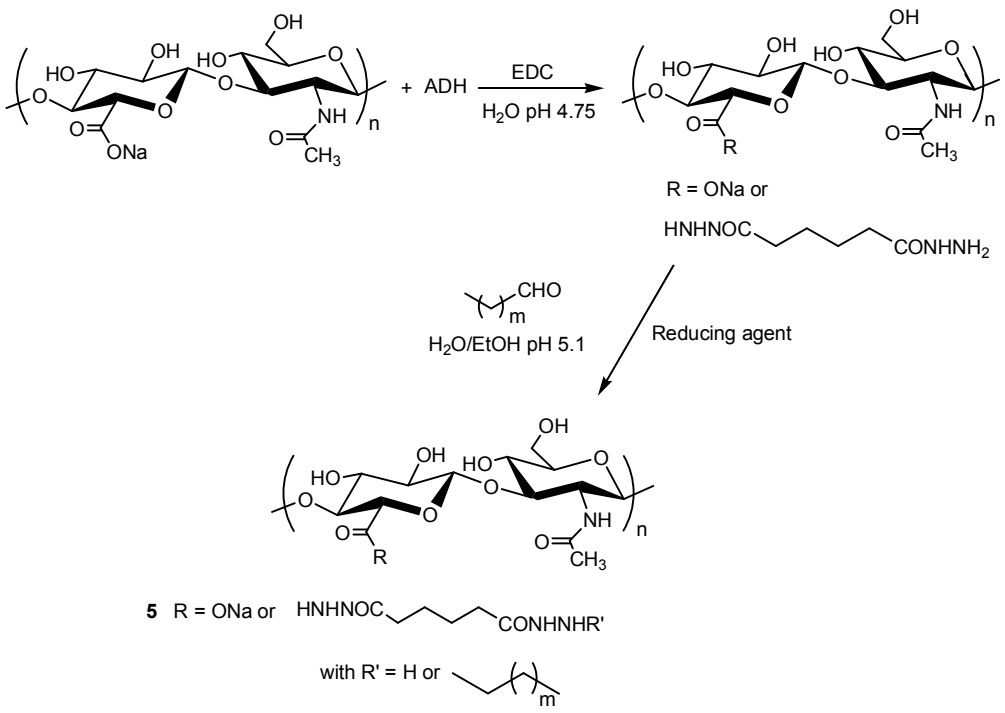


Figure 1.12: Synthesis of alkylamino hydrazide HA derivatives. HA is first modified by hydrazide groups based on its coupling reaction with adipic acid dihydrazide (ADH) using 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide (EDC). The resulting

HA derivative is then reacted with aldehydic alkyl chains (m = 4, 6, 8) under reductive amination conditions.

1.3 Associating properties in aqueous solution

As mentioned in the literatures [6,23,87-90], hydrophobically modified polysaccharides that associate in solution are often efficient rheology modifiers. Despite the fact that the substitution degree of hydrophobic groups is low, aqueous solutions of the modified polysaccharide derivatives usually exhibit interesting rheological behaviors such as a much stronger viscosity enhancement with increasing polysaccharide concentration as compared with the corresponding system with the unmodified parent polysaccharide.

As used as thickener, their solution properties have been largely examined on different hydrophobic substituent, on different molar substitution levels [91], on the effects of operating parameters and so on [92,93].

It should be noted that for a given polymer concentration radical changes in inter- and intramolecular associations may be observed by modifying the nature of the hydrophobic group or the molar mass of the polysaccharide. Moreover, the strength of the associations can be also modulated by adding cosolute molecules such as surfactants or cyclodextrins to the polymer solution. In aqueous mixtures of a hydrophobically modified polysaccharide and a surfactant, the association strength can be increased or weakened depending on the level of surfactant addition [41]. The addition of cyclodextrin molecules to the polymer solution provides decoupling of hydrophobic interactions via inclusion complex formation with the polymer hydrophobic moieties, and this leads to a dramatic reduction of the viscoelastic response [41]. Since this subject has been developed in a recent article, this section will focus on the behavior in aqueous solution of hydrophobically modified derivatives of neutral and charged polysaccharides alone, i.e. cellulose, starch, dextran, guar and HA.

1. Cellulose

Cellulose derivative is the mostly important bio-thickener and have been widely used. Landoll (1980) first disclosed that hydrophobically modified nonionic low molecular weight cellulose containing large hydrophobic groups, were capable of producing viscous aqueous solutions [6]. The viscosity of the modified cellulose solution can be influenced by several parameters as discussed below.

Solution viscosity of hydrophobically modified (HM)-HEC was shown to increase with the increase of degree of substitution as shown in **Figure 1.13**. HM-1-HEC and HM-2-HEC are hydroxyethyl celluloses modified with glycidyl hexadecyl ether with DS of 1% and 2 %, respectively.[94]

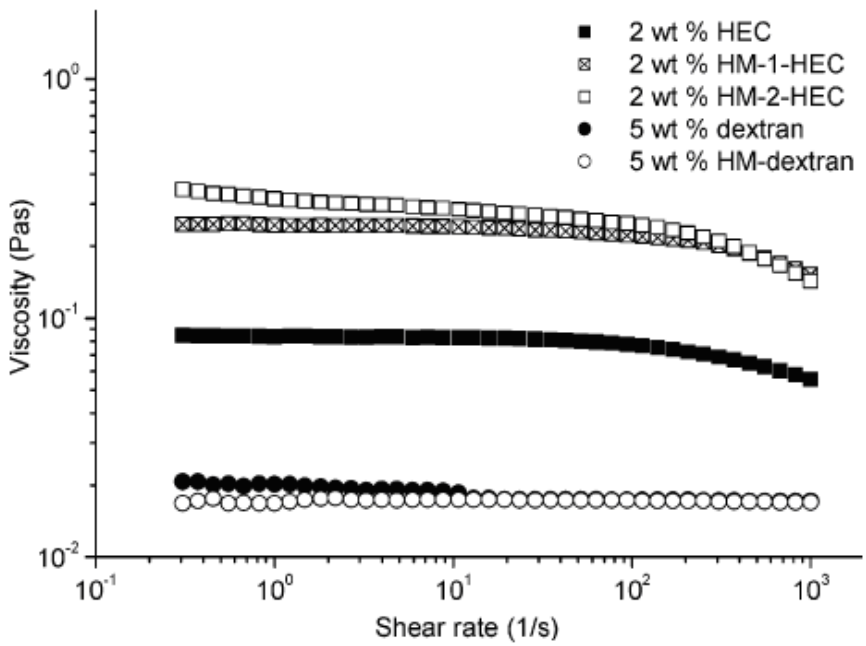


Figure 1.13: Shear rate dependence of the viscosity in 1 M NaOH for 2 wt % solutions of HEC, HM-1-HEC, and HM-2-HEC and for 5 wt % solutions of dextran and HM-dextran. [94]

The viscosity-concentration relationships for cellulosic associative thickeners have been studied a lot [95-98]. **Figure 1.14 a) and b)** showed the steady state viscosity of HMHEC (molar mass Mw=560000, hydrophobes=hexadecyl, DS of hydrophobes was not given) solutions at several concentrations and temperatures. **Figure 1.14 a)** shows

that viscosity increases with the concentration in all the range of shear rates, as expected, since a higher number of micelle-like aggregates favors the formation of bridges and enhances the network [99].

A shear-thinning behavior is observed for all cases, except at the lower concentration, were a slight shear-thickening is observed, that is attributed to loop-to-bridge shear-induced transitions as molecules elongate under shear [100]. At higher concentrations shear-thickening is not observed probably because when micelles are close enough the number of bridges is not limited by the distance between them and elongation of chains does not favors the formation of new bridges [99].

It can be observed in **Figure 1.14 b)** that viscosity at low shear rates decreases with temperature T, and tends to be independent of T at high shear rates. While at low shear (in the Newtonian range), the association structure and, as a consequence, the viscosity, depends on Brownian motion and, consequently, on temperature. In another word, this effect is caused by the loss of hydration water around the polymer molecules when the temperature increases, which leads to the decrease in viscosity. At higher shear rates Brownian motion can be neglected in front of shear. Therefore, viscosity tends to be independent of temperature [96-99,101].

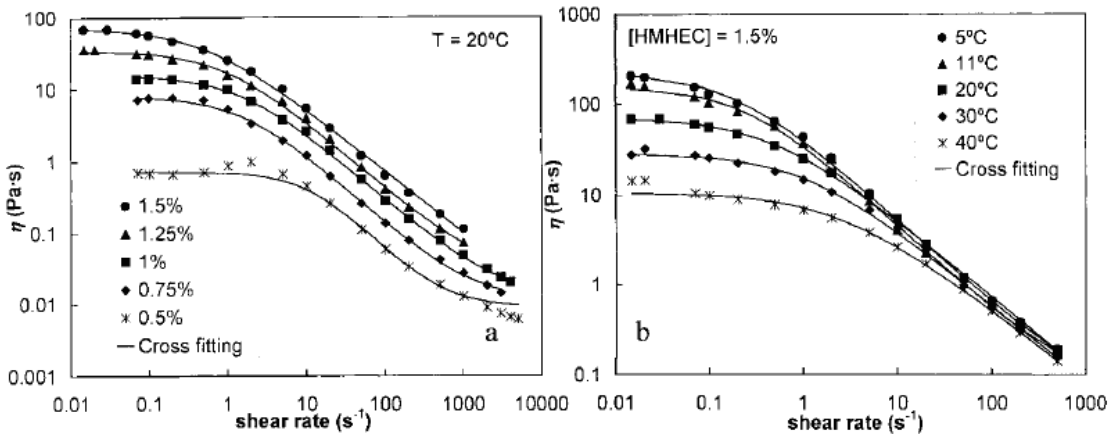


Figure 1.14: Steady state curves of HMHEC aqueous solutions a) at T = 20 °C and several concentrations; b) at [HMHEC] = 1.5 % and several temperatures.[99]

When salt is added to an aqueous polymer solution, the viscosity may either increase or decrease, depending on the identity of the salt [102-105]. As an example shown in **Figure 1.16**, the viscosities were changed for both ethyl(hydroxyethyl) cellulose (EHEC with DS_{ethyl}=0.6-0.7) solution and HM-EHEC solution by adding salt. The chemical structures of EHEC and HM-EHEC are shown in **Figure 1.15**. The viscosity changes for the HM-EHEC solution is more pronounced than for the EHEC solution. Upon addition of NaCl, the viscosity is increased, while a decrease is observed upon addition of NaSCN. NaBr has an intermediate effect and the viscosity is slightly increased. When salt with a small anion (e.g. NaCl) is added to the HM-EHEC solution, the energy cost to break the physical bond between two hydrophobic tails is increased due to the increased surface energy. This is expected to increase the viscosity. While the addition of NaSCN makes the anion enriches at the surface of the hydrophobic junction zones which is accompanied by a decreased energy cost in breaking the hydrophobic bonds, followed by a decreased viscosity. The analogy between surface energies and molecular association has been discussed by Nilsson et. al.[103-105]

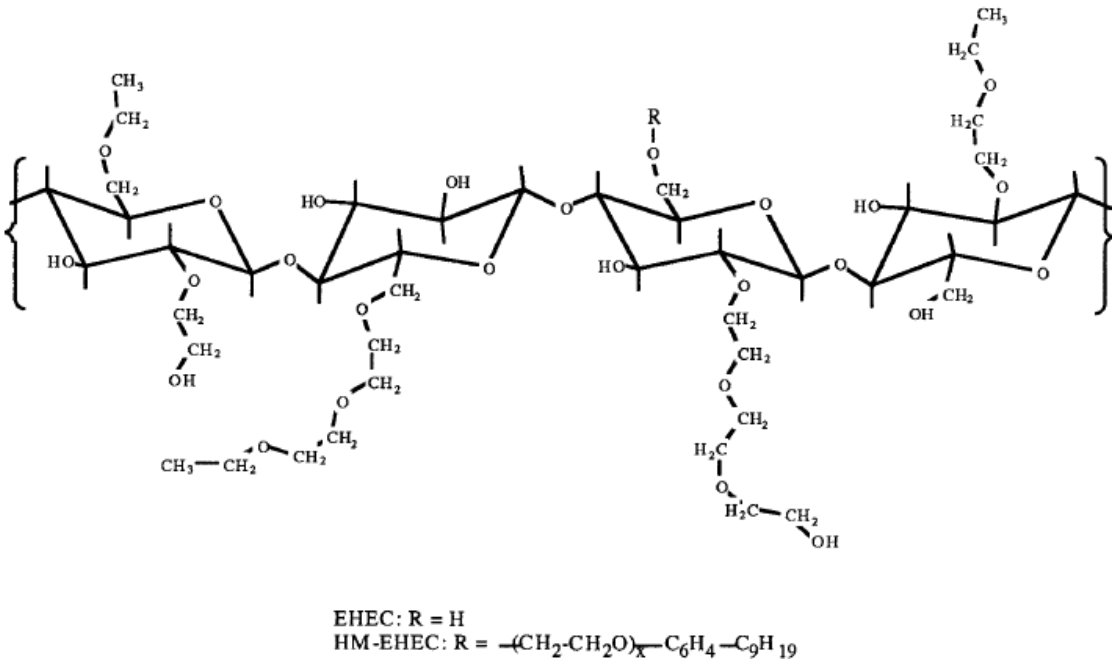


Figure 1.15: Chemical structure of EHEC and HM-EHEC.[103]

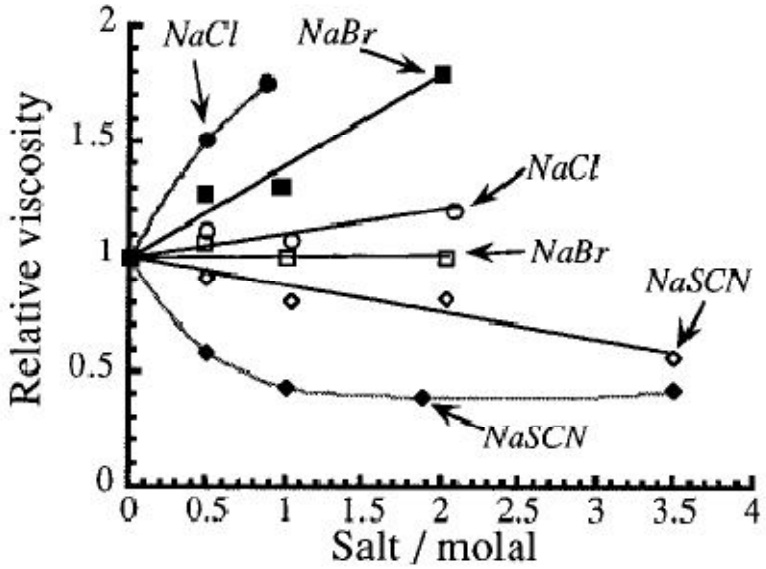


Figure 1.16: Relative viscosity of a 1 % (w/w) EHEC solution (open symbols) or a 1% (w/w) HM-EHEC solution (filled symbols) as a function of added NaCl (○, ●), NaBr (□, ■), or NaSCN (◇, ◆).[103]

Therefore in many reference, the addition of NaCl were studied, and it often leads to an effective thickening, which results from the enhancement of interchain hydrophobic aggregation [102,106].

Consistent with the postulated hydrophobic association in aqueous media, the addition of organic solvents causes a decrease in the viscosity [106].

From the discussion above, it could be found that HMHEC were the object of most studies as a cellulosic associative thickener, and their water solution thickening properties with different parameters are representative.

2. Starch

No associative behavior has been observed when the backbone is formed by starch until 2005 [107]. Indeed, in the United States Patent 7157573 [51], Starch derivatives

bearing alkyl chains with 4–24 carbon atoms were shown to have associative behavior (**Table 1.7**). There is no detailed discussion in the patent, but it the alkyl chain length and the degree of substitution on different types of starch (potato starch, amylopectin potato starch or waxy maize starch) were demonstrated to strongly influence the associative behavior of the polysaccharides. For potato octenylsuccinic ester, as the DS increased, the viscosity increased. Moreover, for all the samples, the viscosity increases upon heating, this was attributed to entropy driven increase in hydrophobic bonding.

Table 1.7: Viscosities of hydrophobic starch solution with a concentration of 2 wt % at 20 ºC.[51]

Brabender viscosity of the different alkylsuccinic esters of different starch types				
		DS	Brabender viscosity (BU)	
Type of starch	Alkylsuccinic ester	(mole/mole)	at 20 °C	at 30° C.
potato	Octenylsuccinic	0.015	960	1690
	Dodecenylsuccinic	0.008	860	1550
Amylopectin potato	none	none	700	960
	Octenylsuccinic	0.013	2040	2370
		0.019	2380	2830
		0.023	2580	3000
	Dodecenylsuccinic	0.005	1400	3220
	Tetrapropenylsuccinic	0.016	2090	2720
Waxy maize	Octenylsuccinic	0.017	1320	1520
	Dodecenylsuccinic	0.005	290	560
	Tetrapropenylsuccinic	0.016	1210	1660

3. Dextran

The covalent fixation of phenoxy groups onto dextran leads to DexP derivatives whose amphiphilic properties were clearly evidenced by viscosity experiments (**Figure 1.7**)[108]. The significant decrease of intrinsic viscosity [η] in water with increasing contents in grafted phenoxy groups were classically interpreted in terms of

hydrophobic interactions (intramolecular or intermolecular) between phenoxy groups, leading to low solvated coils and shrunken conformations in dilute solution. For dextran derivatives containing more than molar 11% phenoxy groups, the evolution of the reduced viscosity is no longer linear over the whole concentration range. At concentrations above 40 and 35 g/L for DexP15 (DS = 15 %) and DexP22 (DS = 22 %) solutions, respectively, the reduced viscosity deviates from a linear variation and starts to increase sharply with polymer concentration. This slope modification is due to the formation of aggregates with higher hydrodynamic volume probably because of intermolecular interactions becoming predominant at concentrations higher than the "overlap concentrations (*C**)" and initiating the formation of a physical network. Note that some intermolecular compact aggregates of DexP22 are formed even in dilute solution. For dextrans with substitution ratios lower than 11%, the formation of a physical network probably occurs for much higher concentrations and cannot be detected in the explored concentration range [34].

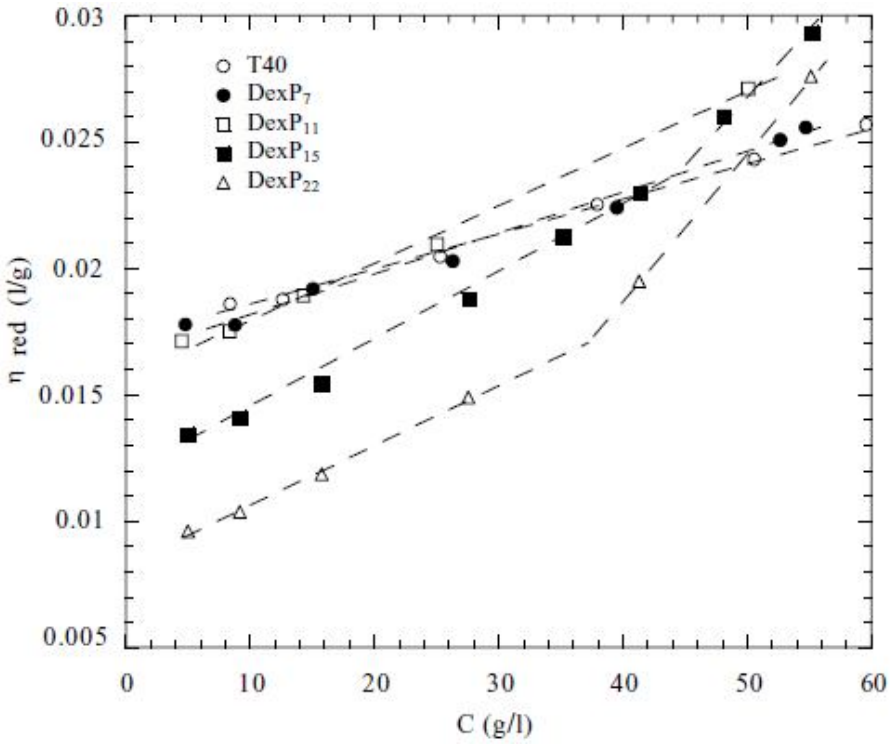


Figure 1.17: Variation of the reduced viscosity of aqueous solutions of dextran T40 and hydrophobically modified dextrans (water, 25◦C).[34]

4. Guar

Very few data concerning the associating behavior of hydrophobically modified guar (HMG) derivatives are reported in the literature.

In U.S. Patent No. 4,870,167 [31], hydroxypropyl guar (HPG) were modified by epoxide containing long alkyl chains or C_{24-28} alpha olefin oxide (MS = 0.1-20 %). The behavior of the hydrophobically modified HPG (HMHPG) in aqueous media was presented, as shown in **Table 1.8**. It was found that HMHPG possessing hydroxyhexadecyl chains (**HMHPG 5**) and C_{24-28} alpha olefin oxide (**HMHPG 6**) exhibit remarkable increase of viscosity compared to initial guar. Such polygalactomannans are particularly useful as thickening agents for aqueous systems. The viscosity of aqueous solutions of the compositions of this invention is enhanced by the addition of surfactants (ammonium lauryl sulfate (ALS)). Any water soluble surfactant can be used in this invention.

Table 1.8: The viscosities of the hydrophobic guar solution with a concentration of 0.5 wt % at 25 ^{o}C [31].

HMHPG	Hydrophobic reagent* (HR)	Ratio of HR/HPG (w/w)	Aqueous Solution Viscosity cps	mls ALS**	Viscosity cpa
Control	--	--	200	0.3	No change
1	1.2-epoxyoctane	18/90	150	1.6	800
2	1.2-epoxydodecane	14/90	150	0.3	4800
3	1.2-epoxyhexadecane	18/90	360	0.3	4900
4	1.2-epoxyoctadecane	20/90	370	0.3	5000
5	C_{24-28} alpha olefin oxide	29/90	1366		
6	1.2-epoxyhexadecane	36/90	1520		

* HPG was reacted with hydrophobic reagent at 60 ^{o}C for 1.5 h under nitrogen atmosphere.

** Ammonium lauryl sulfate - 28 % solution in water

5. Hyaluronic acid

Thickening properties were also observed by esterification of HA with dodecyl (C_{12}) or octadecyl (C_{18}) bromide [19]. The influence of the length of alkyl chain, its content on HA, and of polymer concentration was well identified and it was shown that some derivatives were potential materials useful for the treatment of the osteoarthritis of the knee and cartilage repair. Dynamic rheological measurements demonstrated a gel-like behaviour for aqueous solutions of HA (M_w = 480000 g/mol) modified with C_{18} chains and with a degree of substitution of 0.02 (HA-C18-2) in 0.15 M NaCl (polymer concentration C_p = 4 and 7 g/L), contrary to the solutions of HA-C_{18}-1 (DS = 0.01) and of HA-C_{12}-5 (DS = 0.05) at concentrations in the range 6-12 g/L, which only exhibit a viscous character. Indeed, from **Figure 1.18**, it can be seen that the storage (elastic) modulus (G') is larger than the loss modulus (G'') within the whole range of frequencies covered for the HA-C_{18}-2 solution. Moreover, the values of the storage and loss moduli are much higher than those obtained for the solution of HA-C_{18}-1, although the concentration of the latter is higher than that of HA-C_{18}-2. These rheological data thus demonstrate the formation of physical network made of loosely cross-linked HA.

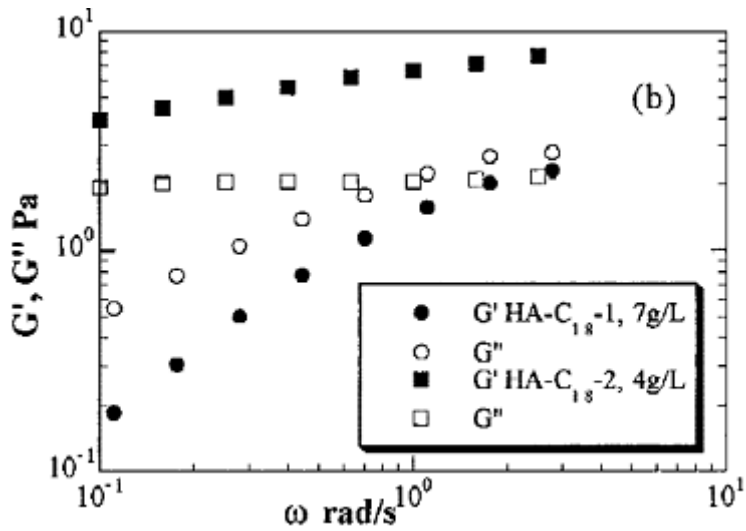


Figure 1.18: Comparison of the storage and loss moduli as a function of frequency for solutions of HA-C_{18}-1 and HA-C_{18}-2 derivatives in 0.15 M NaCl at 37 °C. [19]

Stable hydrogels at polymer concentrations higher than 3 g/L were also obtained from amide derivatives of HA possessing C_{16} side chains with a DS of 0.01-0.03. [80]

It should be noted, that in the case of alkylamino hydrazide derivatives of HA, the formation of physical hydrogels was observed for derivatives possessing shorter chains (C_{10})[109]. The ability of the latter derivatives to self-associate in aqueous solution similarly to those directly modified by longer alkyl chains (C_{16} or C_{18}) may be attributed to the presence of the adipic spacer arm and/or the different experimental conditions used for the grafting of alkyl chains (**Figure 1.19**).

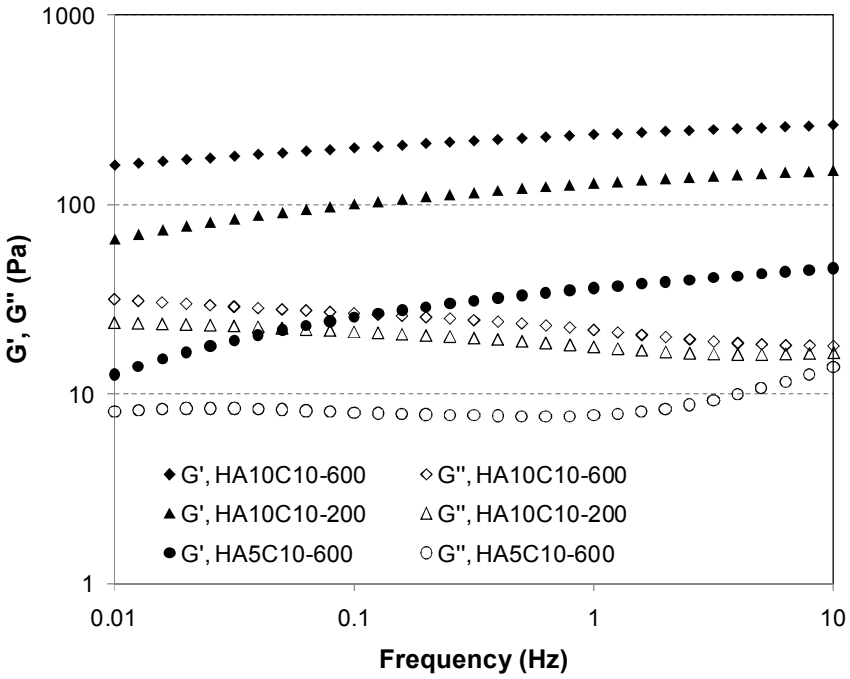


Fig. 1.19: Comparison of the storage and loss moduli as a function of frequency for solutions of decylaminohydrazide derivatives of HA in PBS at 25 °C and at a polymer concentration of 10 g/L. The derivatives are designated as HAxC10, x reflects the degree of substitution (DS; x = 100 DS) of the decyl (C_{10}) chains. [109]

Nevertheless, it can be noticed that all these derivatives have the common feature to be modified with low degrees of substitution (DS ≤ 0.2).

Indeed, functionalization of HA with hydrophobic molecules at high degrees of substitution promotes the formation of aggregates with a compact conformation in aqueous solution as described in the case of HA esters modified with the steroidal anti-inflammatory drug 6-methylprednisolone [110] and benzyl moieties [111]. Thus, the zero-shear viscosity of a semi-dilute solution of HA (M_w=150000 g/mol) benzyl ester (DS=0.5) was found to be lower than that of initial HA[111]. Moreover, nanosized spherical micelles were obtained from HA-paclitaxel conjugates in aqueous solution. The micelles showed an average diameter of 196 ± 9.6 nm with a narrow size distribution. Paclitaxel, which is a very hydrophobic anticancer drug, was directly conjugated to HA in DMSO by forming HA/poly(ethylene glycol) (PEG) nanocomplexes with a size of 120 ± 6.3 nm [112]. This strategy indeed allowed solubilization of HA and other biomacromolecules in DMSO by formation of inter- and intrahydrogen bonds [113.] This solubilization method was also used to graft hydrophobic and biodegradable poly(lactic-co-(glycolic acid) (PLGA) on HA (M_w = 17000 g/mol)[114]. The resulting HA-g-PLGA copolymers (with 0.05 ≤ DS ≤ 0.26) self-assembled in aqueous solution to form nano-sized micellar aggregates with average diameters ranging from 98.4 to 539.4 nm depending on the degree of substitution and molar mass of PLGA. Thus, the size of the micelles was found to decrease when the DS was increased. This phenomena was also observed in the case of HA-NPs which was formed by the self-assembly of HA grafted with 5β-cholanic acid [115]. As developed in the second section of this review, those nanoparticles may be useful as biocompatible and biodegradable carriers for the targeting of anticancer drugs.

1.4 Conclusion

The strategies to synthesis of hydrophobically modified natural polysaccharides (HM-P) have been investigated in different works. The conditions of the currently investigated reactions of these synthetic routes and the grafting efficiency are reviewed. Each synthesis strategy has its advantage and disadvantage, however, etherification seems to provide easy access to a variety of bio-based materials with valuable properties, the etherifying reagents are usually easily handled than the reagents employed for modification via the ester or urethane linkages or amine-acid coupling. The detailed reaction conditions and the obtained degree of substitute give a reference for the further study, three medium systems, IPA/water, DMSO and DMSO/water, are favorable for synthesis of HM-P.

As used as thickener, the rheological properties of the modified polysaccharides in aqueous solution have been largely examined. It was found that the viscosity of the aqueous solution of modified polysaccharide depends on not only the different hydrophobic substituent and different molar substitution levels, but also the effects of operating parameters.

From the literature, still, there are many challenges remaining for synthesis of HM-P, the most intractable one is to maintain of the water solubility of HM-P at the same time to increased viscosity of the aqueous solution of HM-P. Additionally, due to the intrinsic property of each polysaccharide, each work focuses on one kind of polysaccharide; therefore, there is no uniform approach for different polysaccharide.

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CHAPTER 2

Synthesis of hydrophobically modified derivatives of polysaccharides

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Résumé (*Fr*)

Ce chapitre décrit la synthèse et la caractérisation de polysaccharides neutres (guar, hydroxyéthyl guar et hydroxyéthyl cellulose) hydrophobiquement modifiés. Des groupements alkyle ou aryle hydrophobes ont été greffés en utilisant des éthers de glycidyle hydrophobes (″époxydes hydrophobes″) commerciaux (hexadécyl ether de glycidyle, phényl éther de glycidyle) et non commerciaux (Brij 56® époxyde (Brij56E), OP-10 époxyde (OP10E) ou poly(propylène glycol) époxyde (PPGE)). Les dérivés époxydes Brij56E, OP10E et PPGE ont été préparés à partir d'alcools commerciaux et caractérisés par RMN ^{1}H et spectrométrie de masse afin de confirmer leur structure. L'éthérification des polysaccharides par ouverture des époxydes en milieu basique a été effectuée selon différentes conditions réactionnelles : (i) dans le diméthylsulfoxyde en présence d'hydroxyde de tétrabutylammonium comme base, (ii) dans le diméthylsulfoxyde en présence d'hydroxyde de sodium comme base et, (iii) dans un mélange isopropanol/eau en présence d'hydroxyde de sodium. Différents rapports molaires époxyde/polysaccharide ont également été testés afin de faire varier le degré de substitution (DS) des dérivés de polysaccharides. Certains polymères se sont révélés être insolubles dans l'eau en raison vraisemblablement de DS trop élevés (évalués par RMN ^{1}H). Les résultats obtenus indiquent par ailleurs des réactivités différentes des époxydes. L'analyse du comportement en milieu aqueux des polysaccharides hydrophobiquement modifiés par des mesures de viscosité met en évidence soit des augmentations de viscosité, soit des diminutions selon les conditions réactionnelles utilisées.

Summary (*En*)

This chapter is devoted to synthesis and characterization of hydrophobically modified polysaccharide. The hydrophobic alkyl groups are introduced to the polysaccharide chain using hydrophobic epoxides. The epoxide derivatives, PPGE, OP10E and Brij56E, have been prepared from the commercial alcohols respectively in the first part. They are characterized by ^{1}H NMR and MS spectra to confirm their structure.

In the second part, coupling of epoxide derivatives with guar and hydroxyethyl guar are first presented, and hydroxyethyl cellulose are also used to be modified. Three different alkylation reaction conditions including reaction medium, ratio of alkylation epoxide to polysaccharide, reaction temperature and reaction time are investigated. Excess grafting of hydrophobic alkyl groups on polysaccharide cause water insoluble product, therefore control DS through the control of reaction conditions are studied. The products are characterized by ^{1}H NMR to determine their degree of substitution (DS). The results also indicate different reactivity of the alkyl epoxides.

The introduction of alkyl groups along the polysaccharide chain is expected to provide the polysaccharides self-association properties in aqueous solution. The rheological behaviors of the different modified samples in dilute solution are measured using a low shear 30 viscometer. To clarify the procedure effect on the rheological behavior of the modified polysaccharide, blank experiments are performed.

2.1 Introduction

Guar is a natural polysaccharide extracted from the seeds of *Cyamopsis tetragonoloba*. It consists of a linear backbone of β-1,4 linked D-mannose units with randomly attached α-1,6 linked galactose units as side chains (**Figure 2.1 a**). The ratio of mannose to galactose units (M/G ratio) is on average equal to 2.

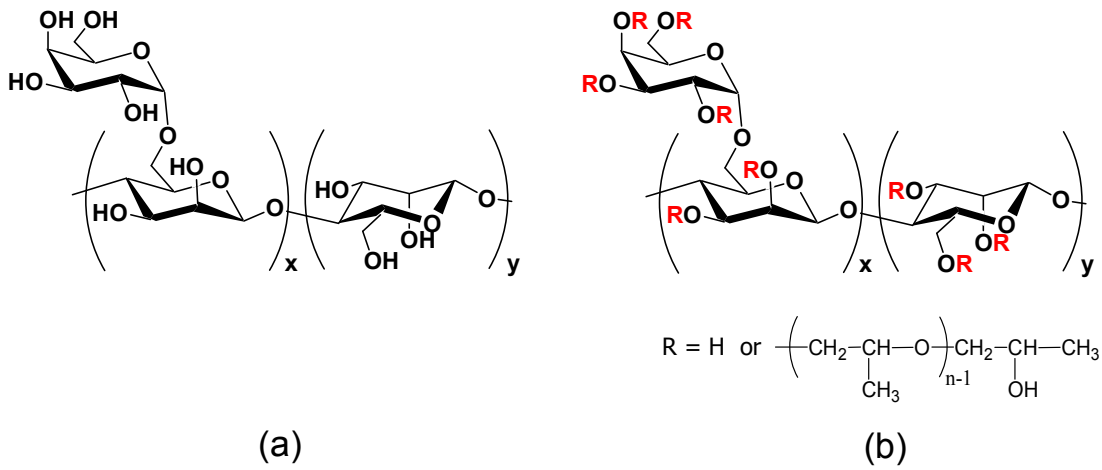


Figure 2.1: Structure of (a) guar and (b) hydroxypropyl-guar (HPG).

This water-soluble polysaccharide which can produce highly viscous aqueous solutions is used in a wide range of industrial applications like oil well drilling, paper and textile sizing, as a binding agent for explosives, as a functional ingredient in the food and cosmetic industries, as dietary fibre, in pharmaceutical formulations, etc. Substituted guar derivatives like hydroxyethyl guar (HEG), hydroxypropyl guar (HPG) and carboxymethyl guar (CMG) find also uses in many industries. Hydroxypropyl guar (**Figure 2.1 b**) is the most widely available derivative of natural guar. It is also more soluble in water and shows better temperature stability with respect to the physical properties in solution, than guar.

The objective of this work was to synthesize new hydrophobically modified guar derivatives (HM-G) based on the grafting of hydrophobic polyether chains. The synthetic strategy we proposed consisted in the alkaline etherification of guar with

hydrophobic epoxide derivatives presented in **Figure 2.2**.

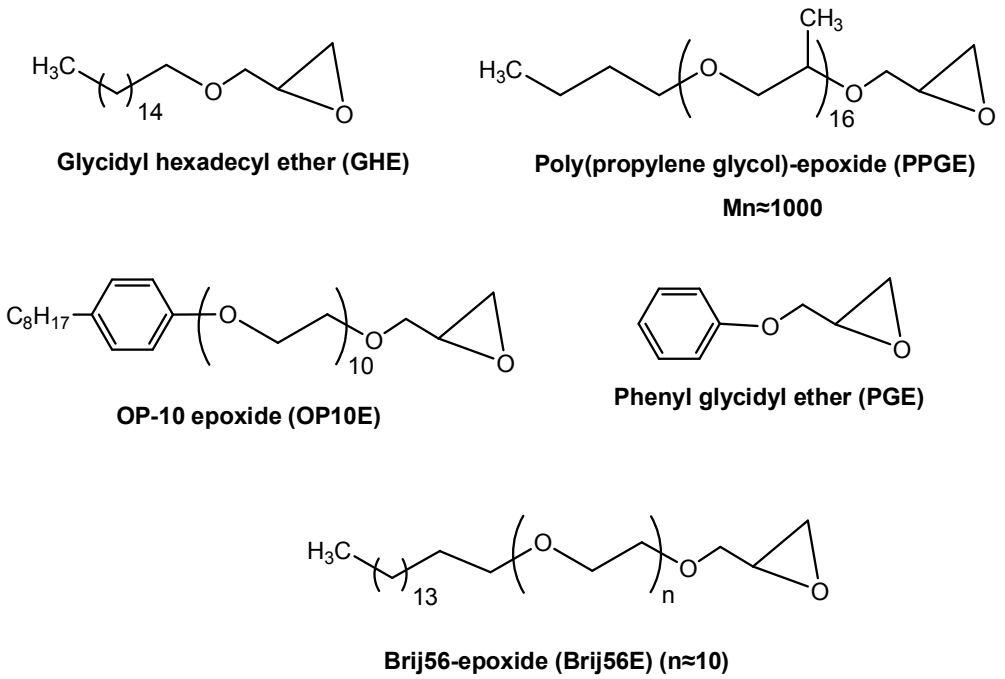


Figure 2.2: Chemical structure of hydrophobic epoxide derivatives used as etherifying agents of guar and derivatives.

GHE has been selected as it is a commercial product which has been successfully used to prepare hydrophobically modified hydroxyethyl cellulose (HM-HEC) derivatives [1]. PGE is a commercial product which has also been used to prepare amphiphilic derivatives of dextran.[2] PPGE, OP10E and Brij56E have been prepared in this study from the commercial alcohols, poly(propylene glycol) (PPG), polyoxyethylene octylphenol ether (OP-10) and polyethylene glycol hexadecyl ether (Brij®56 or Brij56), respectively. To our knowledge, the coupling of the latter epoxide derivatives with guar has never been described. Therefore, the development of conditions for the grafting of such derivatives may pave the way to new products with original properties in aqueous solution. In this chapter, we describe the synthesis of hydrophobically modified derivatives of guar.

Guar exhibits high viscosity in aqueous solution which is related to its high molar mass (up to 2 millions g/mol) and to the presence of extensive entanglements

promoted by hydrogen bonding. The latter may hamper alkylation reaction. Therefore, for comparison, we also studied the alkylation of other neutral water-soluble polysaccharide namely hydroxyethyl cellulose (HEC) and hydroxyethyl guar (HEG). As presented below, several alkylation conditions have been investigated.

2.2 Analysis of the initial polysaccharides by SEC

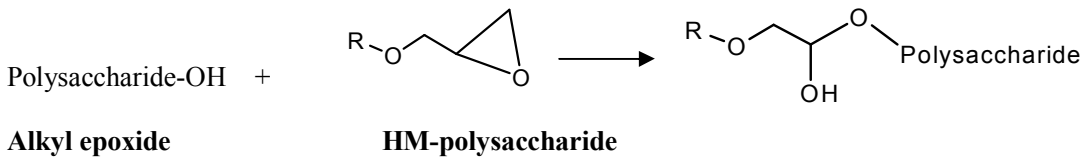
The initial polysaccharide materials we used were characterized by size exclusion chromatography (SEC) (eluant: 0.1 M $NaNO_3$) using a water GPCV Alliance 2000 chromatograph with three online detectors: a differential refrachometer, a viscometer and a light scattering detector (MALLS), the results is summarized in **Table 2.1**.

Table 2.1: Analysis of the initial polysaccharides by SEC.

Entry	Polysaccharide	MS (From providers)	Batch number or Lot	Mw (g/mol)	Ip (Mw/Mn)	% of eluted product
1	G (pure guar 2)	--	Rhodia H0307 594 A	1332000	1.2	59
2	HEG (hydroxyethyl guar)	0.2~0.3	JK0601 08-4-15-13-1	779700	3.2	47
3	HEC (hydroxyethyl cellulose)	2.5	NATROSOL A 1982	104800	5.2	11

2.3 Synthesis of alkyl epoxide

The objective of this project was to develop the synthesis of new alkylated polysaccharide derivatives, exhibiting thickening behaviour in aqueous solution. The alkylating groups were grafted on the polysaccharides through the ring opening of hydrophobic epoxides, as shown below in **Scheme 2.1**.

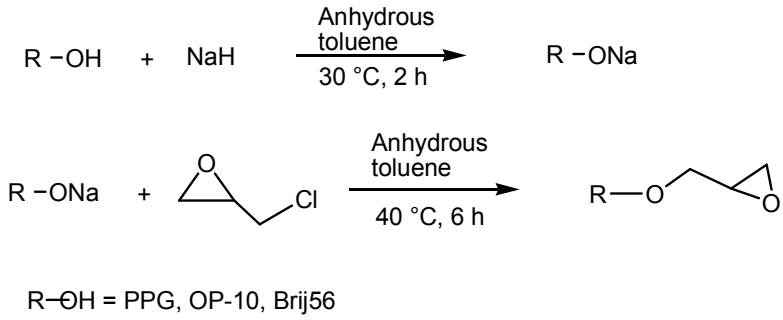


Alkyl epoxide = GHE, PGE, OP10E, PPGE, Brij56E

Polysaccharide = Guar, HEG, HEC

Scheme 2.1: Synthetic route to alkyl derivatives of polysaccharides.

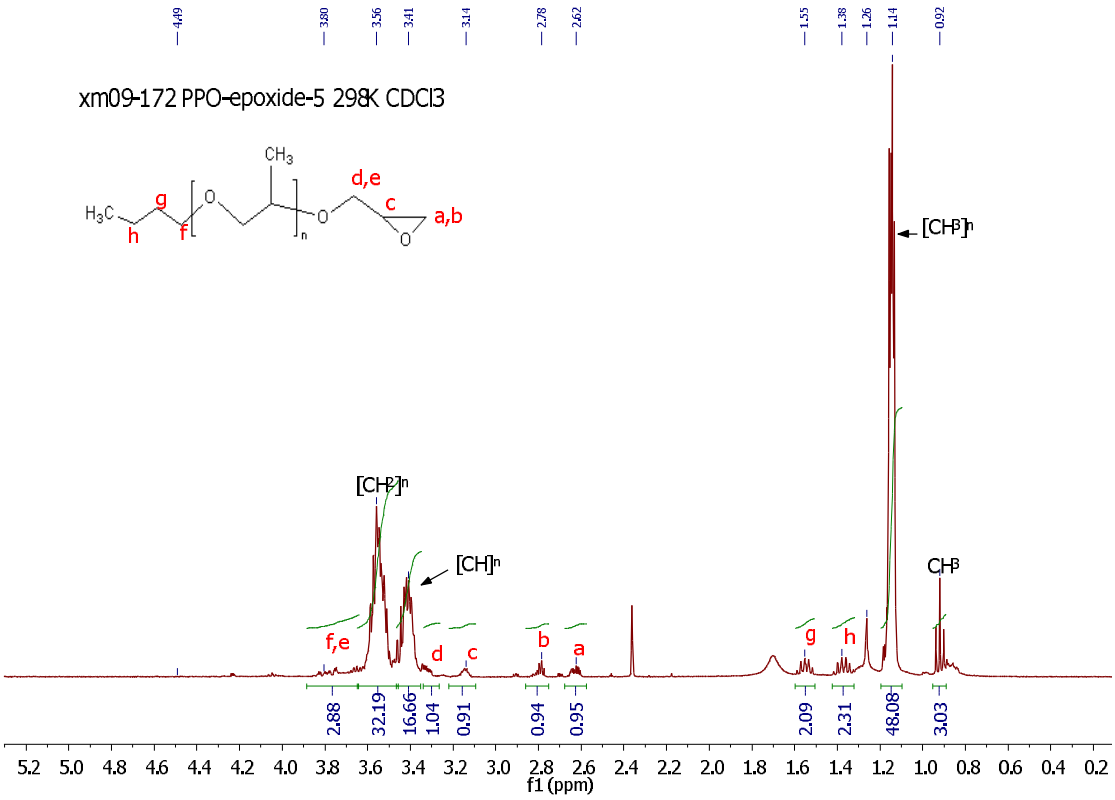
As mentioned above, the **GHE** and **PGE** derivatives are commercially available. The other three epoxides (**PPGE, Brij56E** and **OP10E**) were synthesized from epichlorohydrin and the precursor alcohols as illustrated in **Scheme 2.2**.



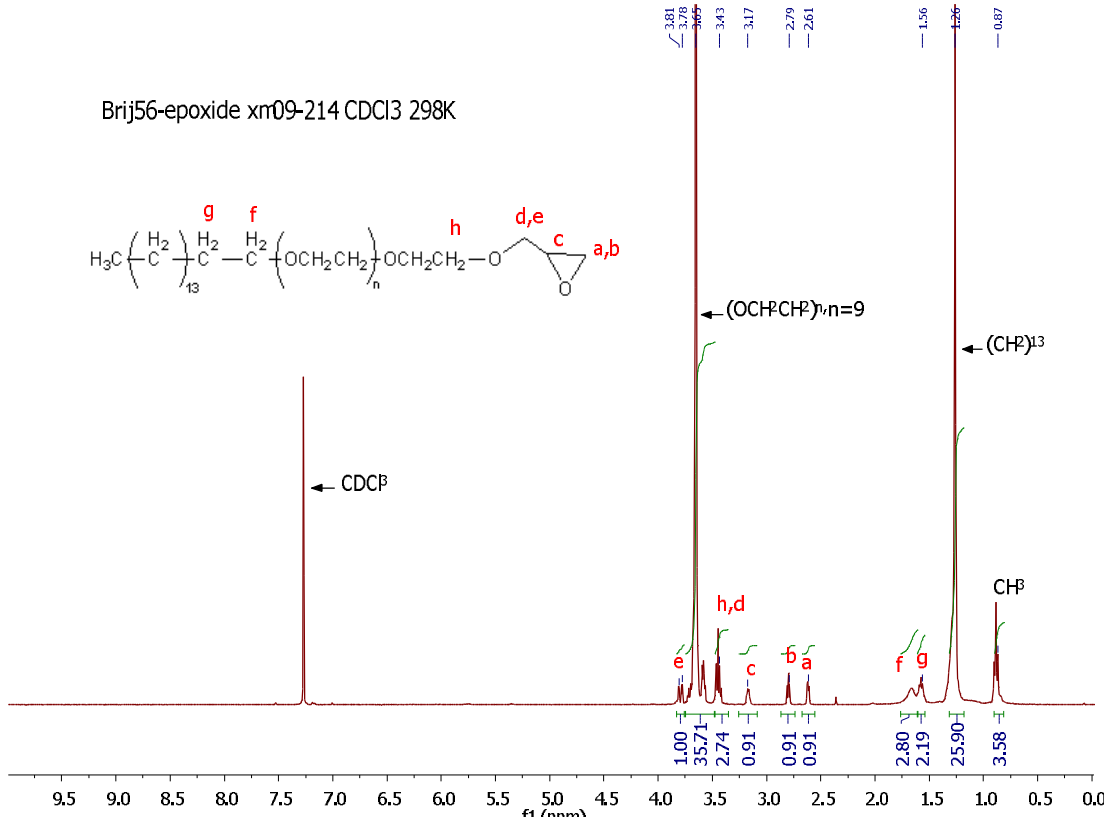
Scheme 2.2: Synthesis of hydrophobic epoxides.

The resulting reaction mixture was left overnight at 4 °C to make the solid (NaCl) deposited from the liquid. The liquid was separated from the solid carefully and then evaporated under reduced pressure to remove the solvent. The epoxide derivatives were isolated without further purification with yields in the range of 90-95 %.

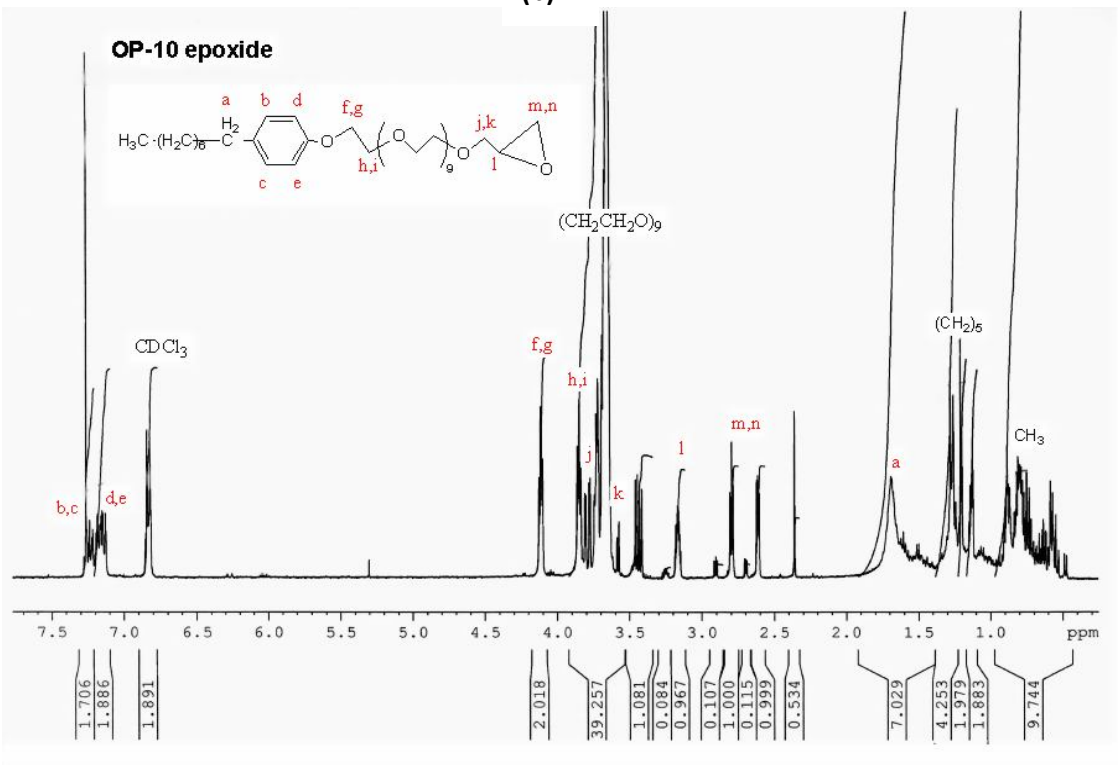
The ^{1}H NMR spectra of PPGE, Brij56E and OP10E are given in **Figure 2.3**. The ^{1}H NMR spectra are recorded in $CDCl_3$. The proton resonances of the epoxide ring moiety appear between 2.5 and 3.3 ppm. From integration of the ^{1}H NMR spectra, the yield of epoxide formation was ~ 100%.



(a)



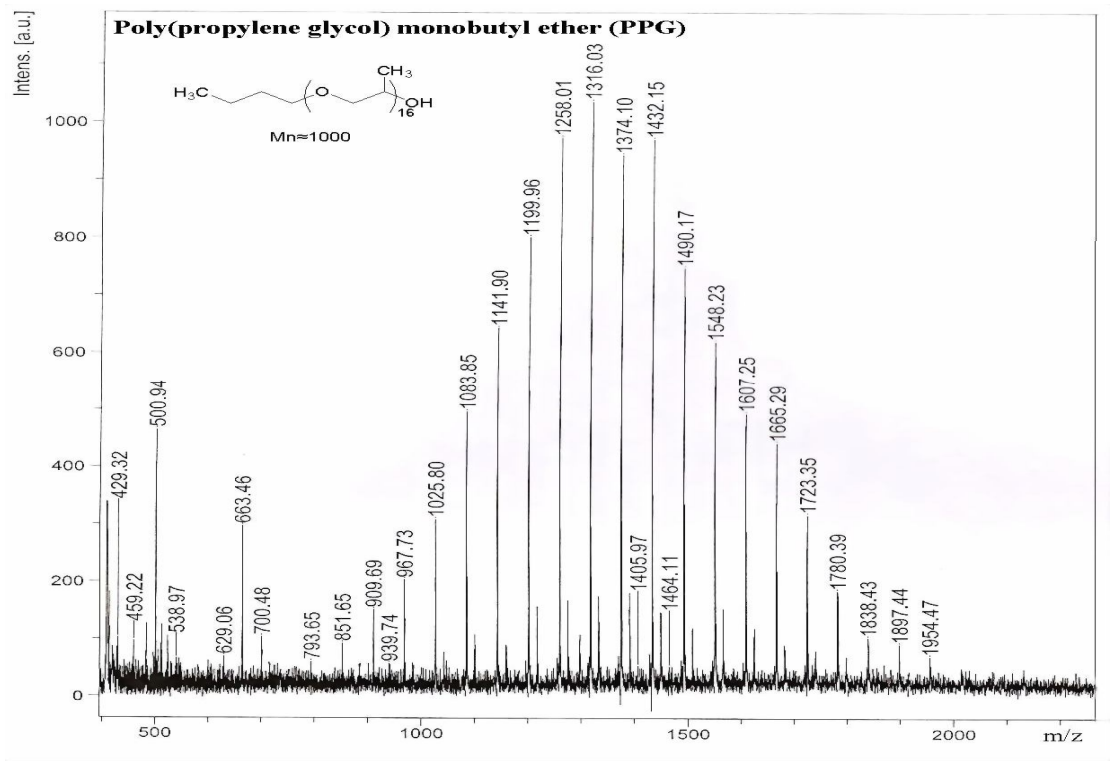
(b)



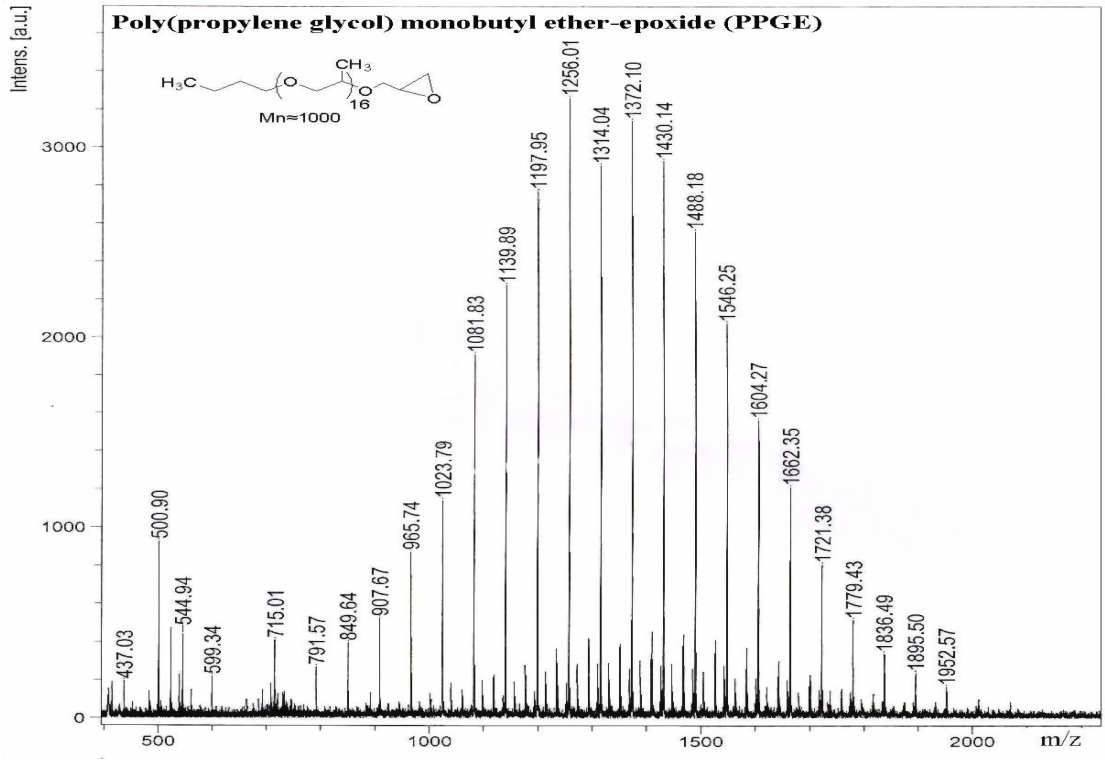
(c)

Figure 2.3: ^{1}H NMR spectra (400M Hz, 80 ^{o}C) of (a) PPG epoxide, (b) Brij56 epoxide and (c) OP-10 epoxide in $CDCl_3$ (6 mg/ml).

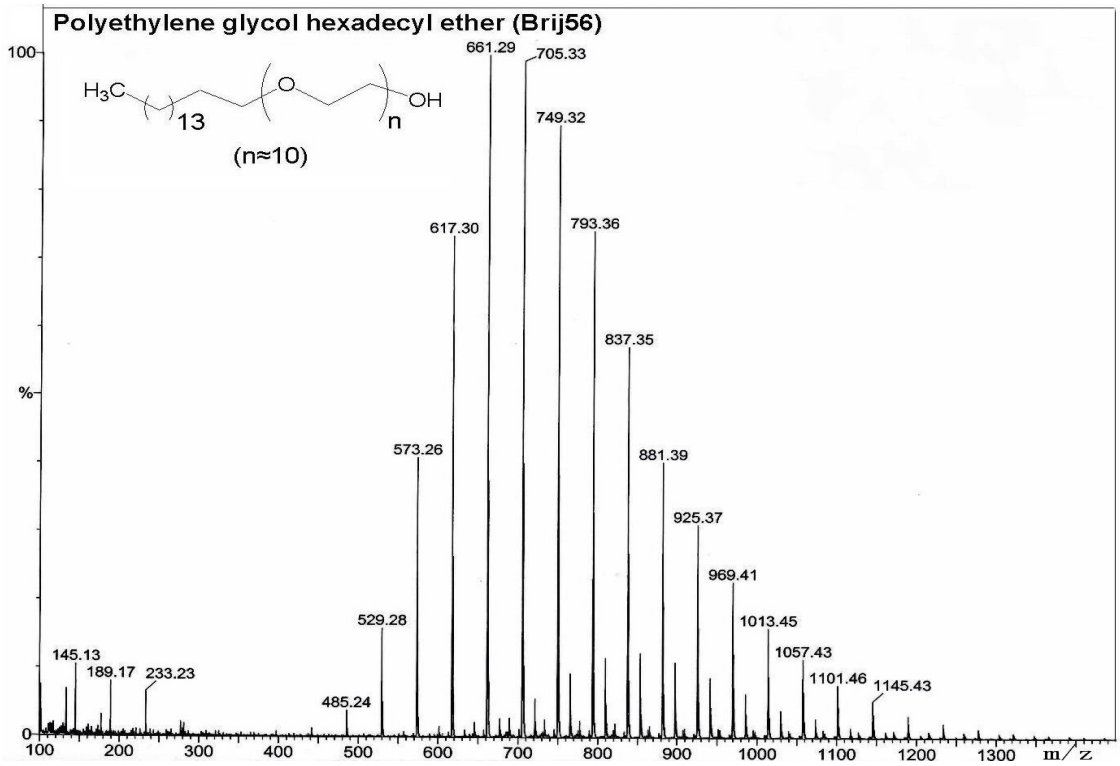
The formation of alkyl glycidyl ethers was also confirmed by Maldi-TOF mass spectrometry. In the mass spectra shown in **Figure 2.4**, the gap of 44 amu between each *m/z* signal in Brij56E and OP10E and, of 58 amu between each *m/z* signal in PPGE, serves to locate the repeat units in the $(OCH_2CH_2)_n$ and $(OCH_2CH(CH_3))_n$ series, while the ring structure is defined by the gap of 56 amu between *m/z* from of initial alcohol and the final epoxide.



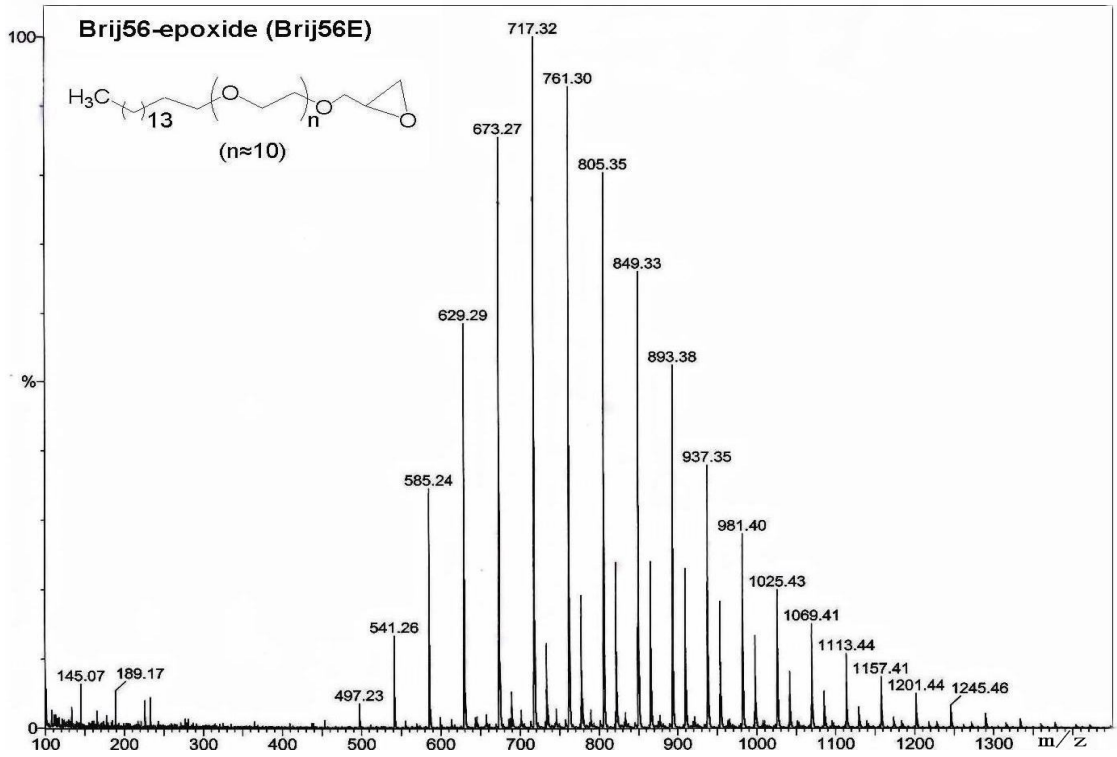
(a)


Poly(propylene glycol) monobutyl ether-epoxide (PPGE)
Intens. [a.u.]
Mn≈1000
437.03
500.90
544.94
599.34
715.01
791.57
849.64
907.67
965.74
1023.79
1081.83
1139.89
1197.95
1256.01
1314.04
1372.10
1430.14
1488.18
1546.25
1604.27
1662.35
1721.38
1779.43
1836.49
1895.50
1952.57
m/z

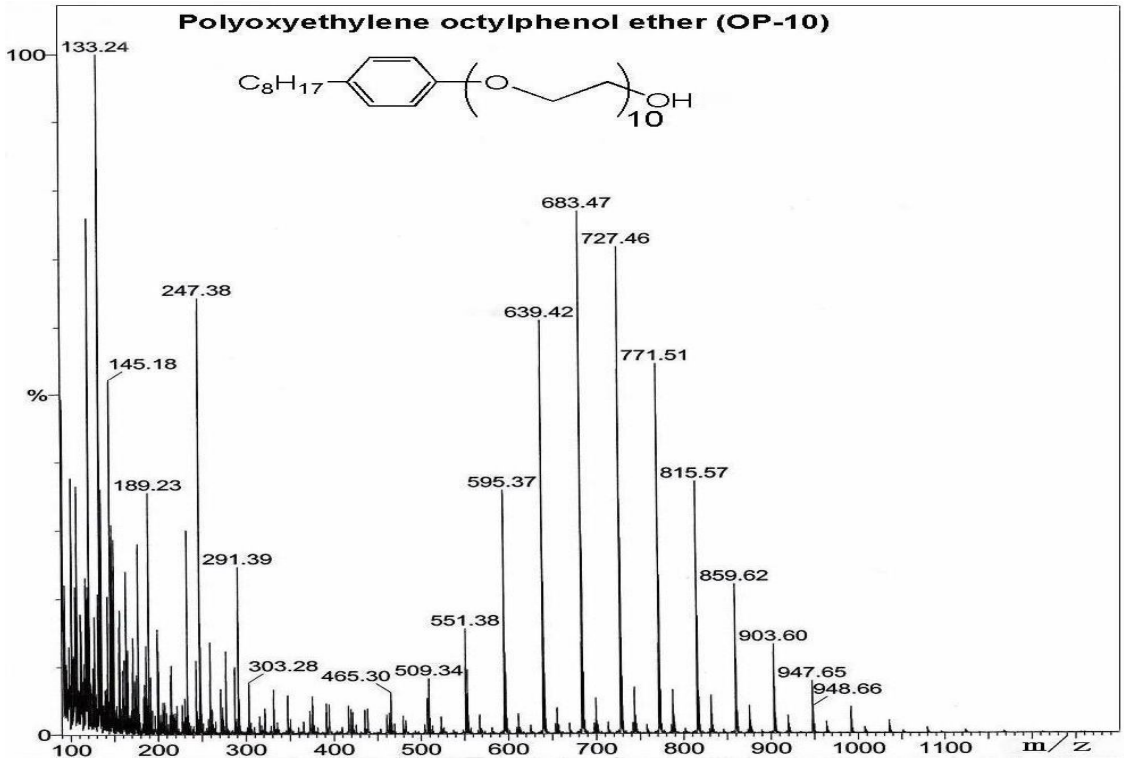
(b)


Polyethylene glycol hexadecyl ether (Brij56)
(n≈10)
661.29
705.33
749.32
617.30
793.36
837.35
573.26
881.39
925.37
969.41
529.28
1013.45
1057.43
1101.46
1145.43
145.13
189.17
233.23
485.24
m/z

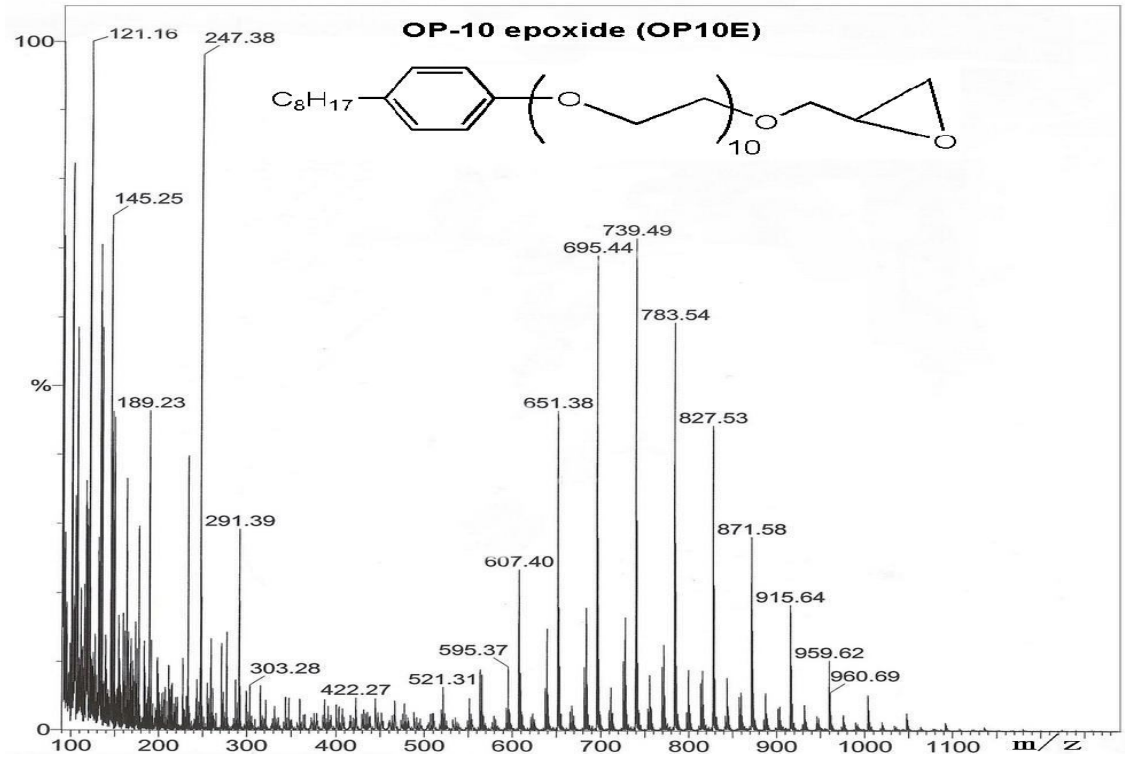
(c)


Brij56-epoxide (Brij56E)
H3C
13
n
(n≈10)
717.32
761.30
673.27
805.35
849.33
629.29
893.38
937.35
585.24
981.40
1025.43
1069.41
541.26
1113.44
1157.41
1201.44
1245.46
145.07
189.17
497.23
100
%
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
m/z

(d)


Polyoxyethylene octylphenol ether (OP-10)
C8H17
OH
10
133.24
683.47
727.46
247.38
639.42
145.18
771.51
815.57
595.37
189.23
291.39
859.62
551.38
903.60
303.28
465.30
509.34
947.65
948.66
100
%
0
100
200
300
400
500
600
700
800
900
1000
1100
m/z

(e)



(f)

Figure 2.4: Maldi-TOF mass spectra of (a) PPG, (b) PPGE, (c) Brij56®, (d) Brij56E, (e) OP-10 and (f) OP10E.

2.4 Synthesis of hydrophobically modified polysaccharide

As mentioned above, the aim of this study was to introduce alkyl groups on the polysaccharide chain using hydrophobic epoxides. Different polysaccharides and reaction conditions were investigated in this part.

Three polysaccharides were chosen: Guar, HEG and HEC.

Different alkylation reaction conditions including solvent, ratio of alkylating epoxide to polysaccharide, reaction temperature and reaction time were investigated. In this part, three different methods according to literature were studied:

1. In DMSO, tetrabutylammonium hydroxide (TBAOH) as alkali[2];
2. In isopropanol (IPA)/water, NaOH as alkali[1];
3. In DMSO/water, NaOH as alkali, pH=13[3].

The products were characterized by ^{1}H NMR to determine their degree of substitution (DS). Their behavior in aqueous solution was also analyzed by viscosity measurements.

2.4.1 Alkylation of HEG and Guar in DMSO in presence of TBAOH

2.4.1.1 Hydrophobically modified hydroxyethyl guar (HM-HEG)

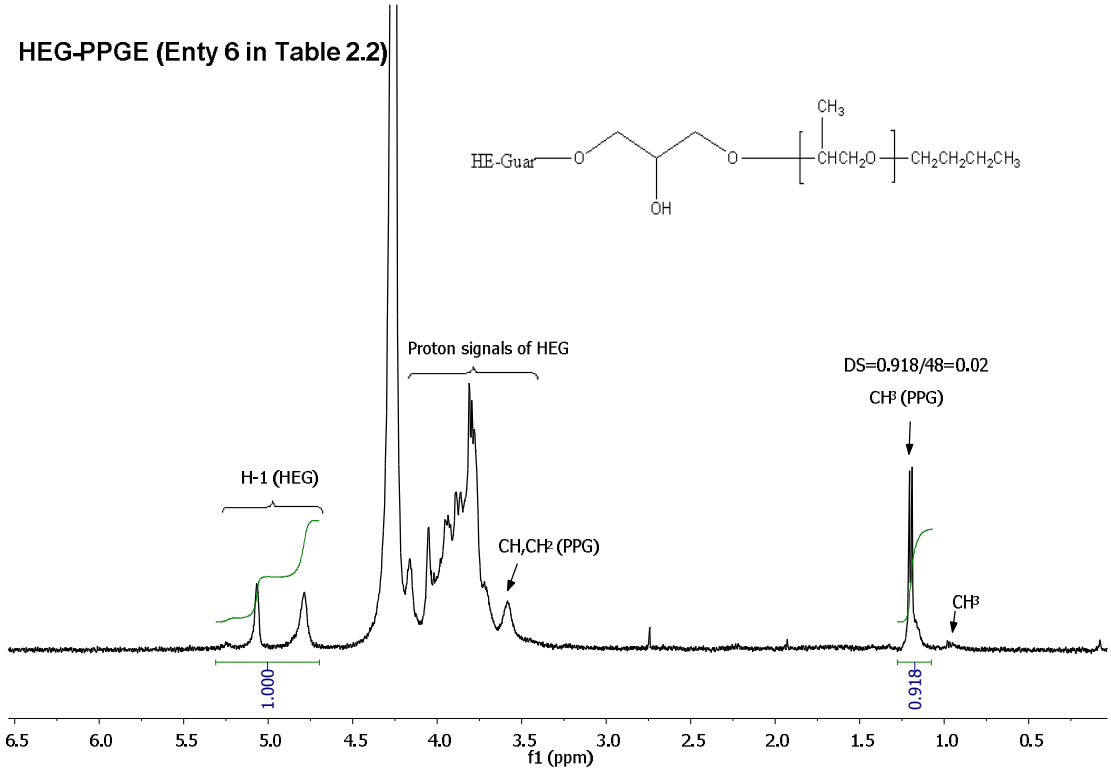
I. Synthesis and NMR characterization of HM-HEG

Five hydrophobic epoxides were used respectively as alkylating agents of HEG (hydroxyethyl guar, DS=0.2~0.3): GHE, PGE, PPGE, Brij56E and OP10E.

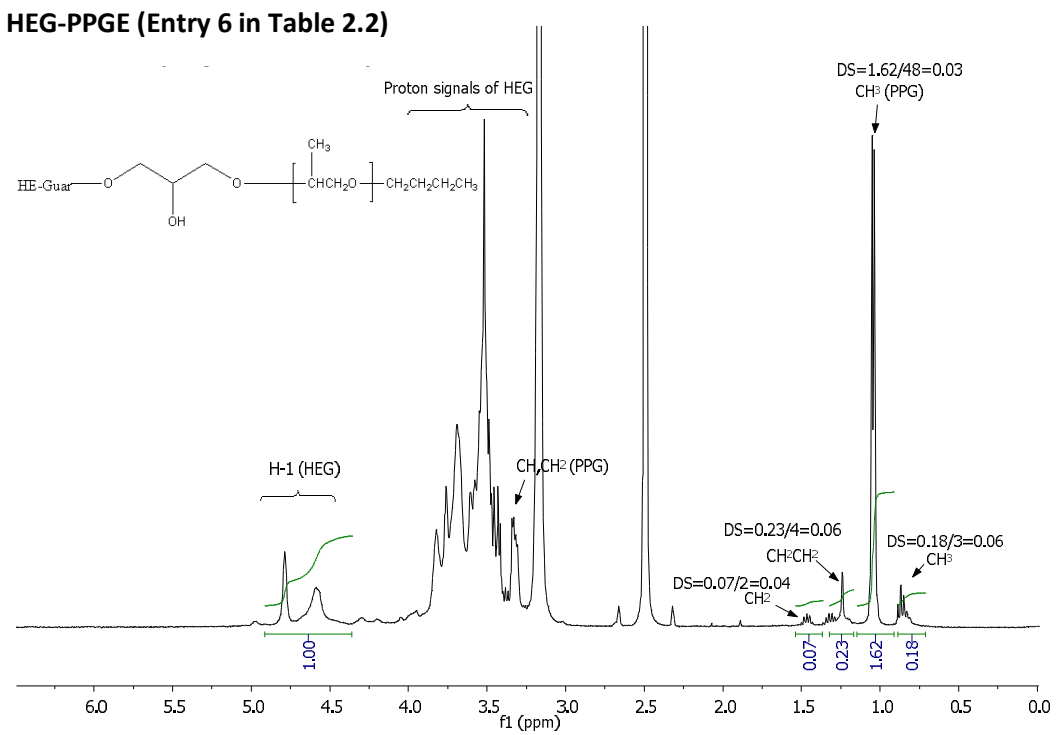
The reaction conditions and the DS are listed in **Table 2.2**. The reaction procedure consists in reacting HEG with the alkyl epoxide in DMSO, in the presence of TBAOH, at room temperature, for 4 days, and then the product is isolated by dialysis

or precipitation. In this thesis, the hydrophobically modified polysaccharides (HM-P) are called by reference to the reactants. For example, reaction of hydroxyethyl guar with glycidyl hexadecyl ether (GHE) leads to HEG-GHE.

The ^{1}H NMR spectra were performed in D_2O and/or in DMSO-d6+D_2O. ^{1}H NMR analysis in both solvents appeared necessary as aggregation due to hydrophobic interactions was observed for some derivatives in aqueous solution leading to some discrepancies in the DS values derived from integration. As an example, **Figure 2.5** compares the ^{1}H NMR spectra of HEG-PPGE recorded in D_2O and in DMSO-d6+D_2O**.** Integration of the two spectra shows discrepancies for some NMR signals. The DS derived from integration of the CH_3 proton signals and the anomeric protons of HEG in the spectrum recorded in D_2O (**Figure 2.5.a**) was found to be 0.02 whereas that obtained from the integration of the same signals in the spectrum recorded in DMSO-d6+D_2O (**Figure 2.5.b**) was 0.03.



(a)



(b)

Figure 2.5: ^{1}H NMR spectrum (400M Hz, 80 ^{o}C) of HEG-PPGE (Entry 6 in Table 2.2) (a) in D_2O (6 mg/ml) and (b) in DMSO-d6+D_2O (6 mg/ml).

Table 2.2: Modification of HE-Guar in DMSO (TBAOH).

Entry	Name of Product	Alkyl epoxide	Ratio of epoxide /HEG	Yield (%)	Solubility in D_2O	Solubility in DMSO	DS in D_2O	DS in DMSO
1	HEG-GHE	GHE	0.15	78	ST, LV	T, V	0.14	0.26*
2			0.05	65	ST, FP, LV	--	0.04	--
3	HEG-PGE	PGE	0.15	56	ST, FP, LV	T,	0.14	0.18*
4			0.05	57	T,	--	0.05	--
5	HEG-PPGE	PPGE	0.15	74	ST, FP,	T,	0.07	0.23*
6			0.05	53	T,	T,	0.02	0.03
7	HEG-Brij56E	Brij56E	0.15	91	T, P, V	T,	0.14	0.20*
8			0.05	28	ST, LP,	T, FP	0.01	0.04
9	HEG-OP10E	OP10E	0.15	70	T, LP,	T,	0.05	0.06
10			0.05	27	T,	--	0.01	--

* : DS was excess to the dosage of alkyl epoxide used, that means it is not correct.

-- : not measured.

T: transparent

ST: semi-transparent

FP: a few of white precipitate

LP: a lot of precipitate

V: viscous

LV: low viscous

From **Table 2.2**, it can be concluded that all the five alkyl epoxides, GHE, PGE, PPGE, Brij56E and OP10E, could be efficiently grafted on HEG. Indeed, for a ratio of epoxide/HEG of 0.15, the obtained DS (in D_2O) of HM-HEG samples were as follows: PGE (0.14) = GHE (0.14) = Brij56E (0.14) > PPGE (0.07) > OP10E (0.05). For a ratio of epoxide/HEG=0.05, the DS (in D_2O) of modified HEG are as follows: PGE (0.05) > GHE (0.04) PPGE (0.02) > Brij56E (0.01) = OP10E (0.01). These results indicate different reactivity of the alkyl epoxides. The PPGE and OP10E

appear to be less reactive, which may be related to their high hydrophobicity.

It should be noted that the balance between hydrophobic interactions favoring precipitation and water-solubility of modified polysaccharides is difficult to control. In DMSO-d6, all products gave clear solutions. In D_2O, HEG-GHE, HEG-PGE and HEG-PPGE also lead to transparent solutions, whereas HEG-Brij56E and HEG-OP10E were only partly dissolved. The water solubility observed for HEG-PPGE may be explained by the low DS obtained.

II. Viscosity of HM-HEG

The steady shear viscosities of solutions of the different HEG derivatives in dilute solution were measured using a low shear 30 viscometer. **Figure 2.6** compares variation of the viscosity as a function of shear rate for some samples of HEG derivatives in water at a concentration of 4.8 g/L.

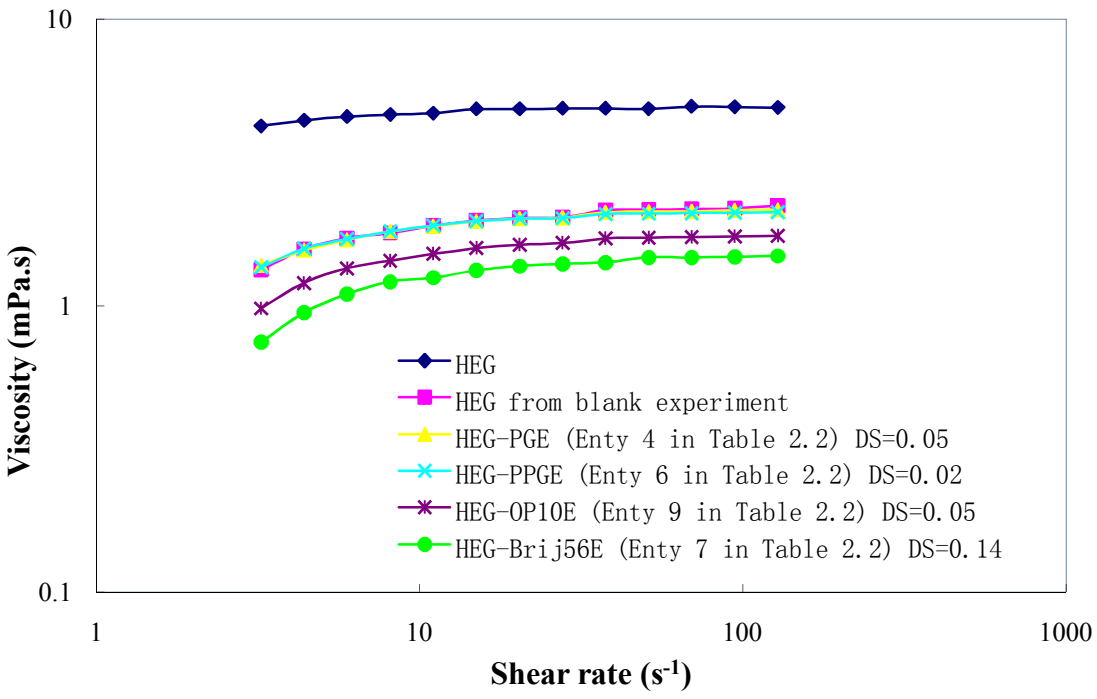


Figure 2.6: Variation of the viscosity as a function of shear rate for some samples of HEG derivatives in water at a concentration of 4.8 g/L at 25 °C.

As can be seen from **Figure 2.6**, the viscosities of all products were lower than the

initial HEG; this was attributed partly to the degradation of modified HEG during the reaction procedure in presence of TBAOH as indicated by the "blank" experiment performed without epoxide. This result may also suggest self-aggregation of the HM-HEG leading to nano-sized particles.

2.4.1.2 Hydrophobically modified guar (HM-G)

I. Synthesis and NMR characterization of HM-G

Five hydrophobic epoxides were used respectively as alkylating agents of guar: GHE, PGE, PPGE, Brij56E, and OP10E.

The reaction conditions and the degree of substitution (DS) derived from ^{1}H NMR are summarized in **Table 2.3.** The reaction procedure consists in reacting guar with the alkyl epoxide in DMSO, in the presence of TBAOH, at room temperature, for 4 days, and then the product is isolated by dialysis or precipitation.

Table 2.3: Modification of Guar in DMSO.

Entry	Name of product	Alkyl epoxide	Ratio of epoxide /Guar	Yield (%)	Solubility		DS	
					in D_2O	in DMSO	in D_2O	in DMSO
1	G-GHE	GHE	0.15	--	ST	--	0.03	--
2			0.05	98	ST	INS	0.02	--
3	G-PGE	PGE	0.15	87	T	--	0.02	--
4			0.05	90	T	INS	0.01	--
5	G-Brij56E	Brij56E	0.15	35	ST	ST, FP	0.02	0.09
6	G-PPGE	PPGE	0.15	91	INS	ST, FP	--	0.03

7	G-OP10E	OP10E	0.05	47	ST	---	0	--

-- : not measured.
T: transparent
ST: semi-transparent
FP: a few of white precipitate
INS: insoluble

From **Table 2.3**, it can be noticed that all alkyl groups could be grafted on guar but the DS of the HM-G derivatives measured by ^{1}H NMR appeared to be lower compared to the HM-HEG derivatives. In other words, HEG seems to be much easier to be reacted with hydrophobic epoxide than Guar. This may be related to results reported in patent US4870167 [4]: under the conditions of alkylation used in this invention (in IPA, KOH as alkali, 60°C), underivatized guar does not react with the long chain alkyl halide or epoxy compound. In order for the reaction to take place, the polygalactomannan must first be alkylated with a short chain alkyl halide or alkylene oxide. It was also mentioned in patent US4960876 [5] that in reactions involving polygalactomannan containing short chain substituent, i.e., less than 4 carbon atoms, at low molar substitution (less than 0.5), it is very difficult to obtain sufficient compatibility with the hydrophobic reagent for any reaction to take place.

Nevertheless, no clear conclusion can be drawn from our experiments concerning the reactivity of HEG and guar. Indeed, as the products obtained exhibited only partial water-solubility, it can be assumed that the precipitates may correspond to polysaccharide chains with higher DS.

II. Viscosity of HM-G

Figure 2.7 compares the shear rate dependencies of viscosity for some samples of guar derivatives in water or 0.1 M NaCl at a concentration of 4.8 g/L.

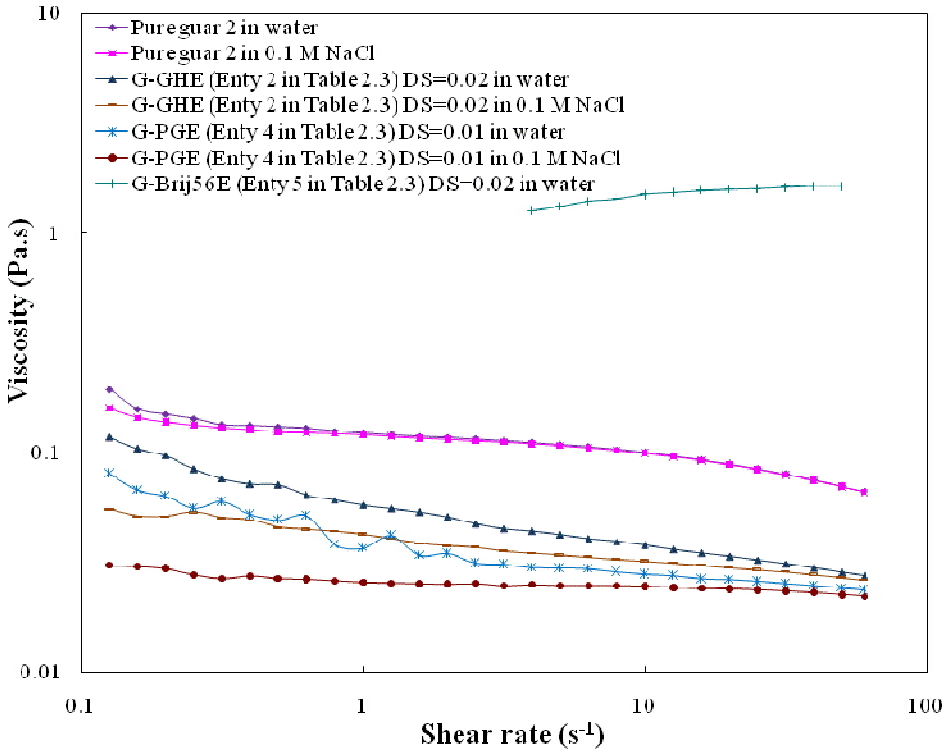


Figure 2.7: Variation of the viscosity as a function of shear rate for some samples of guar derivatives in water and in aqueous solution of NaCl (0.1 M) at a concentration of 4.8 g/L at 25 °C.

As can be seen from **Figure 2.7**, the viscosities of the solutions of the modified guar derivatives appeared to be lower than those of the initial guar except that of G-Brij56E. The lower viscosity values may be related to degradation of modified guar but also to their poor water-solubility indicating strong aggregation of polysaccharide chains due to hydrophobic interactions. The high increase of viscosity observed with the G-Brij56E derivative may be thus explained by the presence of a hydrophilic oligo(ethylene spacer) between the alkyl chain and the guar backbone. Different viscosity values were observed for the modified samples depending on the ionic strength of the solvent. The viscosities measured in 0.1 M NaCl were systematically lower than those measured in water. Hydrophobic interactions thus seem to be promoted by the presence of salt, leading to aggregation of the polysaccharide chains.

2.4.2 Alkylation of HEC, guar and HEG in IPA/water in presence of NaOH

The reaction of alkyl glycidyl ether with HEC, guar and HEG were also performed in heterogeneous conditions, i.e. in a mixture of IPA/water (88/12, v/v). In such a solvent, the alkyl epoxide is soluble whereas the polysaccharide is in a powder form. This solvent is especially used for industrial procedures as the modified polysaccharide can be easily recovered by filtration and washing.

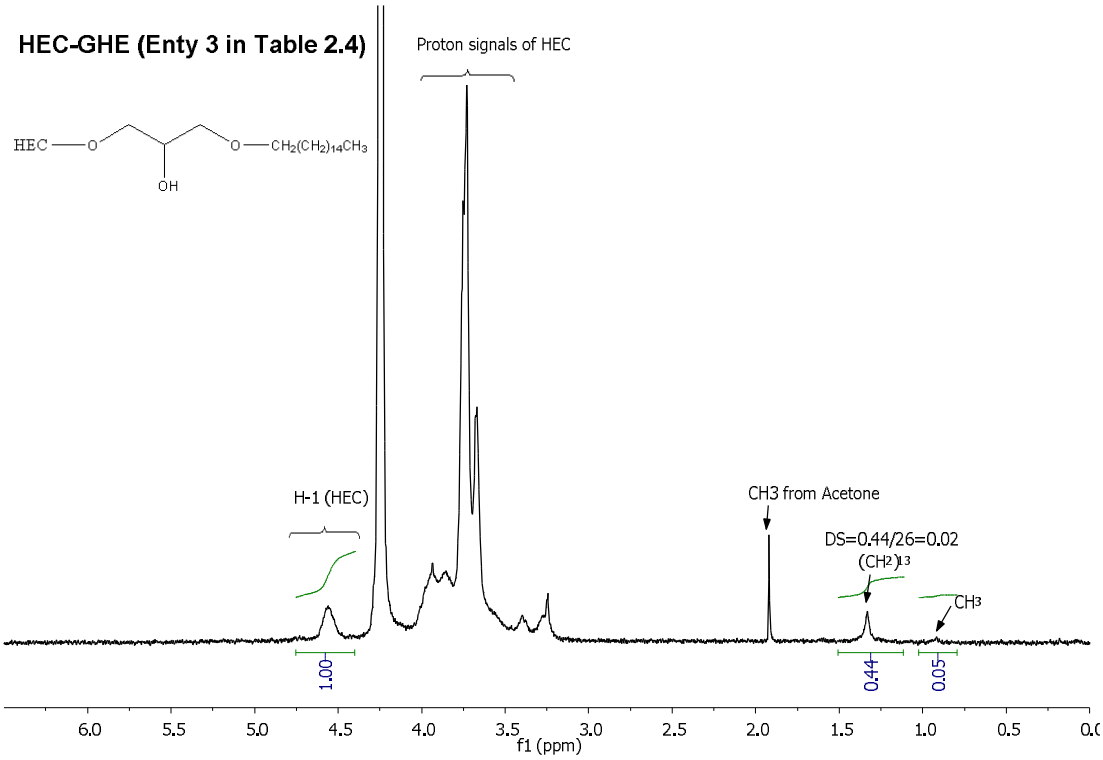
2.4.2.1 Hydrophobically modified hydroxyethyl cellulose (HM-HEC)

I. Synthesis and NMR characterization of HM-HEC

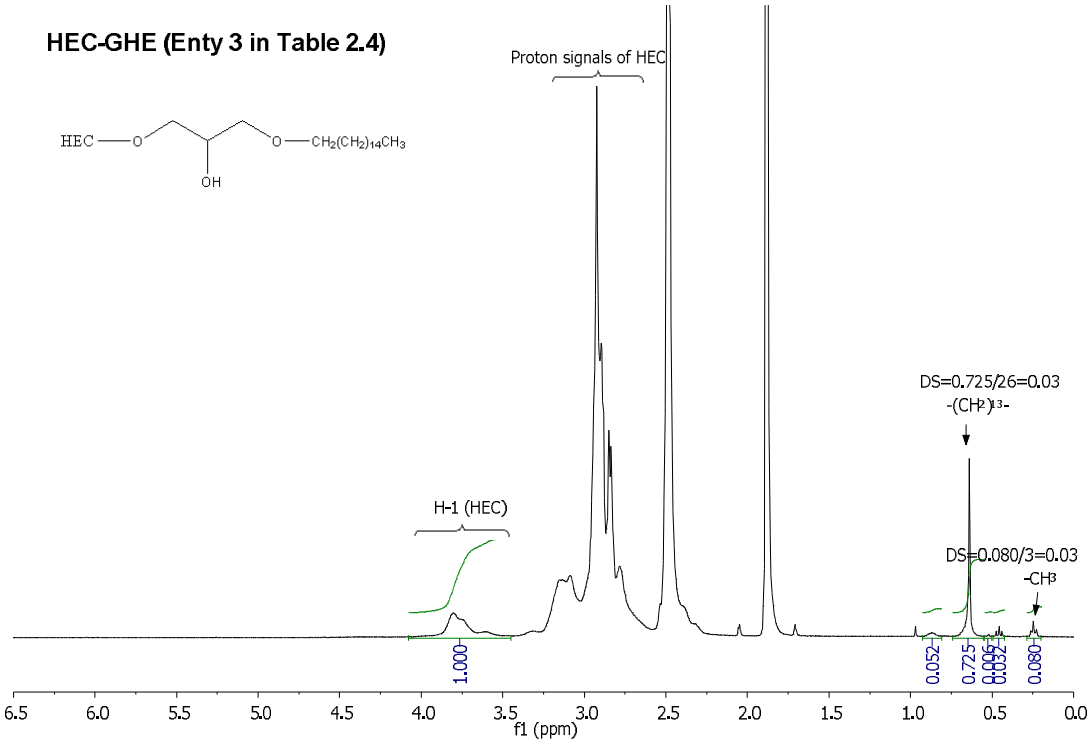
Hydrophobically modified hydroxyethyl cellulose (HM-HEC) derivatives by long chain alkyl epoxides were synthesized and characterized in this section. Five hydrophobic epoxides were used respectively as alkylating agents of HEC: GHE, PGE, PPGE, Brij56E, and OP10E.

The reaction conditions and the degree of substitution (DS) derived from ^{1}H NMR are summarized in **Table 2.4.** The reaction procedure consists in reacting HEC with the alkyl epoxide in IPA/water, in the presence of NaOH, at 80 ^{o}C, for 8 h, and then the product is isolated by filtration and washing.

^{1}H NMR analysis was performed in D_2O and/or DMSO-d6+D_2O. As already mentioned, some discrepancies were observed depending on the solvent. As illustrated in **Figure 2.8**, the DS measured from the spectrum recorded in D_2O (0.02, **Figure 2.8.a**) was lower than that derived from the spectrum recorded in DMSO-d6+D_2O (0.03, **Figure 2.8.a**).



(a)



(b)

Figure 2.8: ^{1}H NMR spectrum of HEC-GHE (Entry 3 in Table 2.4) recorded (a) in D_2O and (b) in DMSO-d6 (+1 drop of D_2O).

Table 2.4 gives the DS calculated from ^{1}H NMR integration for the different HM-HEC together with the qualitative observation of their behavior in water at a concentration of 6 g/L.

Table 2.4: Modification of HEC in IPA/water.

Entry	Name of product	Alkyl epoxide	Ratio of epoxide /HEC	Yield (%)	Solubility		DS	
					in D_2O	in DMSO	in D_2O	in DMSO
1	HEC-OP10E	OP10E	0.20	65	S	S	0.02	0.01
2			0.61	74	S	--	0.04	--
3	HEC-GHE	GHE	0.18	68	S	S	0.02	0.03
4	HEC-PGE	PGE	0.23	56	S	--	0.13	--
5	HEC-PPGE	PPGE	0.23	82	INS	INS	--	--
6			0.23	82	INS	INS	--	--
7	HEC-Brij56E	Brij56E	0.10	67	T, V	S	0.01	0.01
8			0.05	62	T, V	S	0.01	0.01

-- : not measured.
S: soluble
T: transparent
ST: semi-transparent
INS: insoluble

From **Table 2.4**, it can be noticed that most of the derivatives appear to retain water-solubility. The grafting efficiency seems to be rather low. Indeed ~ 10 % of the epoxide appeared to be reacted with the polysaccharide chain which can be related to

the heterogeneity of the reaction conditions. These indeed my not favor the reaction. However, this methodology has the advantage of using environmentally friendly conditions.

Insoluble products were observed for the grafting of Brij56E and PPGE on HEC. The water-insolubility may be due to the excess grafting of the long hydrophobic chain of Brij56E and PPGE.

II. Viscosity of HM-HEC

Figure 2.9 compares the shear rate dependencies of viscosity for some samples of HEC derivatives in water at a concentration of 4.8 g/L.

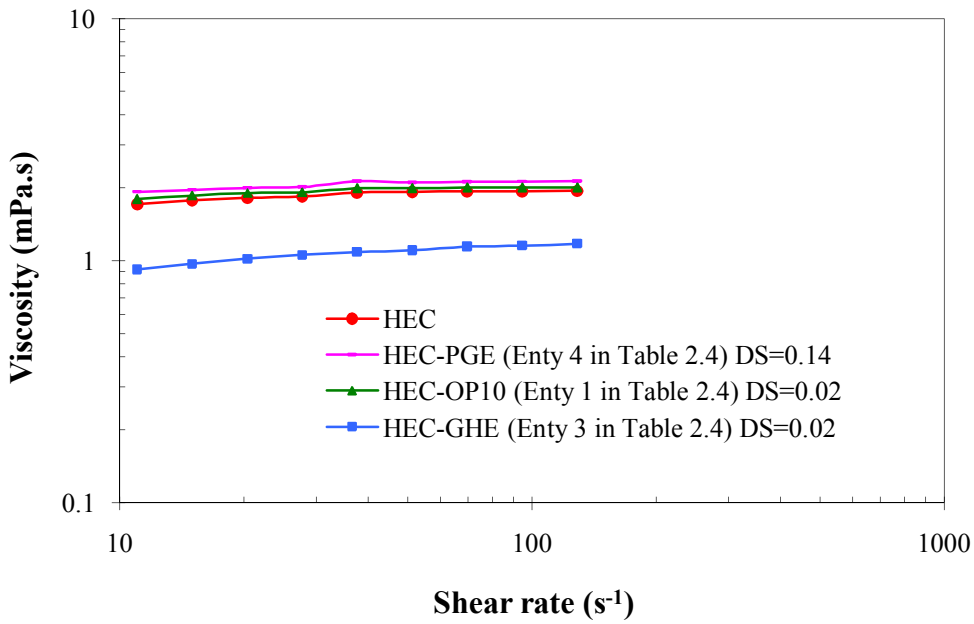


Figure 2.9: Variation of the viscosity as a function of shear rate for some samples of HEC derivatives in water at a concentration of 4.8 g/L at 25 °C.

Figure 2.9 shows that contrary to the viscosity of the solution of HEC-GHE which is much lower than that of initial HEC, the viscosity of solutions of HEC modified with PGE and OP-10 is similar to that of initial HEC.

On the other hand, the reverse situation can be observed for higher polymer

concentrations (25g/L). Indeed, in **Figure 2.10**, it can be observed that the viscosity of HEC-GHE is much higher than that of initial HEC.

Figure 2.10 also shows an increase of viscosity for the HEC-Brij56E derivatives compared to the initial HEC solution. It indicates that at low polymer concentrations, the HM-HEC coils contract due to intramolecular interactions, whereas at higher concentrations, molecules associate in aqueous solution through their hydrophobic groups. As a consequence, a three-dimensional network begins to extend which highly increases viscosity. Moreover, comparing HEC-Brij56E (Entry 7 in Table 2.4) with HEC-Brij56E (Entry 8 in Table 2.4), it could be found that the viscosity appears to increase with the theoretical DS value (derived from the epoxide/HEC ratio), which suggests that intermolecular association phenomena in the polymer solutions are promoted by not only increasing polymer concentration but also by increasing hydrophobicity.

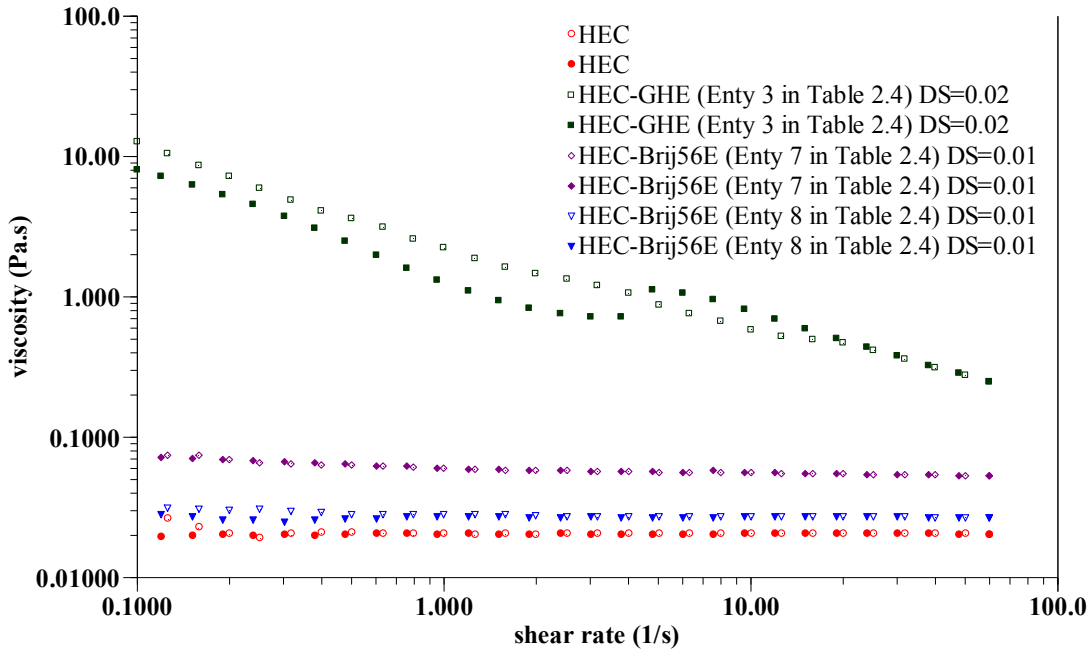


Figure 2.10: Variation of the viscosity as a function of shear rate for some samples of HEC derivatives in water at a concentration of 25 g/L at 25 °C. Open symbols: curves obtained from increasing shear rates; closed symbols: curves obtained from decreasing shear rates.

In fact, previous work[1,6] reported the modification of HEC by grafting of GHE (glycidyl hexadecyl ether). The DS and viscosity were similar to those found in this study. However, there is no paper reporting the modification of HEC by Brij56E group. These results are consistent with those obtained with guar (in section 2.4.2.2) which indicating that associative properties can be achieved through the grafting of Brij56E.

2.4.2.2 Hydrophobically modified guar (HM-G)

I. Synthesis and NMR characterization of HM-G

Based on the result obtained from alkylation reactions of HEC, we focused on the alkylation of guar with GHE and Brij56E. We used GHE and Brij56E as hydrophobic epoxides to prepare hydrophobically modified derivatives in IPA/water. Similar to HEC, the reactions were carried out in IPA/water (88/12, v/v) in the presence of NaOH, at 80 ^{o}C for 8 h.

Table 2.5: Modification of guar in IPA/water.

Entry	Name of product	Alkyl epoxide	Ratio of epoxide /guar	Solubility in water	DS in D_2O	DS in DMSO
1	G-GHE	GHE	0.65	S	0	0.07
2			0.18	S	0	--
3			0.13	S	0	0.04
4	G-Brij56E	Brij56E	0.18	S	0	--
5			0.10	S	0	--

6		0.05	S	0	--

-- not measured.
S: soluble
T: transparent
ST: semi-transparent
INS: insoluble

From **Table 2.5**, it was found that by ^{1}H NMR analysis in DMSO-d6, GHE could be grafted on guar under these reaction conditions. But under these conditions, the degree of substitute was low. In the case of reactions performed with Brij56E, the grafting of Brij56 could be detected by ^{1}H NMR.

II. Viscosity of HM-G

Although grafting of Brij56 could be hardly demonstrated by NMR analysis, we investigated the behavior in aqueous solution of guar modified with GHE and Brij56E. **Figure 2.11** compares the variation of viscosity as a function of shear rate for two samples of G-GHE and G-Brij56E (Entry 2 and 4 in Table 2.5). For G-GHE (Entry 2 in Table 2.5, DS=0.007) and G-Brij56E (Entry 2 in Table 2.5, DS=0.01), the viscosity increased than the initial guar.

The steady shear viscosities of solutions of the different guar samples clearly demonstrate a thickening effect resulting from the grafting of Brij56 on guar. Indeed, a two-fold increase of the viscosity at the lower shear rate can be observed. On the other hand, no clear conclusion can be drawn from the behavior of Guar-GHE as the viscosity values obtained from increasing and decreasing shear rate are different. Additional experiments need to be performed for better understanding of this behavior.

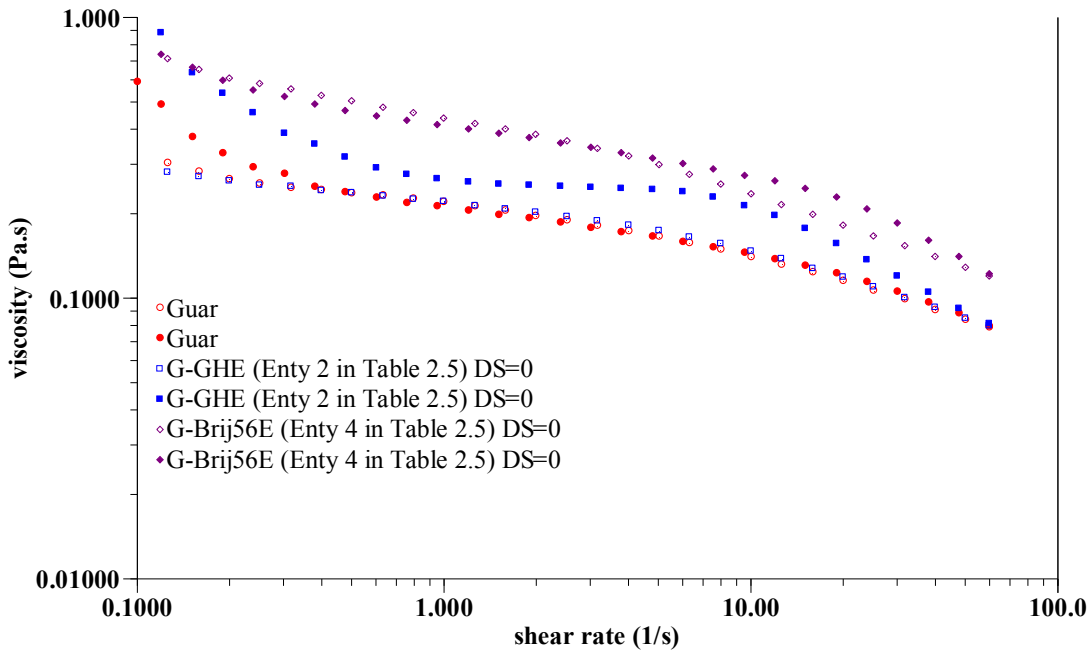


Figure 2.11: Variation of the viscosity as a function of shear rate for some samples of guar derivatives in water at a concentration of 4.8 g/L at 25 °C. Open symbols: curves obtained from increasing shear rates; closed symbols: curves obtained from decreasing shear rates.

As addition of 0.1 M NaCl appeared to have a strong effect of the viscosity values of solution of modified guar samples prepared in DMSO, we also investigated the role of salt in the case of the Guar-Brij56E derivative prepared in IPA/water. As illustrated in **Figure 2.12**, addition of NaCl at a concentration of 0.1 M leads to a strong decrease of the storage and loss moduli, G' and G", respectively. It can be thus concluded that salt promotes hydrophobic interactions leading to strong aggregation of the guar derivatives in aqueous solution.

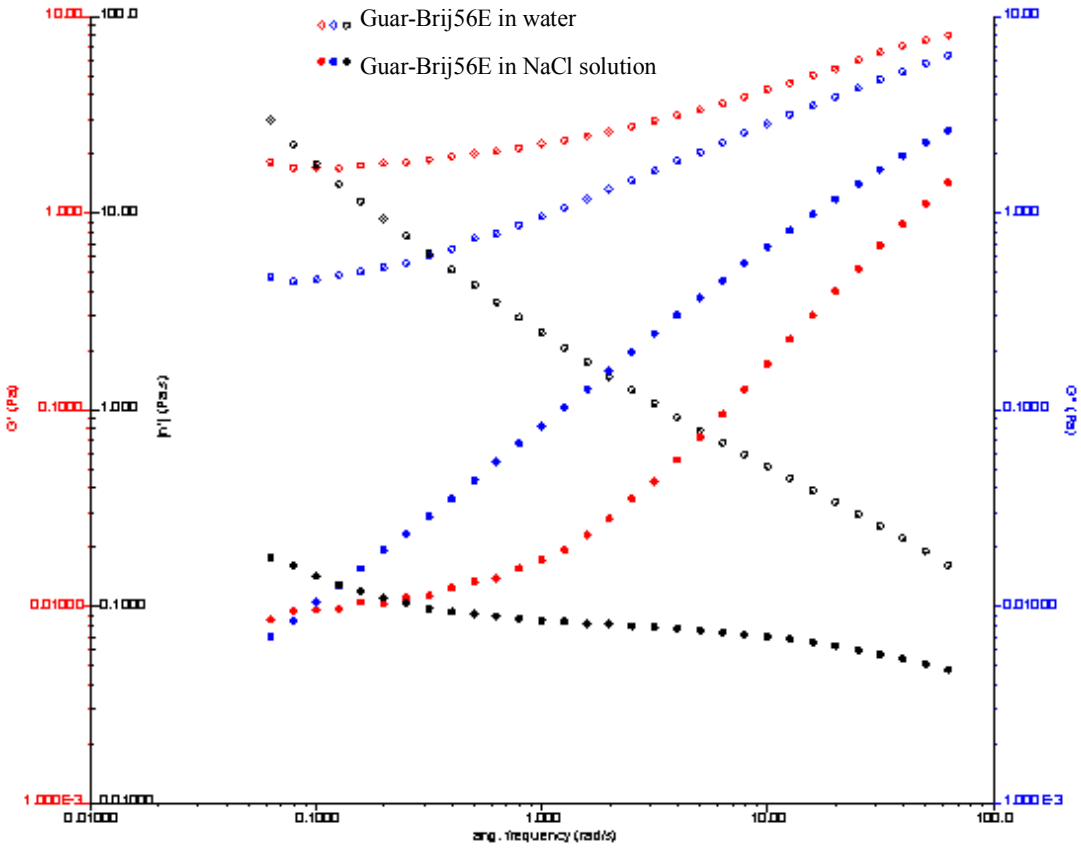


Figure 2.12: Comparison of the storage and loss moduli as a function of frequency for solutions of Guar-Brij56E (4.8g/L) in pure water and in 0.1 M NaCl at 25 °C.

2.4.2.3 Hydrophobically modified hydroxyethyl guar (HM-HEG)

I. Synthesis and NMR characterization of HM-HEG

The same condition reactions were then extended to hydroxyethyl guar (HEG, DS=0.2~0.3) using different epoxides: GHE, PGE, PPGE, Brij56E. The reactions were performed in IPA/water, in the presence of NaOH, at 80 °C for 8 hours. The chemical reaction conditions and the degree of substitution (DS) derived from ^{1}H NMR are given in **Table 2.6.**

Table 2.6: Modification of HEG in IPA/water.

Entry	Name of product	Alkyl epoxide	Ratio of epoxide/	Yield (%)	Solubility	DS

			HEG		in D_2O	in DMSO	in D_2O	in DMSO
1	HEG-GHE	GHE	0.13	68	S	--	0	0.01
2			0.30	50	S	--	0	0.07
3	HEG-PGE	PGE	0.13	50	S	--	0	--
4			0.65	50	S	--	0.04	--
5	HEG-PPGE	PPGE	0.15	51	INS	INS	--	I
6	HEG-Brij56E	Brij56E	0.15	85	INS	INS	--	
7			0.05	62	S	--	0	0.0055
8			0.10	68	S	--	0	0.0066
9	HEG-GPE	GPE	3.8	61	INS	INS	--	--
10			0.65	52	INS	INS	--	--
11			0.3	58	INS	INS	--	--
12			0.15	39	INS	INS	--	--
13			0.05	42	PS	--	0	--

From **Table 2.6**, it can be noticed that most of the products are insoluble in aqueous solution which may be related to the hydrophobic character of the final product and/or the heterogeneous conditions used for the coupling reaction. One can indeed assume that the water-insolubility is due to high degrees and/or heterogeneity of substitution,

promoting aggregation and precipitation.

II. Viscosity of HM-HEG

Figure 2.13 compares the shear rate dependencies of viscosity for some samples of guar derivatives in water at a concentration of 4.8 g/L.

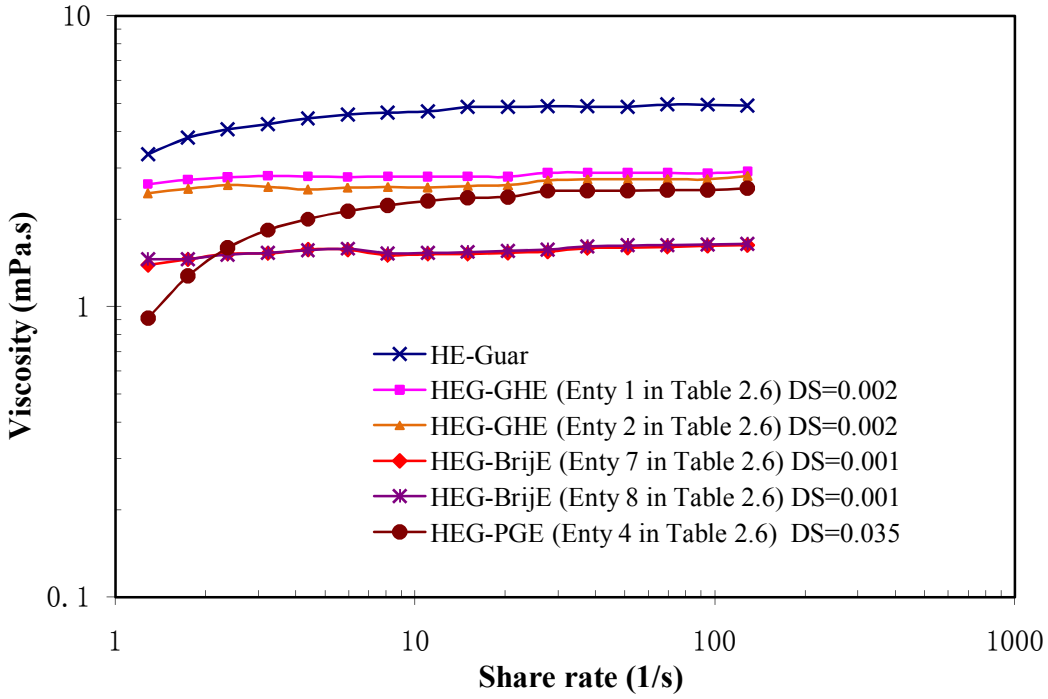


Figure 2.13: Variation of the viscosity as a function of shear rate for some samples of HEG derivatives in water at a concentration of 4.8 g/L at 25 °C.

From **Figure 2.13**, it can be observed that all the viscosities of the modified products decreased compared to initial HEG indicating aggregation of the polysaccharides in aqueous solution.

2.4.3 Modification of HEG in different mediums

From the results obtained from HEG, we decided to assess in more details the role of

the solvent on the properties of the modified HEG for a given hydrophobic epoxide. PGE was thus reacted with HEG in three different medium: 1) in DMSO/water mixture; 2) in water; 3) in DMSO.

The chemical reaction conditions and the degree of substitution (DS) from the result of ^{1}H NMR are summarized in **Table 2.7**.

Table 2.7: Modification of HEG by PGE in different mediums.

Entry	Ratio of PGE /HEG	Solvent condition /volume	Reaction temperature and time	Yield (%)	Solubility		DS	
					in D_2O	in DMSO	in D_2O	in DMSO
1	0.30	H_2O/DMSO=1/1 10 ml pH=13 (NaOH)	RT, 4 days	58	T, V	T, V	0.04	0.04
2	0.30	H_2O 5ml pH=13 (NaOH)	RT, 4 days	62	T, V	T, V	0.03	0.03
3	0.30	DMSO 5ml pH=13 (NaOH)	RT, 4 days	61	T, V	T, V	0.04	0.04
4	0.65	IPA/water=88% 1 ml (NaOH)	80 ^oC, 8 h,	50	S	--	0.04	--

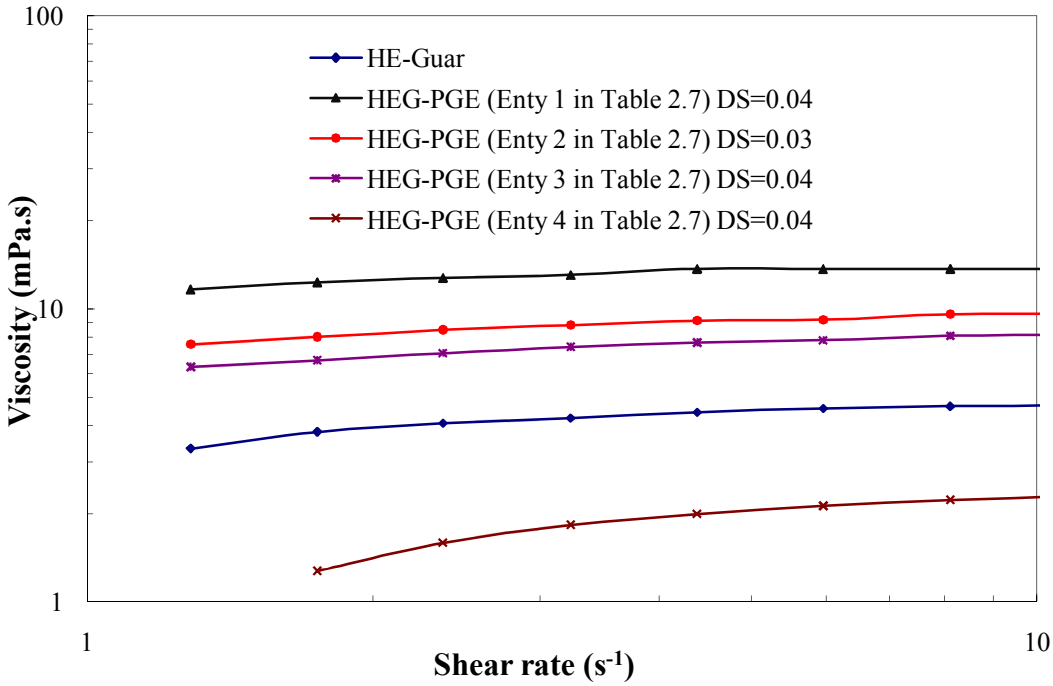


Figure 2.14: Variation of the viscosity as a function of shear rate for some samples of HEG derivatives in water at a concentration of 4.8 g/L at 25 °C.

From **Figure 2.14**, HEG-PGE samples prepared under homogeneous conditions (Entries 1, 2 and 3 in **Table 2.7**) lead to a slight increase of viscosity in aqueous solution, contrary to the sample synthesized in IPA/water. In the latter case, a decrease o viscosity can be observed although the DS is similar to that of the other samples. These different associative properties suggest different distributions of the alkyl groups along the HEG chains.

2.4.4 Blank experiment

To clarify the procedure effect on the rheological behavior of the polysaccharide, blank experiments using the same reaction conditions used for the synthesis of the HM-HEG derivatives but without epoxide were performed. The molecular weight of the final products was measured by size exclusion chromatography (SEC) and the results were summarized in **Table 2.8.**

Table 2.8: Molecular weight of blank experiment products.

Entry	Reaction	Solvent condition /volume	Reaction temperature and time	Mw （GPC） （g/mol）	Polydispersity (Mw/Mn)	% of eluted product
--	HEG	--	--	594900	2.28±0.08	45
1	HEG blank in presence of TBAOH	DMSO 4 ml	RT, 4 days	80210	1.25±0.06	108
2	HEG blank	H_2O/DMSO=1/1 10 ml	RT, 4 days	574000	2.15±0.03	73
3	HEG blank	H_2O 5 ml	RT, 4 days	597300	2.45±0.03	81
4	HEG blank	DMSO 5ml	RT, 4 days	573800	2.80±0.04	84

As shown by **Table 2.8**, the molecular weight of Entry 1 in Table 2.8 decreased significantly than that of initial HEG, indicating that the procedure using TBAOH caused the degradation of HEG. For other three products, it was found that the reaction procedure including dissolving, stirring, dialysis and freeze-drying did not cause the degradation of HEG. **Figure 2.15** compares the variation of viscosity as a function of shear rate for some samples of HEG derivatives. It is not easy to explain the slight increase of viscosity after the blank experiment. These results are also not consistent with the results described in section 2.4.3 (modification of HEG by PGE in different mediums). Additional experiments are required to clarify this.

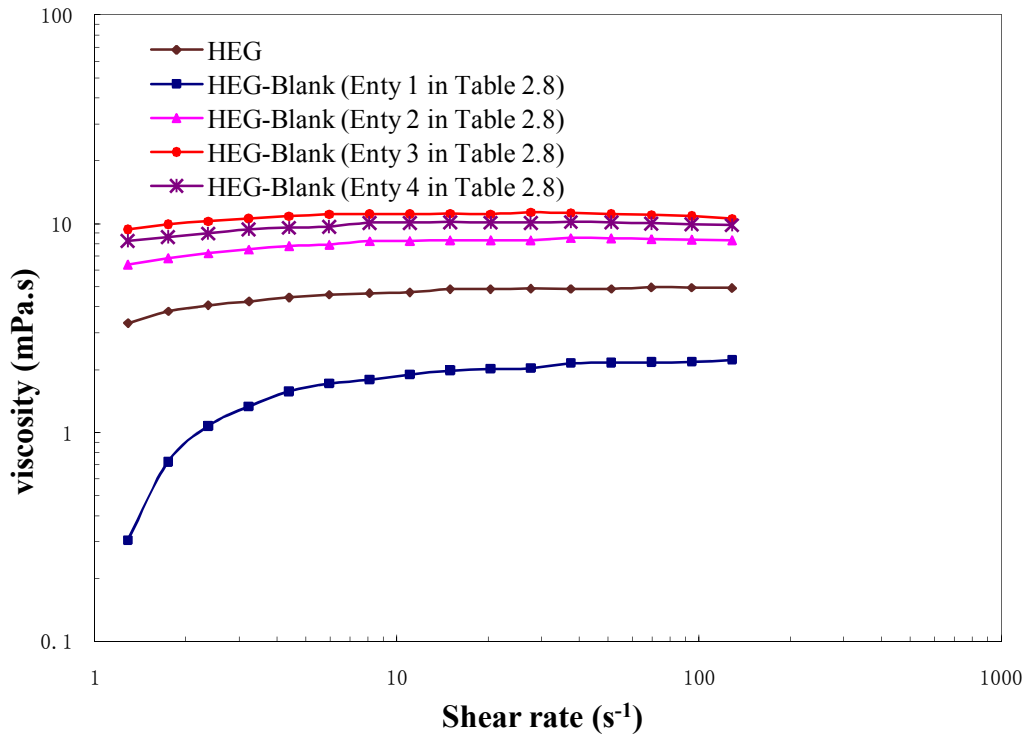


Figure 2.15: Variation of the viscosity as a function of shear rate for some samples of HEG derivatives from blank experiments in water at a concentration of 4.8 g/L at 25 °C.

2.5 Conclusion

In order to obtain the amphiphilic polysaccharide which is expected to exhibit original rheological properties in aqueous solution, three strategies to synthesis of the hydrophobically polysaccharide are investigated. It is found that IPA/water reaction system could give several aimed products. The viscosities of solutions of the modified samples clearly demonstrate a thickening effect resulting from the grafting of GHE or Brij56 on HEC or guar. It is the first time that modification of HEC and guar by Brij56E group are represented, which indicated that associative properties can be achieved through the grafting of Brij56E. Further more, the intermolecular association phenomena in the polysaccharide solutions are promoted by not only the increasing hydrophobicity but also the increasing polysaccharide concentration (from 4.8g/L to 25 g/L). However, in IPA/water reaction system, the grafting efficiency seems to be rather low, ~ 10 % of the epoxide appeared to be reacted with the polysaccharide chain which can be related to the heterogeneity of the reaction conditions. However, this methodology has the advantage of using environmentally friendly conditions.

On the contrary, DMSO (with TBAOH) system could not give the anticipated product, even though all the five alkyl epoxides, GHE, PGE, PPGE, Brij56E and OP10E, could be efficiently grafted on HEG and guar under this system, the viscosity of the solution of modified polysaccharide decreased than that of initial polysaccharide. Indeed, for a ratio of epoxide/polysaccharide of 0.15, the obtained DS of hydrophobically modified samples are between 0.14 and 0.05; for a ratio of epoxide/polysaccharide of 0.05, the obtained DS are between 0.05 and 0.01. The various obtained DS indicate, on one hand, different reactivity of the alkyl epoxides as follows: PGE ≥ GHE ≥ Brij56E >PPGE > OP10E. The PPGE and OP10E appear to be less reactive, which may be related to their high hydrophobicity. On the other hand, HEG seems to be much easier to be reacted with hydrophobic epoxide than Guar. The low viscosity of the solution of product is related to degradation of modified guar and maybe also to their poor water-solubility indicating strong

aggregation of polysaccharide chains due to hydrophobic interactions.

It should be noted that the balance between hydrophobic interactions favoring precipitation and water-solubility of modified polysaccharides is difficult to control under all the three reaction systems. Water insoluble products are observed in many cases, which may be due to the high degrees and/or heterogeneity of substitution of the long hydrophobic chain, promoting aggregation and precipitation.

The addition of NaCl at a concentration of 0.1 M have a strong effect of the viscosity values of solution of modified guar samples prepared in either IPA/water or DMSO. It leads to obvious decrease of the storage and loss moduli, G' and G'', respectively. It can be concluded that salt promotes hydrophobic interactions leading to strong aggregation of the guar derivatives in aqueous solution.

HEG-PGE samples prepared under the third strategy: in DMSO/water in presence of NaOH, homogeneous conditions, lead to a slight increase of viscosities in aqueous solutions, contrary to the HEG-PGE sample prepared in IPA/water. In the latter case, a decrease of viscosity can be observed although the DS is similar to that of the other samples prepared under the third strategy. These different associative properties suggest different distributions of the alkyl groups along the HEG chains.

Based on the above results, IPA/water reaction system is successful to introduce the long alkyl chain along HEC and guar and give the aimed products.

2.6 Materials and Methods

Materials. Guar and hydroxypropyl guar were supplied by Rhodia (Saint-Fons, France). Hydroxyethyl guar and hydroxyethyl cellulose were provided by Hercules-Aqualon (Rueil-Malmaison, France) and Jingkun chemistry company (Kunshan, China), respectively. The molar mass distribution and the weight-average molar mass of the initial polysaccharides and blanks were determined by size exclusion chromatography using a Waters GPCV Alliance 2000 chromatograph (USA) equipped with three on-line detectors: a differential refractometer, a viscometer and a light scattering detector (MALLS) from Wyatt (USA); the solutions were injected at a concentration of 5×10^{-4} g/mL in 0.1 M $NaNO_3$. Polyoxyethylene octylphenol ether (OP10) was purchased from Kelong (Chengdu, China). Glycidyl hexadecyl ether (GHE), phenyl glycidyl ether (PGE), Brij®56 and all other chemicals were purchased from Sigma-Aldrich-Fluka.

The water used in all experiments was purified by a Millipore Milli-Q Plus purification system, with a resistivity of 18.2 MΩ cm.

NMR spectroscopy. ^{1}H NMR spectra of the hydrophobically modified polysaccharides dissolved in deuterium oxide (6 mg/mL) were performed at 80 °C using a Bruker DRX400 spectrometer operating at 400 MHz. Deuterium oxide was obtained from SDS (Vitry, France).

Mass spectrometry. MALDI-TOF measurements were performed on a Bruker Daltonics Autoflex apparatus using 2,5-dihydroxybenzoic acid as matrix for the analysis of modified CD.

Rheological experiments. Flow experiments were carried out with the AR2000 rheometer. The polysaccharide derivatives were first dissolved in water or 0.1 M NaCl at a concentration of 10 g/L. After stirring for 12 hours at room temperature, the samples were kept in the refrigerator for ~ 12 hours.

Synthesis.

Synthesis of Brij56 glycidyl ether (Brij56E). Brij®56 (2 g, 2.93 mmol) was dissolved in 10 mL of anhydrous toluene and dried by azeotropic distillation of the solvent. Sodium hydride (0.21 g, 8.80 mmol) was added in a flask, and then 5 mL of anhydrous toluene was added, the mixture was stirred under nitrogen atmosphere. The dried Brij®56 was redissolved in 10 mL of anhydrous toluene, and then added in the flask under nitrogen atmosphere. The reaction mixture was stirred at 30 °C for 2 h under nitrogen atmosphere. Epichlorohydrin (0.75 mL, 11.73 mmol) was added to the reaction mixture and stirred at 40 °C for 6 h. The result mixture was left overnight in refrigerator (4 °C) to make the solid deposited from the liquid. The liquid was separated from the solid carefully and then evaporated to remove the solvent (toluene) by reduced pressure distillation. The product (Brij56E) was not purified further.

The ^{1}H NMR spectrum of Brij56E was given in **Figure 2.3.b**. The mass spectra of Brij®56 and Brij56E were given in **Figure 2.4**.

The same procedure was applied for the synthesis of OP10E and PPGE.

The protocols described below are typical examples of those used for the synthesis of hydrophobically modified guar, HEG and HEC.

Synthesis of HEG-PPG in DMSO in the presence of TBAOH (Table 2.2, entry 6). HEG (MS=0.2~0.3, 0.1 g) was dissolved in an aqueous solution (4 mL) for 24 hours. Tetrabutylammonium hydroxide (TBAOH, 1.39 g, the molar ratio of TBAOH to sugar unit is 3:1) was added to the solution, stirred at room temperature for 1 hour. Then, the aqueous solution is freeze-dried. The resulting solid was dissolved in DMSO (4 mL) at room temperature. After 4 hours, the required amount of poly(propylene glycol) monobutyl ether epoxide (PPGE, 0.031 g, the molar ratio of PPGE to sugar unit is 0.05:1) was added to the solution. The reaction medium was stirred at room temperature for 4 days. After that time, the reaction mixture was diluted with water

(12 mL) and dialyzed against a water/ethanol mixture (50:50 v/v) and finally against water. The resulting aqueous solution was freeze-dried and the final product was isolated with a yield of 53 %. The degree of substitution was determined by ^{1}H NMR in D_2O and/or DMSO-d_6+D_2O as described above.

Synthesis of HEC-GHE in IPA/water in the presence of NaOH (Table 2.4, e.g. entry 3). 0.5 g of HEC (MS=2.5) and 4 g of isopropyl alcohol, and 0.035 g of a 48% aqueous solution of sodium hydroxide was added in a 10 mL glass flask to prepare a slurry. The slurry was stirred at room temperature for 30 min under a nitrogen atmosphere. To this mixture, glycidyl hexadecyl ether (GHE, 0.0097 g, the molar ratio of GHE to sugar unit is 0.18:1) was added to conduct a reaction at 80 °C for 8 h. After completion of the hydrophobization reaction, adding acetic acid neutralized the liquid reaction mixture and the product was collected by filtration. The product was washed twice with 5 g of 80% of acetone and then twice with 5 g of acetone, and dried at 70 °C for 24 h under reduced pressure to remove remaining acetone. By this procedure, 0.34 g of the HEC-GHE was obtained (yield of 68 %). The chemical structure (see **Figure 2.8**) and purity of the HEC-GHE was ascertained by ^{1}H NMR (in D_2O and/or DMSO-d_6+D_2O). The degree of substitution of the hydrophobic groups determined from NMR analysis was 2 % in D_2O and 3 % in DMSO-d_6+D_2O.

Synthesis of HEG-PGE in DMSO/water (1/1, v/v) mixture in the presence of NaOH (Table 2.7, entry 1). 0.1 g of HEG was dissolved in 5 mL of water through stirring overnight at room temperature. 5 mL of DMSO was added to the solution, and the pH of solution was adjusted to 13 with 0.1 M NaOH. Phenyl glycidyl ether (PGE, 0.026 g, the molar ratio of PGE to sugar unit is 0.3:1) was added to the HEG solution. The mixture was stirred at room temperature for 4 days. The reaction mixture was diluted with water (10 mL) and dialyzed against a water/ethanol mixture (50:50 v/v) and finally against water. The resulting aqueous solution was freeze-dried and the final product was isolated with a yield of 58 %. The degree of substitution was determined by ^{1}H NMR in D_2O and/or DMSO-d_6+D_2O as described above.

Reference:

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CHAPTER 3

Synthesis of hyaluronic acid-Brij56 conjugates

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Résumé (*Fr*)

De nouveaux dérivés de l'acide hyaluronique (HA) amphiphiles ont été synthétisés par une réaction d'éthérification en utilisant un dérivé Brij®56 époxide. Différentes conditions de synthèse ont été testées. Parmi les dérivés hydrophobiquement modifiés obtenus, certains se sont avérés former des agrégats de taille nanométrique en milieu aqueux, résultant de l'association entre les chaînes hexadécyle des motifs Brij56. Ce phénomène d'agrégation a été mis en évidence par viscosimétrie, diffusion dynamique de la lumière et spectroscopie de fluorescence en utilisant deux sondes hydrophobes, le nile red et le pyrène. D'après les mesures de fluorescence effectuées à partir d'un dérivé HA-Brij56, la formation d'agrégats de taille nanométrique apparaîtrait pour une concentration autour de 0.1 g/L, concentration environ 20 fois inférieure à la concentration critique du polysaccharide non modifié. Cette étude suggère la possibilité d'obtenir des nanoparticules de type gel à base de HA en une seule étape par un procédé simple en milieu aqueux. Des analyses par miscroscopie s'avèrent néanmoins nécessaires pour confirmer l'existence de ces nanoobjets et déterminer leur morphologie.

Summary (*En*)

New amphiphilic derivatives of hyaluronic acid (HA) were synthesized by an etherification reaction using an epoxide derivative Brij56. Different synthesis conditions were tested. Among the derivatives obtained by hydrophobical modification, some were found to form aggregates of nanometer size in an aqueous medium, resulting from the association between the chains hexadecyl group of Brij56. This aggregation phenomenon was shown by viscometry, dynamic scattering of light and fluorescence spectroscopy using two hydrophobic probes, the nile red and pyrene. Based on fluorescence measurements made from a derivative of HA-Brij56, the formation of aggregates of nanometer size appear for a concentration around 0.1 g / L, concentration about 20 times lower than the critical concentration of unmodified polysaccharide. This study suggests the possibility of obtaining nanoparticles of type HA-based gel in one step by a simple process in aqueous media. Analysis by microscope nevertheless proves necessary to confirm the existence of these nano-objects and determine their morphology.

3.1 Introduction

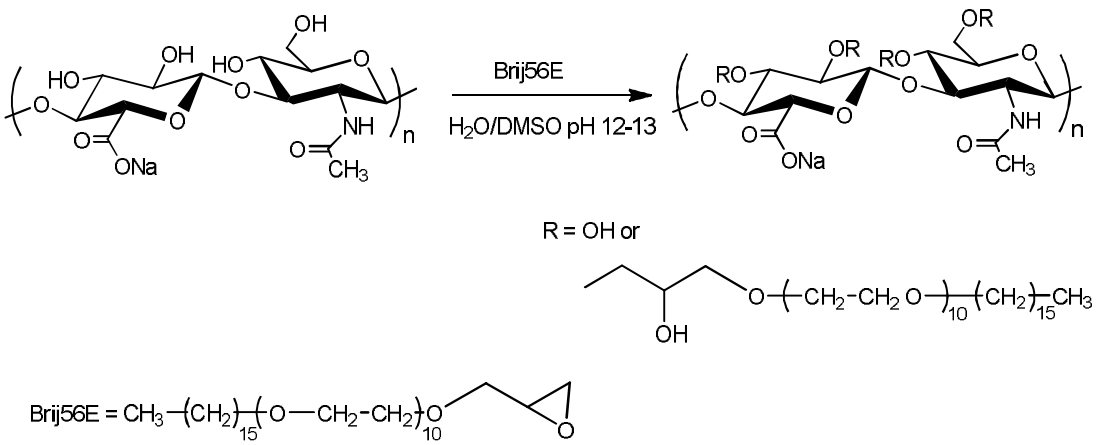
As reported in a recent review on alkyl (aryl) derivatives of hyaluronic acid (HA), several methodologies have been proposed for the synthesis of alkyl derivatives of HA [1]. Among these methods, the alkylation (arylation) of HA based on the reaction in polar organic solvents such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF), of an alkyl halide (i.e. ethyl, propyl, pentryl, benzyl, dodecyl or octadecyl bromide) with the carboxylic group of HA has been extensively used [2-4]. To make HA soluble in DMSO or DMF, HA was preliminary transformed into the tetrabutylammonium (TBA) salt to shield highly anionic carboxylic acid groups and to disrupt inter/intramolecular hydrogen bonds in HA. Alternatively, the preparation of esters was based on the reaction of HA with palmitoyl chloride in DMF in the presence of pyridine [5]. Other methods of hydrophobization in DMSO or DMF leading to the formation ethers [6, 7], carbamoyl [8] and amides [9-11] derivatives of HA were also developed. New alkylated derivatives of HA were also obtained in our laboratory by the reaction of the carboxylic acid group of HA with adipic dihydrazide followed by the coupling with an aldehydic chain (8, 10, 12, 14, 16 carbon atoms) in the presence of $NaCNBH_3$ [12]. The alkylamino hydrazide derivatives of HA were shown to have different thickening properties in aqueous solution depending on the nature of the hydrophobic group and the degree of substitution. The best thickening properties were obtained from decylamino hydrazide derivatives, leading to the formation of elastic hydrogels in aqueous solution as reported in Chapter 1. On the other hand, derivatives with longer alkyl chains (dodecyl (C_{12}), hexadecyl (C_{16}) chains) appeared to be only partly soluble in aqueous solution.

The aim of this study was the preparation of water-soluble hydrophobically modified derivatives of HA with Brij®56 moieties in hydroxyl positions (excluding carboxyl group) and investigation of the effect of reaction conditions on the behavior in aqueous solution of the products. Brij®56 possesses a C_{16} alkyl chain but the presence

of ~ 10 ethylene glycol units is expected to provide hydrophilicity, thereby maintaining water-solubility of modified HA. In this respect, we prepared several new alkylated derivatives by the reaction of HA with Brij®56 epoxide (Brij56E) described in chapter 2. The behavior of the resulting derivatives in aqueous solution was then investigated by viscosity measurements, dynamic light scattering and fluorescence measurements.

3.2 Alkylation of hyaluronic acid by Brij®56 epoxide

The alkylation of HA was performed under basic conditions in a mixture of water/DMSO (1/1, v/v) in order to solubilize both HA and Brij56E (**Scheme 3.1**).



Scheme 3.1: Synthesis of HA-Brij56E.

In a first series of experiments, we adjusted the pH at a value of 12 by adding an aqueous solution of NaOH to a mixture of water/DMSO containing HA. Brij56E was then added under stirring and the reaction medium was heated at 50 °C for 2-2h30. The modified HA samples were isolated by precipitation with ethanol, followed by ultrafiltration. The reaction conditions are summarized in **Table 3.1**. As HA is sensitive to basic conditions due to β-elimination reactions involving the glucuronic acid units which result in the polysaccharide chain cleavage, a "blank" reaction was carried out without adding Brij56E to check the impact of the reaction conditions on the molar mass of the polysaccharide.

Table 3.1 First series of alkylation reactions of HA in H_2O/DMSO (1/1, v/v) at 50 °C using Brij56E.

Entry	Ratio of Brij56E/HA[a]	pH	Reaction time (h)	DS (%)[b]
1	0.24	12	50/ 2	1

2	0.3	11.6	50/ 2.5	1,6
3	-- (Blank)	12.1	50/2	--

[a] Number of molar equivalents of Brij56E with respect to the repeating disaccharide unit;

[b] Determined by ^{1}H NMR in D_2O at 80 °C

At this step, we noticed problems with the electrode for controlling the pH in the hydro-organic mixture. Therefore, we changed the alkylation procedure. In a next series of experiments, we dissolved HA in water alone and added a fixed volume of an aqueous solution of NaOH (2 M) to reach a pH value ~ 12.5-13. The volume was selected by monitoring the variations of the pH of the aqueous solution of HA upon addition of NaOH (entries 1 and 2) or from theoretical calculations to obtain pH = 12.5 (entries 3-5). DMSO was then added followed by Brij56E. The latter was added either in only one time before heating the reaction medium (entries 1-2) or slowly during the reaction in order to limit potential hydrolysis of Brij56E (solubilization of the epoxide in 10 mL DMSO (entries 3 and 4)). The reaction medium was then heated to 50 °C and stirred for 2-3 h. The modified HA samples were isolated by precipitation with ethanol, followed by ultrafiltration. The reactions conditions are summarized in **Table 3.2**. "Blank" reactions were also performed (entries 1 and 5).

Table 3.2: Second series of alkylation reactions of HA using Brij56E (reactions performed from 0.5 g of HA solubilized in water (25 mL)).

Entry	Ratio of Brij56E/HA[a]	$V_{NaOH\ 2M}$ (mL)	pH	Reaction time (h)	DS (%)[b]
1	--(Blank)	3	12.71	2	--
2	0.3	3	12.63	2	0.07
3[c]	0.5	1.32	12.51	2.5	0.2
4[d]	0.5	1.39	12.50	3	0.3

5[e]	--	1.32	12.52	2.5	--

[a] Number of molar equivalents of Brij56E with respect to the repeating disaccharide unit;

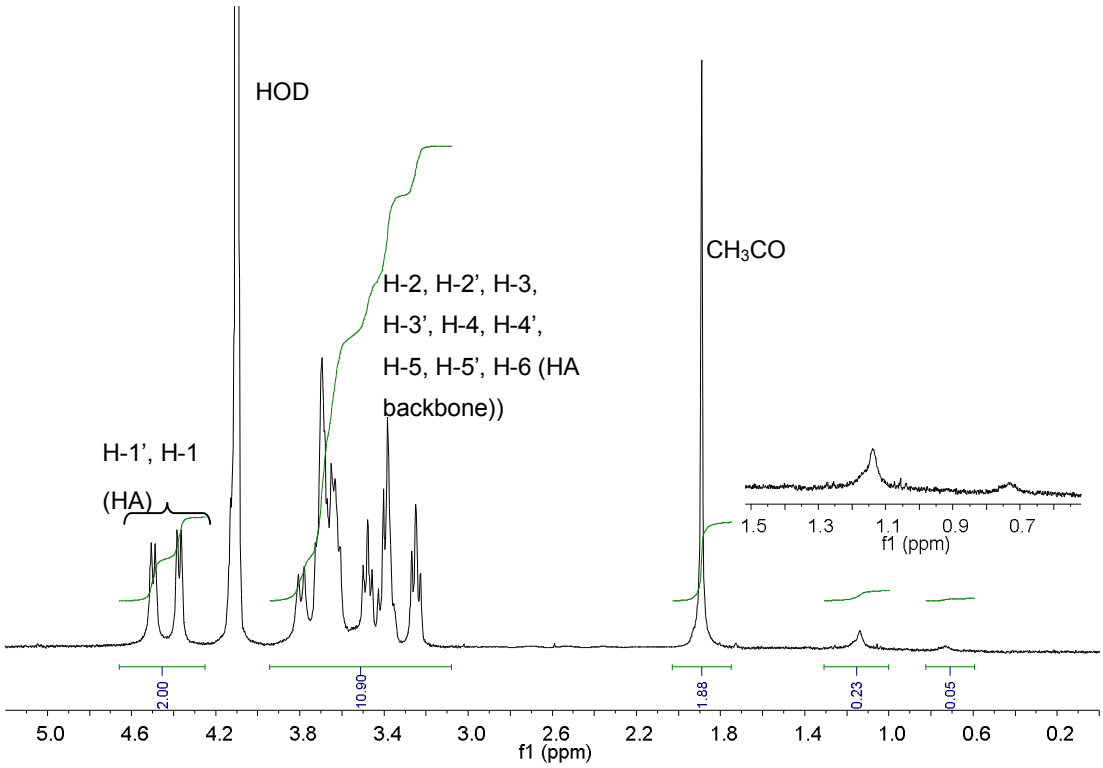
[b] Determined by ^{1}H NMR in D_2O at 80 °C;

[c] Addition of Brij56E (dissolved in DMSO) for 30 min;

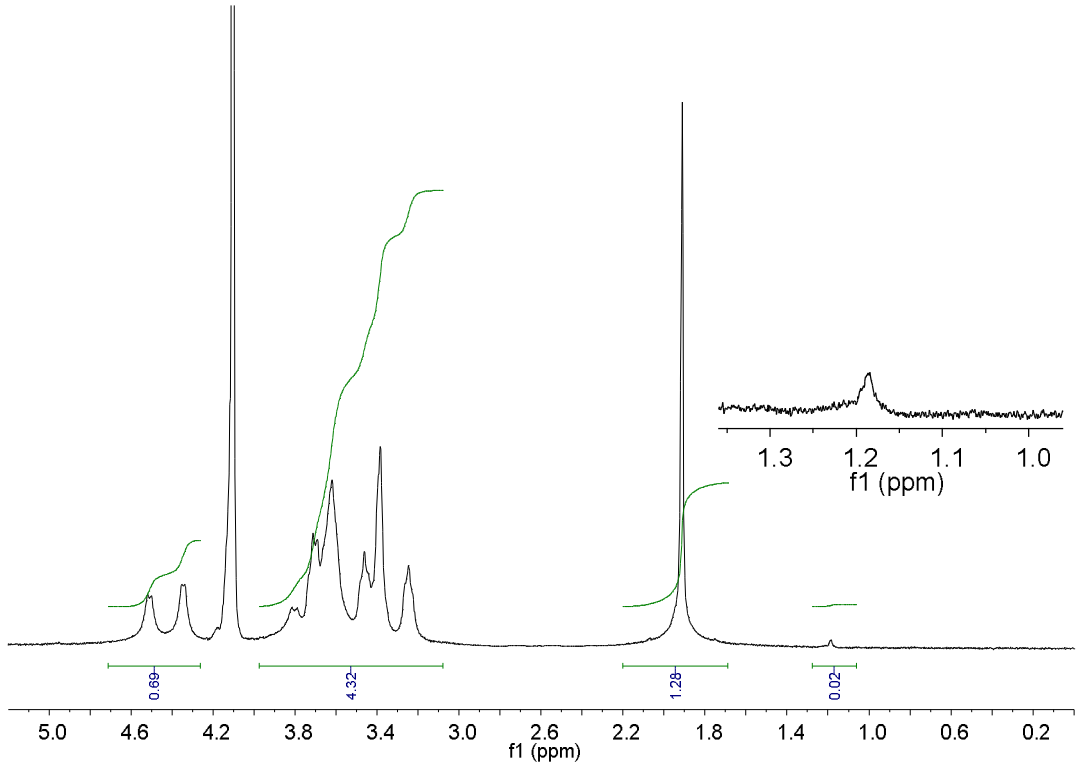
[d] Addition of Brij56E (dissolved in 10 mL DMSO) for 1 h;

[e] Reaction performed following conditions described in entry 3.

We used ^{1}H NMR spectroscopy to check the grafting of Brij56. As examples, **Figure 3.1** shows the ^{1}H NMR spectra of HA-Brij56E prepared under conditions given in entries 2 of Tables 1 and 2. As can be seen from these spectra, the signals provided by the methyl (0.8 ppm) and methylene (1.1 ppm) groups of Brij56 appeared to be broad.



a)



b)

Figure 3.1: ^{1}H NMR spectra (400 MHz, 80 °C, 6 mg/mL in D_2O) of HA-Brij56E prepared according to conditions of entry 2 in Table 3.1 (a) and entry 2 in Table 3.2 (b).

Moreover, the methyl protons signals could not be observed on the spectrum of **Figure 3.1.b**. This suggests that, when dispersed in water, part of the alkyl chains were exposed to hydrophobic microdomains decreasing their mobility [13]. As a result, ^{1}H NMR integration could not provide reliable values of the degree of substitution of the HA derivatives. Nevertheless, as it was not possible to dissolve the HA derivatives in another solvent preventing hydrophobic association, we calculated "apparent" values of the DS by digital integration of the signals of the CH_2 or CH_3 protons of the alkyl chain and the anomeric protons of HA. The values are reported in **Tables 3.1** and **Table 3.2**.

Finally, in order to assess the molar masses of the derivatives prepared in this study, the "blank" samples were analyzed by size exclusion chromatography (SEC) equipped

with a differential refractometer and a multi-angle light scattering detector. **Table 3.3** gives the weight-average molar mass (M_w) and the polydispersity (I_p) index obtained for the different samples.

Table 3.3: Weight-average molar mass (M_w) and the polydispersity index (I_p) of the blank samples derived from SEC analysis.

Blank	Entry	Sample	M_w (g/mol)	$I_p = M_w/M_n$	% eluted product
/	**1**	HA 600000 g/mol	517500	1.3	86
Tab3.1, entry 3	**2**	Blank of sample Table 3.1, entry 1	151900	1.5	78
Tab3.2, entry 1	**3**	Blank of sample Table 3.2, entry 2	226100	1,5	71
Tab3.2, entry 5	**4**	Blank of sample Table 3.2, entry 3	282100	1.5	82

As can be seen from **Table 3.3**, the alkylation reactions lead to partial hydrolysis of the HA chains. The M_w values are about two-fold decreased. It should be noted however that previous work performed in our laboratory demonstrated remarkable thickening properties in aqueous solution of alkylaminohydrazide derivatives prepared from a HA sample of 200000 g/mol[12]. These were additionally superior to those of alkyl derivatives prepared from a HA sample of 600000 g/mol which exhibited a much higher tendency to self-aggregate.

3.3 Behavior in aqueous solution of the HA-Brij56E derivatives

In the next step, we investigated the behavior of the new alkylated HA-derivatives in aqueous solution. Contrary to the decylaminohydrazide derivatives reported previously [12], which gave rise to physical gels in phosphate buffer saline (PBS, pH 7.4, 0.15 M NaCl) at a concentration of 10 g/L, solutions of low viscosity were obtained under the same conditions with the HA-Brij56E derivatives. As shown by **Figure 3.2**, the viscosity of the solutions of most of the modified HA was found to be lower than that of the blank. As hydrolysis of the HA chain varied according to the reaction conditions, resulting in different M_w, the viscosity of each HA-Brij56E was divided by that of "its blank" to assess the impact of the grafted alkyl chains as well as to allow comparison of the behavior between the HA-Brij56E derivatives. These data suggest the tendency of most of the HA-Brij56E to self-aggregate in aqueous solution.

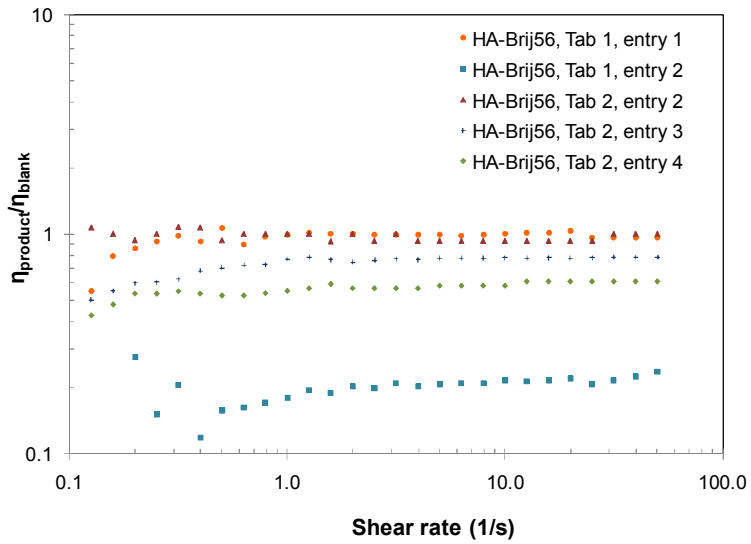


Figure 3.2: Variation of the viscosity of HA-Brij56E derivatives (10 g/L in PBS) divided by that of "their blank" as a function of shear rate. Temperature: 25 °C. (Tab 1 is Table 3.1, Tab 2 is Table 3.2,)

In order to have more information about the behavior of these derivatives in PBS, dynamic light scattering experiments were performed in PBS at a concentration of 2 g/L. This concentration is slightly below the critical overlap concentration of HA with a molar mass M_w of 200000 g/mol assuming $C^*[\eta] \approx 1$ ($C^* \approx 2.4$ g/L) [12].

Figure 3.3 compares the size distributions obtained from DLS measurements at an angle of 90° of the different HA-Brij56E samples. Among the latter, three derivatives (entry 2 in Table 1, and entries 2 and 4 in Table 2,) appeared to form particles with hydrodynamic radii (R_h) centered at ~ 105, 160 and 210 nm. Interestingly, among the latter, those forming aggregates with the lowest radii give the lowest viscosity in PBS (see entry 2 in Table 3.1, entry 4 in Table 3.2 and **Figure 3.2**).

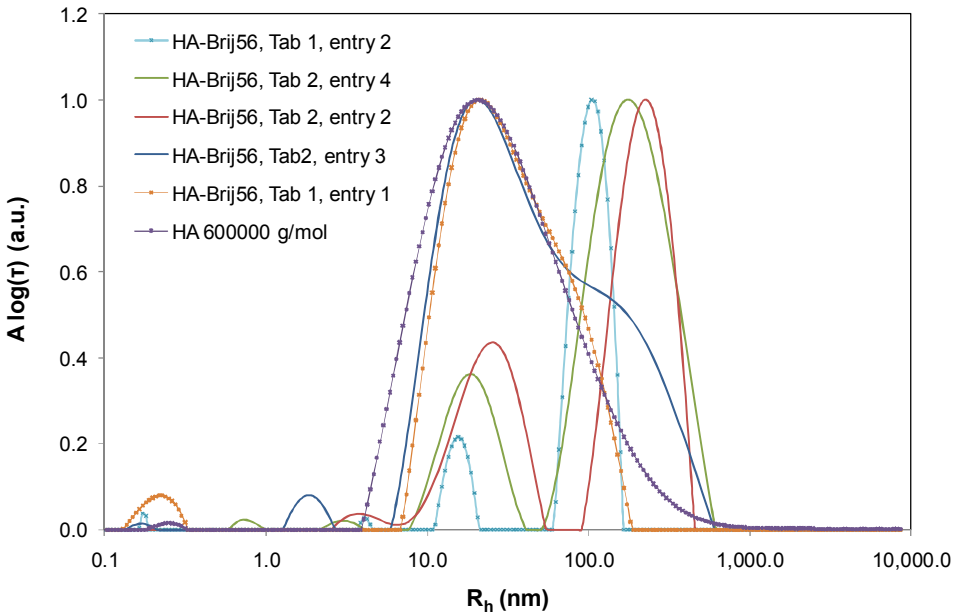


Figure 3.3: Analysis of HA-Brij56E derivatives in PBS (C_p = 2 g/L) by dynamic light scattering. Angle fixed at 90° and temperature fixed at 25 °C. (Tab 1 is Table 3.1, Tab 2 is Table 3.2,)

The self-assembly of HA-Brij56E derivatives in aqueous solution was also studied using hydrophobic dyes, Nile red (NR)[14-16], and pyrene (Py) [17, 18], which are poorly soluble and weakly fluorescent in water.

In contrast, their solubility and fluorescence dramatically increase in a hydrophobic medium. The fluorescence measurements in **Figure 3.4.A** showed that for lower concentrations (in the range 0.001-0.1 g/L) of HA-Brij56E (entry 2 in Table 3.1,), amphiphilic molecules exist in aqueous solutions as individual molecules (premicelle aqueous environment); the fluorescence intensity of NR remained constant, without any shift in the maximum emission wavelength (**Figure 3.4.B**). For higher concentrations (above the critical aggregation concentration (cac)), an increase in intensity associated with a strong blue shift was observed, which is attributed to NR being close to (or inside) HA-Brij56E hydrophobic domains.

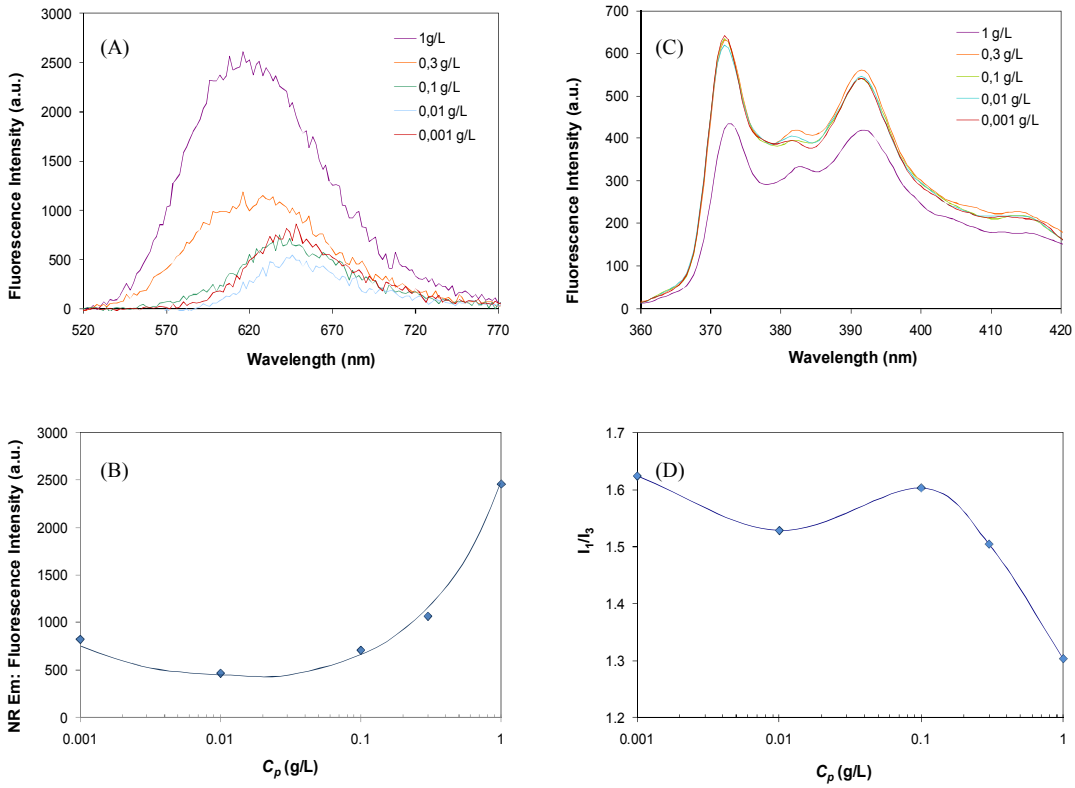


Figure 3.4 : Analysis of the self-assembly of HA-Brij56E by fluorescence spectroscopy: area-normalized fluorescence emission (λ_{ex}=505 nm) spectra of Nile red (1.5 × 10^{-6} M) in the HA-Brij56E /PBS system as a function of HA-Brij56E concentration obtained for reaction conditions of Table 3.1, entry 2, as an example (A,B); fluorescence emission (λ_{ex}=336 nm) spectra of pyrene (3 × 10^{-6} M) in the

HA-Brij56E/PBS system as a function of HA-Brij56E concentration obtained for reaction conditions of Table 3.1, entry 2, as an example (C,D).

In the case of Py fluorescence, the spectra obtained in this study were typical of Py photophysical behavior. However, as can be seen in **Figure 3.4.C**, contrary to our expectations, the intensity decreased for the higher concentration (1 g/L), which may be due to problem of solubility of this dye in aqueous solution. The I_1/I_3 intensity ratio of the first and third vibronic peaks in the fluorescence emission spectrum of Py, which is sensitive to the polarity of the microenvironment of the dye, was measured as a function of polymer concentration. As shown in **Figure 3.4.D**, the I_1/I_3 ratio oscillated with a linear trend below C_p = 0.1 g/L, but a I_1/I_3 decrease above this value was observed. This transition of intensity reflected the transference of Py to a less polar micellar domain, which was coincident with that observed with NR.

3.4 Conclusion

In conclusion, in this study, we investigated different conditions for the synthesis of new hydrophobically modified derivatives of HA from Brij56 epoxide. The grafting of Brij56 was performed in homogeneous conditions at basic pH. Under some conditions, it was possible to obtain HA-Brij56E derivatives giving rise to nanosized aggregates under aqueous conditions by the association of the hydrophobic C_{16} chains according to DLS and fluorescence spectroscopy. However, further work is required to clarify the characteristics of these “nanogels” by microscopy observations as well to optimize the synthesis of HA-Brij56E.

3.5 Materials and Methods

Materials. The sample of bacterial sodium hyaluronate with a molar mass of 6×10^5 g/mol (HA-600) was supplied by ARD (Pomacle, France). The molar mass distribution and the weight-average molar mass of initial HA and blanks were determined by size exclusion chromatography using a Waters GPCV Alliance 2000 chromatograph (USA) equipped with three on-line detectors: a differential refractometer, a viscometer and a light scattering detector (MALLS) from Wyatt (USA); the solutions were injected at a concentration of 5×10^{-4} g/mL in 0.1 M $NaNO_3$. The overlap concentration C^* of HA-600 in 0.01 M phosphate buffered saline (PBS, pH 7.4) solution containing 0.154 M NaCl at 25 °C is around 0.9 g/L. This value was derived from the intrinsic viscosity assuming that $C^*[\eta]$ is about unity [19]. Brij56 glycidyl ether was synthesized according to the procedure described in chapter 2. Pyrene (Py), and 9-(diethylamino)-5H-benzo[R]phenoxazin-5-one (Nile red, NR) and all other chemicals were purchased from Sigma-Aldrich-Fluka. The water used in all experiments was purified by a Millipore Milli-Q Plus purification system, with a resistivity of 18.2 MΩ cm.

NMR spectroscopy. ^{1}H NMR spectra of the HA-Brij56E derivatives dissolved in deuterium oxide (6 mg/mL) were performed at 80 °C using a Bruker DRX400 spectrometer operating at 400 MHz. Deuterium oxide was obtained from SDS (Vitry, France).

Rheological experiments. Flow experiments were carried out with the AR2000 rheometer. The HA-Brij56 derivatives were first dissolved in PBS (0.154 M NaCl, pH 7.4) at a concentration of 10 g/L. After stirring for 4-6 hours at room temperature, the samples were kept in the refrigerator for ~ 12 hours.

Light Scattering Measurements. Scattering measurements were performed using an ALV laser goniometer, which consists of a 22 mW HeNe linear polarized laser

operating at a wavelength of 632.8 nm, an ALV-5000/EPP multiple τ digital correlator with 125 ns initial sampling time, and a temperature controller. The accessible scattering angles range from 50° to 140°. The aqueous solutions of HA-Brij56E (2 g/L in PBS) were filtered directly into the glass cells through 0.45 μm MILLIPORE Millex LCR filter. Data were collected using digital ALV Correlator Control software and the counting time for measuring the elastic or the quasi-elastic scattering intensities varied for each sample from 180 to 300 sec. The relaxation time distributions, $A(t)$, were in the sequence obtained using CONTIN analysis of the autocorrelation function, $C(q,t)$. Diffusion coefficients D were calculated from following equation:

$$\frac{\Gamma}{q^2}\Big|_{q\to 0} = D \quad (1)$$

where Γ is relaxation frequency ($\Gamma = \tau^{-1}$), and q is the wave vector defined as following equation:

$$q = \frac{4\pi n}{\lambda}\sin\left(\frac{\theta}{2}\right) \qquad (2)$$

where λ is the wavelength of the incident laser beam (632.8 nm), θ is the scattering angle, and n is the refractive index of the media. Consequently, the hydrodynamic radius (R_h) was calculated from the Stokes-Einstein relation as follows:

$$R_h = \frac{k_B T}{6\pi\eta\Gamma}q^2 = \frac{k_B T}{6\pi\eta D} \quad (3)$$

where k_B is the Boltzmann constant, T is the temperature, and η is the viscosity of the medium.

Fluorescence Spectroscopy. The self-assembly of HA-Brij56E derivatives in aqueous solution was fluorometrically investigated using hydrophobic guest molecules such as NR and Py. The fluorescence intensity change of these guest molecules was monitored as a function of HA-Brij56E concentration. The HA-Brij56E was dispersed in PBS (1 g/L) with stirring for 12 h at 4 °C. Consecutive

dilutions of 1 mL of each sample were prepared in PBS where NR and Py were injected. A volume of 10 μL of a 1.5×10^{-4} M NR stock solution in ethanol was added, giving a constant concentration of 1.5×10^{-6} M in 1 % ethanol/PBS for all NR fluorescence measurements. A volume of 10 μL of a 3×10^{-4} M Py stock solution in ethanol was added, giving a constant concentration of 3×10^{-6} Min 1 % ethanol/PBS for all Py fluorescence measurements. Samples were stirred overnight before fluorescence measurements. Py emission spectra were measured on a Perkin Elmer luminescence LS 50B spectrometer between 360 and 420 nm. The slit width was set at 5 nm for excitation and 5 nm for emission. The NR emission spectra were measured on a fluorescence microplate reader (Infinite 1000, Tecan, Austria) with excitation and emission wavelengths set at 505 ± 5 nm and 650 ± 5 nm respectively. All spectra were corrected for the instrumental response of the system.

The self-aggregation of HA-Brij56E was analyzed from both the maximum mission intensity of NR (λ_{ex} = 505 nm) and the Py fluorescence intensity ratio of the third (384-385 nm) and first vibrational bands (372-374 nm) (I_3/I_1) of the emission spectra (λ_{ex}=336 nm) in the HA-Brij56E / PBS system as a function of HA-Brij56E concentration.

Synthesis.

The protocol described below has been applied for the synthesis of HA-Brij56E reported in Table 1, entry 2. Similar protocols have been applied for the synthesis of the other derivatives. Changes can occur at the beginning and/or during addition of Brij56E. Indeed, as discussed above, in some experiments, we dissolved HA in water alone and added a fixed volume of an aqueous solution of NaOH (2 M) to reach a pH value ~ 12.5-13. DMSO was then added followed by Brij56E. The latter was added either in only one time before heating the reaction medium or slowly during the reaction. The reaction medium was then heated to 50 °C and stirred for 2-3 h.

HA-Brij56E (Table 1, entry 2). 0.5 g of HA was dissolved in water (25 mL) and the resulting mixture was stirred overnight at room temperature. The pH of the resulting solution was then adjusted to ~ 13 by addition of 2 M NaOH (3 mL) and DMSO (15 mL) was slowly added. After being solubilized in DMSO (10 mL), Brij56E (0.278 g corresponding to [Brij56E]/[HA] = 0.3) was added to the HA solution. The reaction mixture was stirred at 50 °C for 2 h. The aqueous solution was then added with NaCl to have [NaCl] = 0.5 M and the modified polysaccharide was precipitated by addition of EtOH (to have 3/2 EtOH/water, v/v). The precipitate was then washed with EtOH, dissolved in water (100 mL) and purified by ultrafiltration through an ultramembrane Amicon YM 30. The ultrafiltration was stopped when the filtrate conductivity was lower than 10 µS, and the HA-Brij56E derivative was recovered by freeze-drying with a yield of 80 %. The apparent degree of substitution was determined by ^{1}H NMR in D_2O.

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General conclusion and perspectives (*En*)

Up to now, due to the potential thickening applications of natural polysaccharide, the requirement of their modification raised up. Recent patents demonstrated that hydrophobically modified polysaccharides (HM-P) showed excellent thickening effect and could be used in various fields. However, there are many challenges remaining for synthesis of HM-P, including the maintaining of the water solubility of HM-P by controlling the degree of substitution of hydrophobic groups and the increased viscosity of the aqueous solution of HM-P. This clearly motivated this study. In this work, we studied the synthesis of HM-P under different reaction conditions. We focused on the chemical modification of hydroxyethyl cellulose, guar, hydroxyethyl guar and hyaluronic acid in order to prepare amphiphilic polysaccharide derivatives exhibiting the thickening properties in aqueous solution.

To this end, the modification of HEC and guar by Brij56 epoxide (Brij56E) in IPA/water reaction system was investigated for the first time, and the viscosities of solutions of the modified samples clearly demonstrated a thickening effect. Grafting GHE on HEC or guar in IPA/water condition also gave derivatives of which aqueous solution with increased viscosity. We also found that though all the hydrophobic alkyl epoxides could be efficiently grafted on HEG and guar under DMSO (TBAOH) reaction system, the modified product were degraded. It can be concluded that IPA/water reaction system is successful to introduce the long alkyl chain along HEC and guar, while DMSO/water reaction system can be applied to the synthesis of hydrophobically modified HEG derivatives.

Finally, we investigated different conditions for the synthesis of new hydrophobically modified derivatives of HA from Brij56E. The grafting of Brij56 was performed in homogeneous conditions at basic pH. Under some conditions, it was possible to obtain HA-Brij56E derivatives giving rise to nanosized aggregates under aqueous

conditions by the association of the hydrophobic C_{16} chains according to DLS and fluorescence spectroscopy. However, further work is required to clarify the characteristics of these “nanogels” by microscopy observations as well to optimize the synthesis of HA-Brij56E.

Overall, this work showed strategies to prepare HM-P based on the grafting of hydrophobic alkyl groups on polysaccharides by the ring opening of epoxides but further improvements are required. The reaction conditions should be optimized in order to obtained water soluble products with more evidently thickening effect. Additionally, considering the huge applications of hydrophobically modified polysaccharides in industrial field, the reaction conditions should be facile to industrialization. However, the current systems could not satisfy these requirements, the balance between hydrophobic interactions favoring precipitation and water-solubility of modified polysaccharides is difficult to control under all the three reaction systems we used. Thus, one of the challenges in the future is to avoid precipitation and degradation, and at meanwhile to increase the viscosity of the aqueous solution of the modified polysaccharide.

Conclusion générale et perspectives (*Fr*)

La modification chimique de polysaccharides hydrosolubles visant à obtenir des agents épaississants est un domaine de recherche actuellement important compte tenu de leurs nombreuses applications potentielles comme décrit dans des brevets récents. Cependant, des verrous restent à lever en ce qui concerne la synthèse de polysaccharides hydrophobiquement modifiés (HM-P). Le maintien de la solubilité en milieu aqueux, le contrôle du degré de substitution et l'effet épaississant des dérivés sont en effet des paramètres délicats à contrôler. Ce travail de thèse s'inscrivait dans cette problématique. Il avait pour principal objectif d'étudier la synthèse de HM-P dans différentes conditions réactionnelles. Nous nous sommes intéressés à la modification chimique de l'hydroxyéthyl cellulose, du guar, de l'hydroxyéthyl guar et de l'acide hyaluronique afin d'obtenir de nouveaux polysaccharides amphiphiles présentant des propriétés épaississantes en milieu aqueux.

Dans cet objectif, le greffage de groupements Brij56E sur l'HEC et le guar en milieu hétérogène (IPA/eau) a été étudié pour la première fois. Les viscosités obtenues à partir de solutions de polysaccharides modifiés ont révélé un caractère épaississant. Des résultats similaires ont été observés suite au greffage de groupements GHE sur l'HEC et le guar dans les mêmes conditions. Les réactions effectuées en milieu homogène (DMSO, TBAOH) se sont avérées également efficaces mais une dégradation importante des polymères a été observée. Les réactions effectuées dans un milieu IPA/eau se sont ainsi avérées plus adaptées à la synthèse de polysaccharides en tant qu'agents épaississants.

Nous avons finalement étudié différentes conditions de synthèse de dérivés hydrophobiquement modifiés du HA par réaction avec le dérivé époxide Brij56 en milieu basique. Nous avons montré la possibilité d'obtenir dans certaines conditions réactionnelles des dérivés capables de s'autoassocier en nanoparticules du fait de la

formation de nanodomaines de chaînes hexadécyle (C16). Cette étude a reposé sur des expériences de diffusion dynamique de la lumière et de spectroscopie de fluorescence. Cependant, des expériences supplémentaires s'appuyant sur des observations microscopiques s'avèrent nécessaires pour déterminer les caractéristiques morphologiques de ces nanogels.

Ce travail a ainsi conduit à divers dérivés de polysaccharides neutres et chargés porteurs de chaînes hexadécyle/Brij56. Les conditions de réaction, reposant sur l'ouverture d'époxydes en milieu basique, doivent cependant être encore améliorées. L'un des défis dans le futur sera d'optimiser ces conditions afin notamment d'éviter la précipitation des polymères modifiés et leur dégradation.

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Abbreviations

ADH	adipic acid dihydrazide
ALS	ammonium lauryl sulfate
Brij®56	polyethylene glycol hexadecyl ether
Brij56E	polyethylene glycol hexadecyl ether epoxide
cac	critical aggregation concentration
CDI	*N,N'*-carbonyldiimidazole
CMG	carboxymethyl guar
DBTL	dibutyltin dilaurate
DCC	*N,N'*-dicyclohexylcarbodiimide
DMAc	*N,N*-dimethylacetamide
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DS	degree of substitution
EDC	1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide
EHEC	ethyl(hydroxyethyl) cellulose
EtOCOCl	isobutylchloroformate
GHE	glycidyl hexadecyl ether
HA	hyaluronic acid
HEC	hydroxyethyl cellulose
HEG	hydroxyethyl guar
HM-HEC	hydrophobically modified hydroxyethyl cellulose
HM-HEG	hydrophobically modified hydroxyethyl guar
HM-G	hydrophobically modified guar
HM-P	hydrophobically modified polysaccharides
HMHEC	hydrophobically modified HEC
HMHPG	hydrophobically modified HPG
HPG	hydroxypropyl guar

MCMG	methyl carboxymethyl guar
NaCMG	sodium carboxymethyl guar
NMM	*N*-methylmorpholine
NMP	*N*-Methy1-2-pyrrolidone
NR	nile red
OP-10	polyoxyethylene octylphenol ether
OP10E	polyoxyethylene octylphenol ether epoxide
PBS	phosphate buffer saline
PEG	poly(ethylene glycol)
PGE	phenyl glycidyl ether
PLGA	poly(lactic-co-(glycolic acid)
PPG	poly(propylene glycol)
PPGE	poly(propylene glycol) epoxide
PTSA	p-toluenesulfonic acid
Py	pyridine
SEC	size exclusion chromatography
TBA	tetrabutylammonium
TBAOH	tetrabutylammonium hydroxide
TEA	triethylamine
TMAC	phase transfer catalysts
TsCl	4-toluenesulfonyl chloride