Thermoresponsive Starch Nanoparticles

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Abstract

Thermosensitive or thermoresponsive polymers (TRPs) that undergo an abrupt change in aqueous solubility with temperature have numerous potential commercial applications. The vast majority of TRPs reported to date are synthetic, petroleum-based polymers. TRPs based on benign natural polymers such as cellulose and starch have garnered some attention recently due to their biodegradability, biocompatibility, low toxicity and low production costs. Hydroxyalkylated starch nanoparticles were synthesized and their thermosensitive behaviour was examined. Hydroxypropylation of EcoSphere® starch nanoparticles (ENPs) with propylene oxide did not yield thermoresponsive particles even at high molar substitution (MS). Hydroxybutylation of ENPs with butene oxide did yield thermoresponsive ENPs (TRENPs). The MS of the hydroxybutylated ENPs (HBENPs) had to be greater than 1 in order for them to exhibit thermosensitivity in pure water. The lower critical solution temperatures (LCSTs) of the HBENPs decreased with increasing MS with LCSTs ranging from 49.3-37.8 ^{o}C for MSs ranging from 1.16-1.38. The LCSTs of the HBENPs decreased with increasing NaCl concentration and with increasing propanol and butanol concentration. Low concentrations of methanol and ethanol decreased the LCST. Higher concentrations of these alcohols broadened the thermal transitions and increased the LCST. The thermal transitions disappeared at 35% ethanol and 50% methanol. TRENPs could also be obtained by hydroxypentylation of ENPs with pentene oxide. The MS of

the hydroxypentylated ENPs did not have to be as high as the MS of the HBENPs in order to exhibit a thermoresponsive behaviour.

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Chapter 1: Thermoresponsive Polymer Systems

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List of Abbreviations

Abbreviation	Full Name
1-BuOH	*n*-Butanol
EtOH	Ethanol
HBENP	Hydroxybutylated EcoSphere™ Starch Nanoparticles
HPENP	Hydroxypropyl EcoSphere™ Starch Nanoparticles
MeOH	Methanol
AGU	Anhydroglucose Units
BE_0	*n*-Butyl Glycidyl Ether
BE_1	3-(2-*n*-Butoxyethyl) Glycidyl Ether
BE_2	3-[2-(2-*n*-Butoxyethoxy)Ethyl] Glycidyl Ether
BE_nS	3-[2-Butoxy(Ethoxy)$_n$]-2-Hydroxypropyl Starch
BO	1-Butene Oxide
DLS	Dynamic Light Scattering
DMSO	Dimethyl Sulfoxide
DS	Degree of Substitution
EHEC	Ethyl Hydroxyethyl Cellulose
ENP	EcoSphere™ Starch Nanoparticle

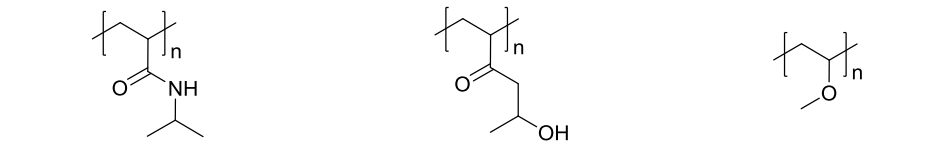
HBENP	Hydroxybutylated EcoSphere™ Starch Nanoparticle
HBPS	2-Hydro-3-Butoxypropoxypropylstarch
HIPS	2-Hydroxy-3-Isopropylpropoxpropyl Starch
HPC	Hydroxypropylcellulose
HPMC	Hydroxypropylmethylcellulose
HPS	Hydroxypropyl Starch
***i*PrOH**	Isopropanol
LCST	Lower Critical Solution Temperature
MC	Methylcellulose
MS	Molar Substitution
pNIPAM	poly(*N*-isopropylacrylamide)
PO	Propylene Oxide
SB	Styrene-Butadiene
S_N2	Bimolecular nucleophilic substitution
TRENP	Thermoresponsive EcoSphere™ Starch Nanoparticle
TRP	Thermoresponsive Polymer
UCST	Upper Critical Solution Temperature

Chapter 1

Thermoresponsive Polymer Systems

1.1 Introduction

Thermosensitive or thermoresponsive polymers (TRPs) are polymers that exhibit an abrupt change of their physical properties with temperature.[1] The vast majority of TRPs developed to date undergo an abrupt change in aqueous solubility with temperature. Hence, for the purposes of this thesis, when we refer to a thermosensitive or thermoresponsive polymer we are referring to a polymer that undergoes an abrupt change in aqueous solubility with temperature.[1] Since the first report of a TRP (poly(*N*-isopropylacrylamide) (pNIPAM)) in 1967 by Scarpa et al.[2] a large number of TRPs have been developed.[1] The vast majority of TRPs reported to date are synthetic, petroleum-based polymers such as pNIPAM, poly(2-hydroxypropylacrylate), and poly(vinyl methyl ether) (Figure 1.1).[1]



poly(N-isopropylacrylamide) poly(2-hydroxypropylacrylate) poly(methyl vinyl ether)

Figure 1.1 Examples of thermoresponsive polymers

Although TRPs have promising applications in such fields as drug delivery, tissue engineering, and separations technology,[1] commercial applications of TRP's have been limited to only a few low-volume applications such as in cell-culture plate coatings.[3] Some of the reasons for this are the cost of preparing TRPs, and environmental and health concerns such as biodegradability and toxicity. Thus, TRPs based on benign carbohydrate polymers such as

cellulose and starch have been of interest due to their biodegradability, biocompatibility, low toxicity and low production costs.[4]

1.2 Thermal Transitions

1.2.1 Introduction to Theory

TRPs undergo a transition from miscible to immiscible upon reaching a critical temperature. There are two types of thermoresponsive behaviors. Systems that exhibit a lower critical solution temperature (LCST) are miscible below their critical temperature and are immiscible above it. Conversely, polymers that exhibit an upper critical solution temperature (UCST) are miscible above their critical temperature and immiscible below it.[5] In this thesis, we are specifically interested in polymers exhibiting LCSTs in aqueous solution.

For polymers exhibiting LCSTs when dispersed in water (pNIPAM is an example of such a polymer), the formation of hydrogen bonds between water and the polymer allows the polymer to be solvated below the LCST.[6] This results in a large negative enthalpy of mixing and is the dominant contributing factor to the favorable free energy of mixing below the LCST. At the same time, a hydration shell forms around the hydrophobic groups of the polymer (for example, the isopropyl groups of pNIPAM). The ordered structure adopted by water molecules reduces the entropy of the system. The loss of entropy is minimised through partial association of hydrophobic groups. As long as the negative enthalpy of mixing is large enough to compensate for the loss of entropy, the polymer remains solvated. As the temperature increases, the H-bonding between the polymer and water is disrupted and intra- and intermolecular H-bonding,

as well as hydrophobic interactions dominate. Increasing the temperature causes the strength of the polymer-water hydrogen bonds to decrease, resulting in a smaller negative enthalpy of mixing. At the LCST, the negative entropic contribution overwhelms the negative enthalpic contribution, resulting in phase separation.[5,6]

1.2.2 Effect of Salt on Thermal Transitions

Salts are classified into two categories based on their effects on thermoresponsive polymers: cosmotropic and chaotropic.[7-10] Cosmotropic salts have small or polyvalent anions (i.e. SO_4^{2-},$H_2PO_4^-$, F^-, Cl^-, Br^-). The anions of cosmotropic salts are strongly hydrated (interact strongly with water) in aqueous solution. This interaction is stronger than the interaction of polymers with water molecules. Thus, when cosmotropic salts are added to a TRP in aq. solution, some of the hydrogen bonds between the polymer and water are destroyed by the salt. This results in a decrease in solubility of the polymer and a decrease in the LCST. The hydration strength of cosmotropic anions decreases in the order $SO_4^{2-} > H_2PO_4^- > F^- > Cl^- > Br^-$. Therefore, the ability of cosmotropic anions to reduce LCSTs decreases from left to right in this series.

Chaotropic salts have anions that are typically large or monovalent (i.e. SCN^-, I^-, NO_3^-). Their effect on the LCSTs of TSPs depends upon salt concentration. At low concentrations, chaotropic salts interact directly with the polymer chains. This results in an increase in polymer solubility and an increase in LCST. At high concentrations, chaotropic salts decrease LCSTs by increasing the surface tension at the polymer/water interface, which promotes the formation of aggregates.[7]

1.2.3 Effect of Alcohols on Thermal Transitions

The effect of alcohol-water mixtures on pNIPAM has been researched extensively.[11-18] pNIPAM is known to be soluble in organic solvents that are capable of hydrogen bonding, including acetone, tetrahydrofuran, dimethylformamaide, methanol, ethanol, and propanol. pNIPAM does not display a miscibility gap if it is only dispersed in any one of these solvents. Based on this observation, it would be reasonable to expect that pNIPAM might be miscible in aqueous dispersions containing any of these solvents, and that an increasing amount of these solvents would tend to raise the LCST. Surprisingly, the opposite was true for small amounts of these solvents. Below a certain threshold percentage of co-solvent, the solvent decreased the critical temperature. This phenomenon is called co-nonsolvency, or antagonistic solvency.[11-18] Co-nonsolvency can be explained in terms of the effect of the organic solvent on the hydrogen bonding network in an aqueous solution. In their discussion of the co-nonsolvency behaviour of pNIPAM in water-alcohol mixtures, Chen et al.[14] suggest that this behaviour is a result of the formation of complexes between water and the organic solvent. The water/alcohol complexes have fewer hydrogen bonding sites. This results in the reduction of the number or the strength of polymer−water interactions. The hydrogen bonding between the pNIPAM and water or solvent-water complexes is overwhelmed by the hydrophobic interactions of the pNIPAM backbone. This results in the desolvation of PNIPAM and a decrease in LCST.

If the polymer is not soluble in the co-solvent, the critical temperature will continue to decrease until the polymer is no longer soluble at any temperature. If the co-solvent can solvate

the polymer, the behaviour of the polymer will respond to an increase in temperature when the fraction of co-solvent exceeds a certain threshold. At some point, enough solvent is added such that not all of it is trapped in complexes with water and there are free solvent molecules available to solvate the polymer. This increases the critical temperature. As more solvent is added, the critical temperature increases further until the polymer no longer displays a miscibility gap.[16,18]

1.3 Applications of TRPs

TRPs have been studied for a variety of applications. The majority of these applications have been in biomedical fields.[19] The TRPs used in biomedical applications are liquid at room temperature, with thermal transitions at or just below human body temperature (37 ^{o}C), like pNIPAM, which has a thermal transition at 32^{o}C.[1] A commonly proposed use for TRPs with LCSTs close to 37^{o}C is as part of a sustained drug release system. For this type of application, a drug-loaded TRP is administered intramuscularly, intraperitoneally, subcutaneously, orally, etc. below its LCST as a liquid.[19] The TRP gels upon administration and slowly releases a therapeutic compound. TRPs have been used as coatings for cell culture plates.[20,21] This represents one of the few commercial applications of TRPs.[3] Cell cultures plates are coated with a TRP like pNIPAM or methylcellulose.[9] Cell cultures are grown above the LCST, where the polymers are more hydrophobic and can adhere to the cells through van der Waals interactions. Upon cooling below the LCST, the polymers become hydrophilic and no longer adhere to the cells. The cultures can be easily recovered without damage to intercellular adhesion structures.[3,20,21]

Non-biomedical applications include column chromatography, where the hydrophobicity of a chromatographic material based on a TRP can be altered by changing the temperature.[22] Sensor technology that employs thermosensitive polymers is also being developed.[23,24]

TRPs have been considered as part of a protocol for oil extraction.[25,26] Above the LCST, the TRP would be more hydrophobic and able to associate with oil. Below the LCST, the oil would be released and separated from the TRP and, the oil could be easily recovered. The TRP, which would remain in the aqueous phase, could also be recovered and reused. This would be particularly useful in the oil sands, where the current extraction protocol involves the use of strong caustic compounds and large amounts of hot water to release bitumen from clay and sand.[27-29] The use of a TRP would reduce the environmental impact of bitumen extraction by reducing the amount of energy required, eliminating the need for strong caustic, and improving the efficiency of the extraction to make the tailings ponds less toxic.

1.4 Thermoresponsive Carbohydrates

As mentioned in Section 1.1, TRPs based on natural polymers, such as polysaccharides, have garnered some attention due to their biodegradability, biocompatibility, low toxicity and low production costs.

1.4.1 Thermoresponsive Cellulose

The first thermosensitive polysaccharides reported were cellulose derivatives. Cellulose is a glucose polymer where glycosyl units are joined by β-1,4-glycosidic bonds (Figure 1.2). It is used by plants as the primary structural component in cell walls.[30]

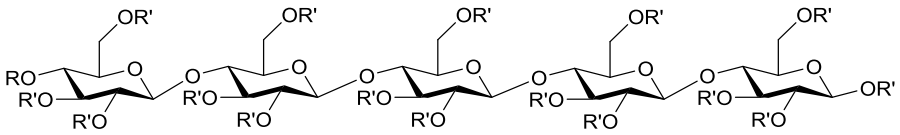


Figure 1.2 Structure of cellulose (R' = H) and derivatives (R' = H or entry in Table 1.1)

Table 1.1 Some examples of thermosensitive cellulose

Cellulose Ethers	R'
Methylcellulose (MC)	$-CH_3$
Hydroxypropyl cellulose (HPC)	$-CH_2CH(OH)CH_3$
Ethyl hydroxyethyl cellulose (EHEC)	$-OCH_3$ or $--CH_2CH_2OH$
Hydroxypropyl methyl cellulose (HPMC)	$-CH_3$ or $CH_2CH(OH)CH_3$

TRPs based on cellulose modified with hydrophobic ether groups have been extensively studied.[30] Some of these cellulose-based TRPs are shown in Table 1.1. Among other factors, the LCST of these derivatives depends upon the molar substitution (MS, the average number of substituent groups per polymer residue) or degree of substitution (DS, average number of positions on a polymer residue that have been modified). The higher the DS or MS, the lower the LCST. For example, methylcellulose (MC) with a DS of 1.21 has an LCST of 63 ^oC[31] while MC with a DS of 1.8 has an LCST of 57 ^oC.[32] Ibbet et al. reported that the distribution of the methyl groups had to be heterogeneous.[33] Derivatives with the same average DS where the methyl groups were homogeneously distributed were not thermosensitive.[33]

Various hydroxypropyl methyl cellulose (HPMC) derivatives have been prepared commercially under the name Metolose®, produced by Shin Etsu Chemical Co.[34] Metolose 60SH has been investigated for biomedical applications, including mucoadhesive eyedrops and as an esophageal drug delivery system.[35,36] In both of these applications, the addition of salts was used to lower the critical temperature of Metolose 60SH from 66°C to 37 °C.[34,35] Hydroxypropyl cellulose (HPC) on its own also displays thermosensitive behaviour: HPC with an MS of 3.9 has an LCST of 43 °C.[36,37]

Ethyl hydroxyethylcellulose (EHEC) is used commercially as a paint thickener. It exhibits an LCST of 30 °C when the DS of the ethoxy group is 1.5 and the MS of the hydroxyethyl group is 0.7. When the DS of the ethoxy group decreases to 0.9 and the MS of the hydroxyethyl group increases to 2.1, the LCST increases to 70 °C.[38] The MS of the hydroxyethyl group of commercially available EHEC is between 0.8 and 2.1 and the DS of the ethyl group is 0.8.[39]

Chitosan (Figure 1.3) is a carbohydrate polymer produced by the partial deacetylation of chitin, a structural component of arthropod exoskeletons.[40] Chitosan exhibits thermosensitive behaviour when it has been neutralized by a buffer such as β-glycerophosphate as shown in Figure 1.3.[41] BioSyntech Inc. developed BST-Gel®, a thermosensitive chitosan-buffer system that gels at physiological temperature. BST-Gel has been investigated for drug delivery. Piramal Healthcare Ltd., which acquired Biosyntech in 2010, further developed this product into BST-Cargel®, which is being investigated in clinical trials for cartilage regeneration.[42]

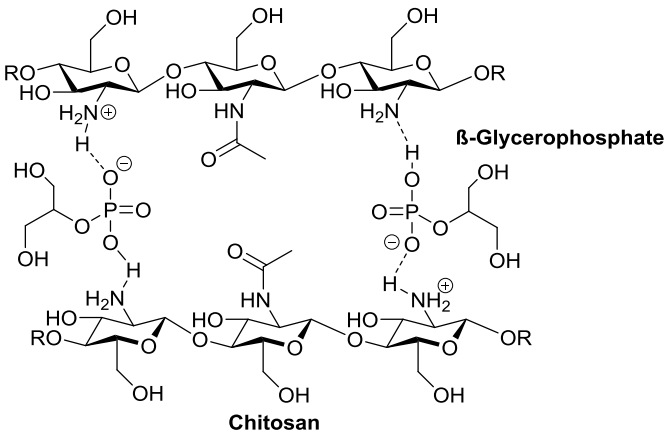


Figure 1.3 Chitosan neutralised with β-glycerophosphate**.**

1.4.2 Thermoresponsive Starch

Starch consists of two naturally occurring glucose polymers, amylose and amylopectin, which are produced and used by plants for energy storage. Starch is stored in plants in a granular structure. The morphology, composition, and size of starch granules vary with the species of the plant and its growing conditions.[43]

The basic structure common to all starch granules is a structure of concentric, alternating amorphous and crystalline layers (Figure 1.4).[43]

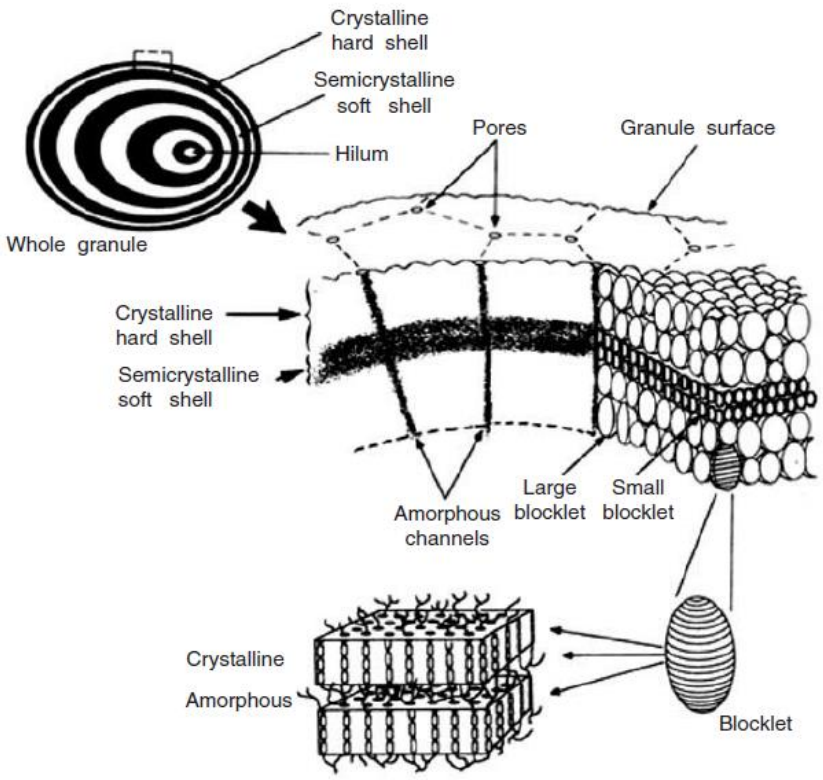


Figure 1.4 Starch granule organisation (Used with permission from Starch Chemistry and Technology, 3rd Ed, p. 183) .[43]

Within the crystalline regions, starch is organized into ellipsoidal blocklets consisting of stacked lamellae which also alternate between crystalline and amorphous regions. The crystalline regions consist primarily of amylopectin, which is arranged into helices that are stabilized by hydrogen bonding. These helices are oriented radially within the starch granule, with the reducing ends oriented towards the centre of the granule. The amorphous regions are primarily amylose, and can contain small amounts of non-starch components such as lipids and proteins.[43]

Glucose monomers in amylose and amylopectin are joined by α-1,4-glycosidic bonds. The branched structure of amylopectin is due to the presence of α-1,6-glycosidic bonds (Figure 1.5).

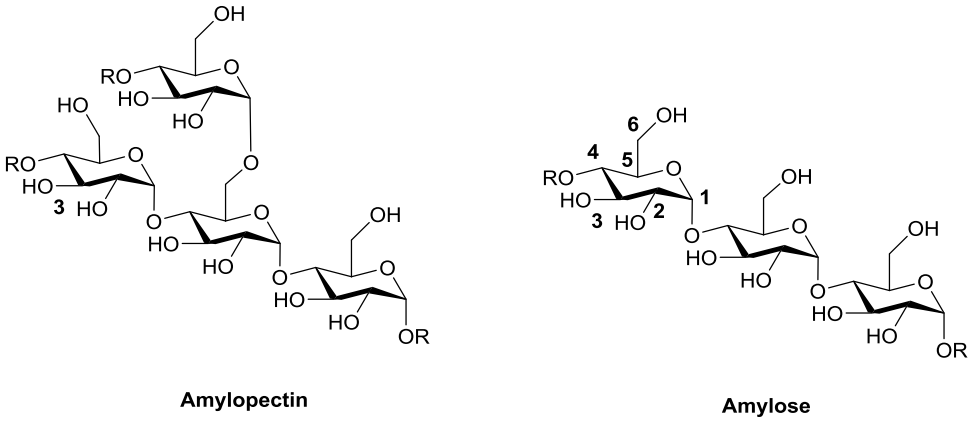


Figure 1.5 Structures of amylose and amylopectin

The highly organized and tightly packed structure of the amylopectin-containing portions of starch granules in their native (unmodified) form helps starch resist degradation when exposed to high temperature, and high or low pH.[43]

Very recently, several reports have appeared describing thermosensitive starch. Ju et al. partially degraded starch in aq. hydrochloric acid at 45 ^{o}C for 5 h and then modified it with butyl glycidyl ether in 1.25 M aq. NaOH at 75 ^{o}C for 5 h. This process gave 2-hydro-3-butoxypropoxypropylstarch (HBPS, Figure 1.6) which exhibited thermoresponsive behaviour.[44] The LCST of HBPS decreased with the MS (moles of butylglycidyl ether substituent to anhydroglucose unit) of the hydrophobic substituent. HBPS with MSs ranging from 0.32 to 0.63 exhibited LCSTs of 32.5-4.5 ^{o}C.[44]

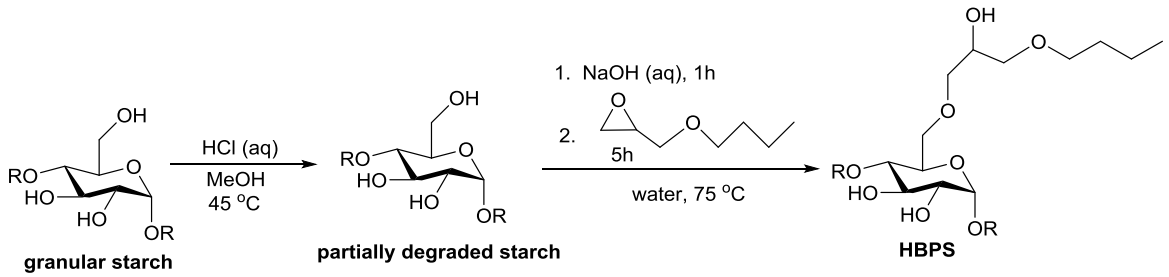


Figure 1.6 Preparation of 2-hydro-3-butoxypropoxypropylstarch (HBPS). Substitution is shown at O-6 though modification may also have occurred at O-2 or O-3.

Shortly thereafter, Ju et al. reported the preparation of thermoresponsive starch derivatives with widely tunable LCSTs by introducing short oligo(ethylene glycol) spacers (Figure 1.7).[45]

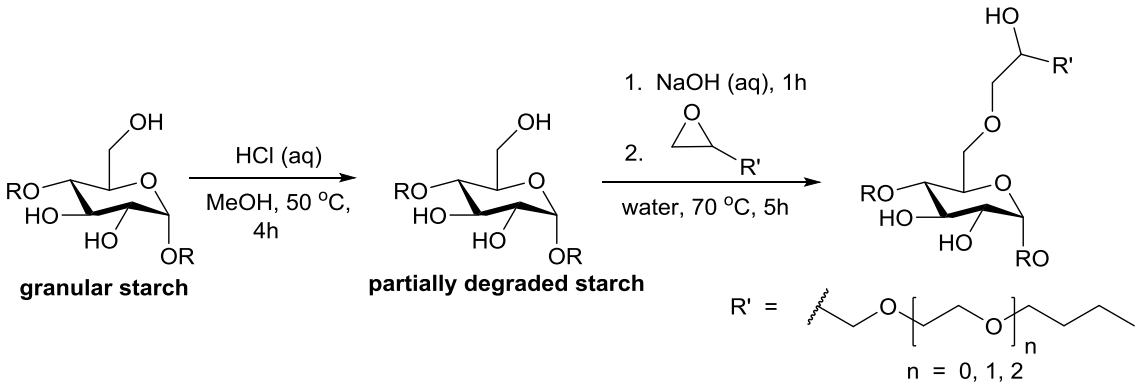


Figure 1.7 Preparation of 3-[2-butoxy(ethoxy)$_n$]-2-hydroxypropyl starch ethers (BE$_n$S) (n = 0, 1, or 2). Substitution is shown at O-6 though modification may also have occurred at O-2 or O-3.

Thermoresponsive 3-[2-butoxy(ethoxy)$_n$]-2-hydroxypropyl starch ethers (BE$_n$S) (n = 0, 1, or 2) were prepared by reacting partially degraded starch with *n*-butyl glycidyl ether (BE$_0$), 3-(2-*n*-butoxyethyl) glycidyl ether (BE$_1$), or 3-[2-(2-*n*-butoxyethoxy)ethyl] glycidyl ether (BE$_2$) in 1.35 M aq. NaOH at 70 °C for 5 h. The LCSTs of the BE$_n$S ranged from 17.5-55.0 ◦C. The LCSTs were tuned by changing the side chain lengths of oligo(ethylene glycol) groups and the MS. The LCSTs increased with increasing side chain length of the oligo(ethylene glycol) groups when BE$_0$S, BE$_1$S,

and BE_2S had similar MS values. An increase in BE_nS concentration and addition of NaCl to the BE_nS solutions led to a decrease in the LCSTs. The effects of NaCl and BE_nS concentration on the LCSTs was weaker when the side chain length of oligo(ethyleneglycol) groups increased.[45]

This same group also prepared thermosensitive starch by reacting granular starch with isopropyl glycidyl ether in aq. NaOH (concentration not specified) at 60 °C for 5 h to give 2-hydroxy-3-isopropylpropoxpropyl starch (HIPS) (Figure 1.8).[46]

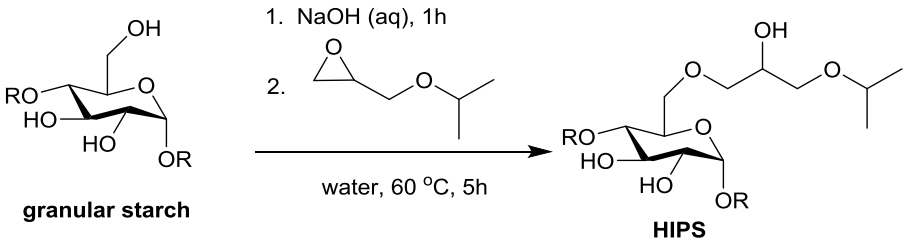


Figure 1.8 Preparation of 2-hydroxy-3-isopropylpropoxpropyl starch (HIPS). Substitution is shown at O-6 though modification may also have occurred at O-2 or O-3.

MS values required for thermosensitivity were quite high; MS values between 0.86-2.77 gave LCST values of 69-28°C. Cosmotropic salts decreased the LCST. The effect of organic solvents (methanol (MeOH), ethanol (EtOH), isopropanol (*i*PrOH), *n*-butanol (1-BuOH), acetone) on the LCST of the HIPS was also examined. In general, at the lower organic solvent concentrations, all organic solvents lowered the LCST. This was attributed to the polymer dehydration caused by the formation of water-solvent complexes as discussed in Section 1.2.3.

The ability of the solvents to lower the LCST of HIPS decreased in the following order: 1-butanol > 2-propanol > ethanol > methanol > acetone. The LCST decreased with increasing hydrophobicity of the alcohol. This was explained in the following manner. The hydroxyl groups

in the alcohols are involved in H-bonding with water. The hydrophobic hydrocarbon groups in the alcohols are also hydrated by water molecules. This results in the formation of a hydration shell with a clathrate-like structure around the nonpolar groups. The amount of water molecules required for the hydrophobic hydration of alcohols increases as the hydrophobicity of the alcohols increases (i.e. MeOH < EtOH < PrOH).[47] If one type of alcohol needs a larger number of water molecules to form a solvent complex than another alcohol, then the addition of the alcohol that requires a larger amount of water molecules to form a solvent complex to an aq. solution of a TRP would produce a more significant disturbance to the hydration of the polymer and have a greater effect on lowering its LCST.[46]

The addition of 1-butanol caused a decrease in LCST at all concentrations studied (up to 5 % v/v for 1-butanol). For the more hydrophilic solvents, acetone and methanol, the results were different. When the water-solvent mixture contained up to 40 % v/v MeOH or acetone, the effect of solvent concentration on the LCST was similar to that of the more hydrophobic solvents. However, above 40% v/v solvent, the LCST began to increase. When the concentration of methanol exceeded 55 % v/v, and the concentration of acetone exceeded 60 % v/v, the HIPS no longer exhibited an LCST. This was explained by proposing that at high MeOH or acetone concentrations, there was sufficient solvent to form solvent/water complexes with all of the water molecules present; no “free” water was available to interact with the HIPS. However, some solvent remained free in solution. Since the HIPS used in this study was very soluble in MeOH and acetone, the MeOH or acetone that remained “free” was able to solubilize the polymer at any temperature at solvent concentrations greater than 55 % v/v for methanol and 60 % v/v for acetone. Isopropanol and ethanol were also good solvents for the HIPS used in this study.

Consequently, the HIPS was soluble at any temperature when the concentration of these solvents was > 45 % v/v.[46]

1.5 Starch Nanoparticles

Nanoparticles are defined as particles with sizes ranging from 10-1000 nm. Starch nanoparticles can be produced by gelatinization and precipitation, treatment with an aqueous solution of sodium hydroxide and urea, or with oil-in-water microemulsions.[47-51]

1.5.1 EcoSphere™ Starch Nanoparticles

EcoSynthetix™, a company based in Burlington, Ontario, has developed a proprietary process for preparing starch nanoparticles on an industrial scale by reactive extrusion.[52, 53] Starch granules are fed continuously into a twin-screw extruder along with various additives at high temperature and shear rates. The addition of glycerol or water disrupts hydrogen bonding in the starch granule, lowering the glass transition temperature and turning the starch into a thermoplastic melt. High shear in the extruder mechanically breaks down the crystalline structures in the granule and depolymerizes the starch. Glyoxal, a dialdehyde, is fed into the extruder which cross-links the starch fragments through the formation of acetal or hemiacetal bonds with starch hydroxyls. The cross-linked ENPs leave the extruder as a spray that settles into 300 μm agglomerates of dry nanoparticles.[46,47] These nanoparticles have the trade name EcoSphere.™ Throughout this thesis, when we refer to EcoSphere starch nanoparticles (ENPs), we are referring to the starch nanoparticles produced by Ecosynthetix.

Aqueous dispersions of ENPs, called Biolatex,™ are used as a wet-end additive in the paper-making industry. The Biolatex™ functions as a rheological modifier in surface sizing and as a binder for ink. Papermaking occurs under conditions of high shear. The standard wet-end additive, styrene-butadiene (SB) latex, is shear-thickening, meaning that its viscosity increases with increasing shear. The viscosity of the Biolatex™ decreases with increasing shear, a behavior termed shear-thinning, improving its performance in papermaking. This feature, as well as its low cost compared to SB latex, has made Biolatex™ an attractive alternative to SB latex as a wet-end additive in the paper-making industry.[52, 53]

1.5.2 Characterization of Ecosphere™ Starch Nanoparticles

ENPs have been characterized, in terms of their size and molecular weight, by static and dynamic light scattering.[54] In water, two dominant particle size distributions at around 50 nm and 300 nm were observed. These were thought to correspond to individual ENPs and aggregates of ENPs. The molecular weight of the ENPs ranged from 2.2-2.6 x 10^6 g/mol. As the temperature increased from 25-65 ^oC, the diameter of the individual particles and the aggregates in water changed negligibly, suggesting that the unmodified particles are not temperature sensitive, and that the aggregation equilibrium is not temperature-dependent.[54]

1.6 Objectives

The objective of this work was to produce thermosensitive starch nanoparticles. This objective was pursued by modifying ENPs with hydroxyalkyl groups.

Thermoresponsive Starch Nanoparticles

2.1 Hydroxyalkylation of starch

It was our desire to prepare thermoresponsive ENPs (TRENPs) using the most economical reagents possible and under the mildest possible conditions. Given the fact that ENPs are only readily dispersible in dimethyl sulfoxide (DMSO) and water, and that DMSO is expensive compared to water, the thermoresponsive TRENPs were prepared in aqueous solution. Moreover, since many of the applications for TRENPs would require them to be dispersed in an aqueous solution for an extended period of time, the bonds between the modifying groups and the starch had to be stable (not readily reversible) in water.

As mentioned in Chapter 1, thermoresponsive cellulose has been prepared earlier by hydroxypropylation of cellulose using propylene oxide (PO).[36,37] Consequently, we anticipated that thermosensitive hydroxyalkylated ENPs could be prepared by reacting ENPs with simple alkene oxides such as PO or 1-butene oxide (BO). These reactions can be carried out in aqueous solution (*vide infra*), and the resulting hydroxypropyl or hydroxybutyl groups would be attached to the starch via ether linkages which are stable in aqueous solution. PO and BO are less expensive than the alkyl glycidyl ethers employed by Ju et al. to prepare thermoresponsive starch as discussed in Chapter 1 (i.e. 1 L of BO from Aldrich is $36.10, while 1 L of butylglycidyl ether is $224.40). Surprisingly, Ju et al. never examined simple alkene oxides as reagents for preparing thermosensitive starch.

Hydroxyalkylation involves the reaction of a hydroxyl group with an epoxide (Figure 2.1). The reaction is typically done under alkaline conditions (pH > 12). It proceeds via a bimolecular

nucleophilic (S_N2) mechanism. Starch hydroxyls attack at the least sterically hindered site on the epoxide. Hydroxyalkylation of starch with ethylene oxide and PO has been extensively studied and is used to produce a large quantity of commercially available etherified starches.[55]

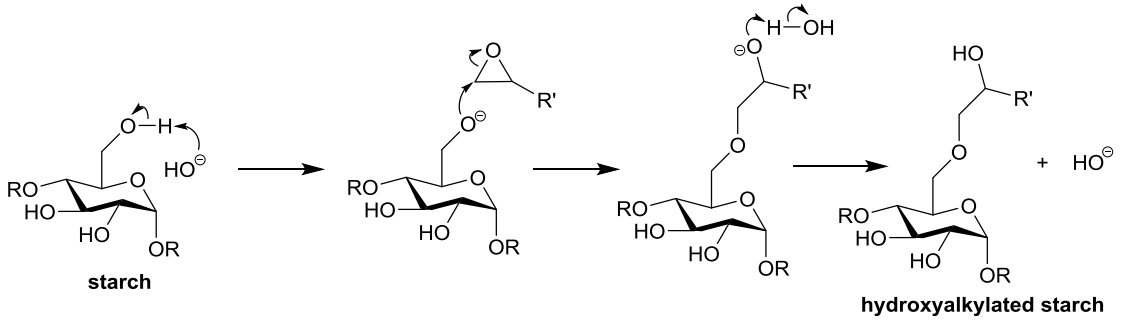


Figure 2.1 Hydroxyalkylation of starch. Substitution is shown at O-6 though modification may also occurr at O-2 or O-3.

Hydroxypropyl starch (HPS) is typically prepared in a sealed reaction vessel and, as the boiling point of PO is 34 ^oC, the reactions are usually carried out at temperatures approaching or above 34 ^oC. The method of preparation of HPS differs depending on whether the target DS is above or below 0.25.[55] Low DS HPS is prepared in an aqueous slurry between 45 and 50 ^oC for 24 h. Higher DS starch is usually prepared in a semi-dry state due to increased swelling of the starch granules in the presence of large amounts of PO. Dry starch granules are sprayed with aqueous sodium hydroxide until the moisture content reaches 20%. This semi-dry mixture is mixed with PO for 24-36 h. Sodium phosphate, sodium sulfate, or sodium chloride may be added to prevent gelatinization of the starch granules and loss of the granular structure. There have been reports of hydroxypropylation of starch via reactive extrusion.[56]

Two major side reactions occur during hydroxyalkylation reactions in basic aq. solution. One is the reaction of water or hydroxide with the alkene oxide to produce the ring-opened

glycol. The second is the polymerization of the glycol into polyglycols. Reaction conditions that minimize these side products include reducing the water content, keeping the temperature below 50°C, and maintaining the alkali concentration below 50%.[55]

2.2 The Stability of ENPs to Alkaline Degradation

Before attempting to modify the ENPs, the ENPs were subjected to dialysis to remove glycerol and glyoxal since these compounds could potentially interfere with the hydroxyalkylation reactions. All hydroxyalkylation reactions were performed on these dialyzed or purified ENPs.

Our initial attempt to modify the ENPs with alkene oxides using the procedure employed by Ju et al. to prepare thermosensitive starch using butyl glycidyl ether[45] demonstrates the problem with using literature procedures to modify ENPs. In Ju et al.'s procedure, partially degraded starch was heated at 75 °C in 1.25 M NaOH for 1 h. Butyl glycidyl ether (BGE) was added and the mixture stirred at 75 °C for 5 h followed by neutralization with aq. HCl and lyophilisation. We tried to modify ENPs with BO using this procedure; however, after 1 h the reaction mixture had turned black, indicating extensive degradation.

The hydroxyalkylation of starch using alkene oxides longer than three carbons requires elevated temperatures and pH's to achieve a high DS.[58,59] These procedures were performed on starch granules, where the crystallinity of the granule confers some protection against these harsh conditions. The hydroxypropylation of starch granules has been found to only take place in the amorphous regions of starch.[43] The ENPs appear to be more susceptible to degradation in

an aqueous alkaline solution than granular starch. Therefore, before any further modifications were attempted, the sensitivity of the ENPs to alkaline degradation was studied. Common degradation reactions that starch undergoes in basic aq. solution are shown in Figure 2.2.

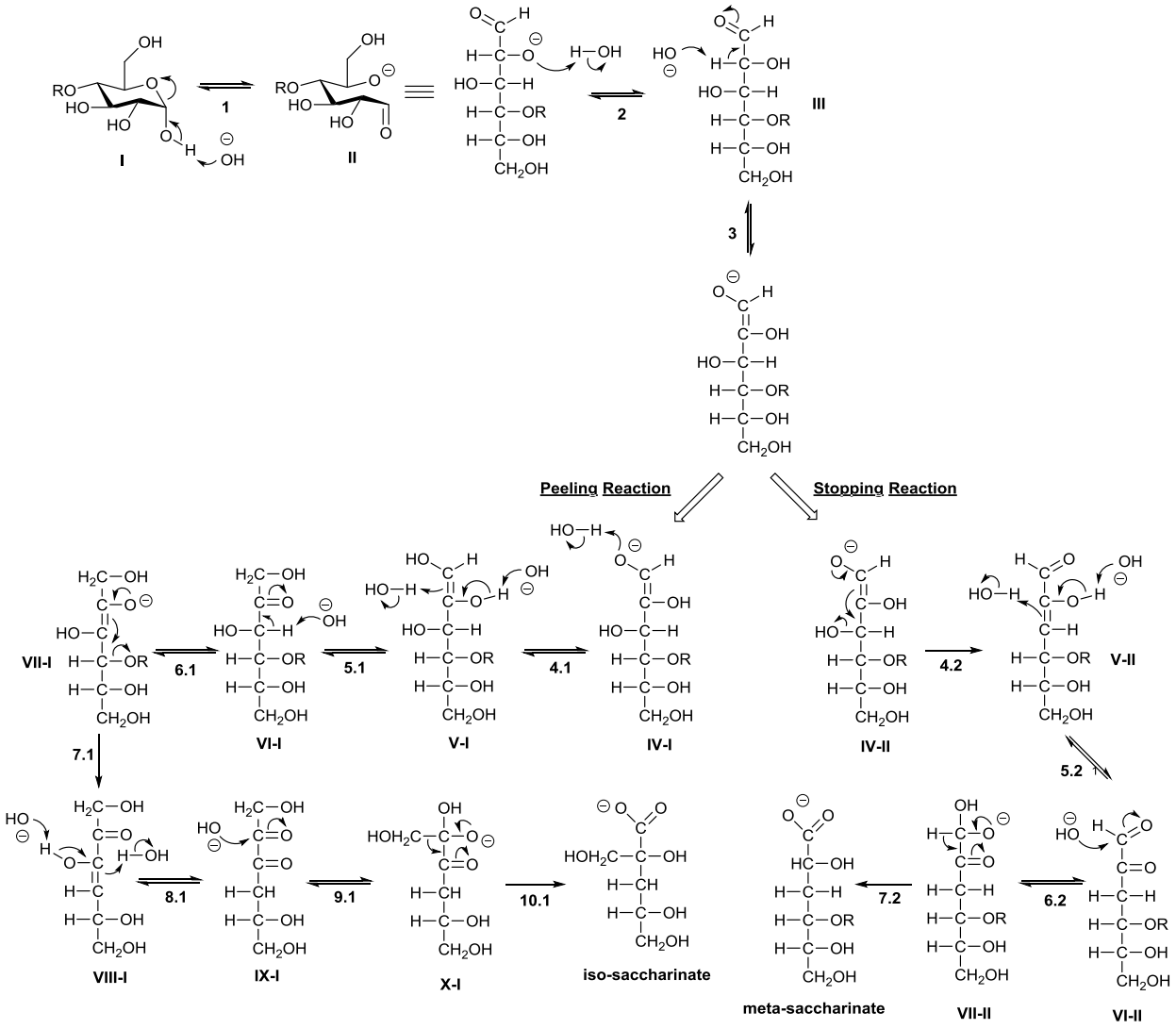


Figure 2.2. Mechanism for the alkaline degradation of starch.[60]

The glycosyl group at the reducing end converts reversibly between the pyranose and aldose forms (step 1). The aldose undergoes a keto-enol tautomerism to give an enolate (**III**). There are two ways that the mechanism can continue from this point. One is the peeling reaction,

which results in the removal of a glucose unit from the end of the chain and, leaves the chain with a reducing end that can undergo the reaction again. The other is the stopping reaction, which produces alkali-stable end groups.

The peeling reaction continues with another keto-enol tautomerism (steps 2-5.1 are known as the Lobry de Bruyn–Alberda–van Ekenstein transformation). No degradation, that is, no irreversible change in starch at the molecular level has really occurred until the elimination (step 7.1) at carbon 4 has taken place. This distinguishes degradation from irreversible physical changes like gelatinization of the starch granule. A benzylic acid rearrangement (step 10.1) produces isosaccharinate.[60-62]

The stopping reaction continues from enolate **III** with elimination of the hydroxyl at C3. A benzylic rearrangement of **VII-II** produces meta-saccharinate, which is alkali-stable. There are many variations on these mechanisms (e.g. a hydroxyl might attack the adjacent carbonyl in steps 9.1 and 6.2 and the product can undergo a benzylic rearrangement to produce different carboxylic acids). Other products that have been isolated from starch degraded under alkaline conditions include formic, acetic, lactic, 2-hydroxy-2-methylpropanoic, and 2-hydroxypentanoic acids.[60-62]

There are a few lessons that can be learned from studying the mechanism of alkaline degradation. Step 1 requires a free anomeric hydroxyl. This means that if more reducing ends exist in the starch sample, the faster it will degrade. It is likely that the harsh conditions under which the ENPs are produced partially degrade starch, increasing the number of reducing ends relative to native starch granules that were fed intro the extruder. The ene-diol rearrangement in step 2 also requires a free hydroxyl at carbon 2. If either the oxygen at the anomeric carbon or the oxygen at carbon 2 are involved in an ether bond, degradation will not occur as readily. The

susceptibility of a particular type of starch to alkaline degradation also depends on the degree of crystallinity and the surface area of the starch granule or derivative.[62]

The alkaline degradation studies and all modifications of the ENPs were done on commercial grade ENPs that had been purified by dialysis. The ^{1}H spectrum of the purified ENPs are shown in Figure 2.3.

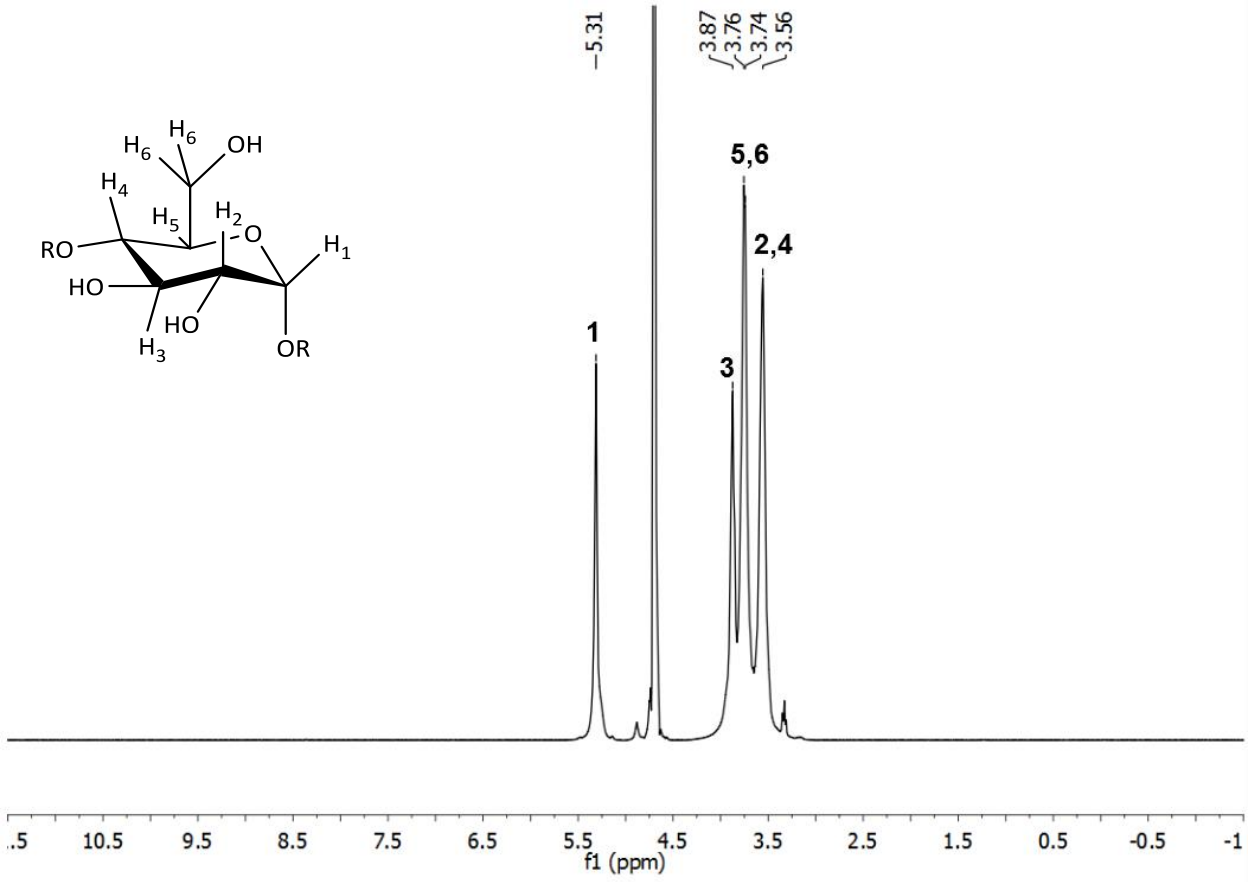


Figure 2.3 ^{1}H-NMR spectrum (500 MHz) of dialyzed commercial grade ENPs (100 mg/mL in D_2O).

This spectrum is similar to that of starch which has been reported in the literature. Peak assignments in Figure 2.3 are based on peak assignments that have been reported in the literature for starch.[64]

The hydroxyalkylation of starch proceeds very slowly at pHs below 12, even at elevated temperatures. Therefore, the hydroxyalkylation of the ENPs would have to be conducted at pH 12 or greater. To determine the extent of degradation of the the ENPs in aq. NaOH solution at pH 12 and 13, a dispersion of dialyzed ENPs in aq. NaOH at pH 12 or 13 were incubated at various temperatures for 24 h. The mixtures were then neutralized with aq. HCl, lyophilized and the resulting white powders were analyzed by ^{1}H-NMR. The ^{1}H-NMR spectrum of ENPs after being subjected to aq. NaOH (pH 13) at 40^{o}C for 24 h followed by neutralization with aq. HCl and lyophilization is shown in Figure 2.4.

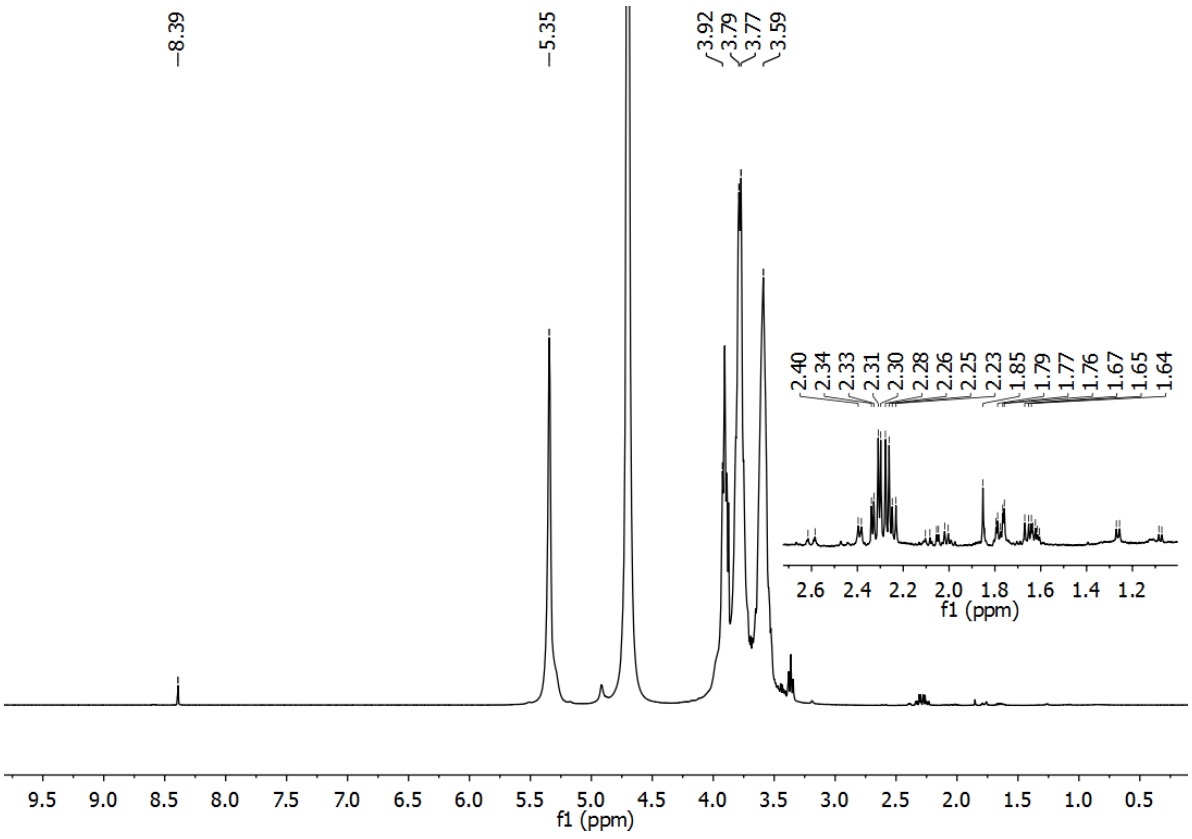


Figure 2.4 ^{1}H-NMR spectrum (500 MHz) of ENPs subjected to aq. NaOH (pH 13) at 40^{o}C for 24 h (100 mg/mL in D_2O).

New peaks due to degradation of the ENPs are readily evident. The appearance of what might be an aldehyde peak at 8.38 ppm suggests the presence of the acyclic form of the reducing end of the carbohydrate chains. However, this is unlikely as the reaction mixtures were neutralized before the NMR spectrum was obtained. The reducing end is almost always present in the ring-closed pyranose form under neutral aq. conditions. This peak might also correspond to an α-dicarbonyl compound with an aldehyde (e.g. **VI-II** in Figure 2.2). It is most likely that this peak is due to formic acid which exhibits a chemical shift in water of 8.30 ppm. A spiking experiment with authentic formic acid would have confirmed this but this experiment was not done. There is a doublet of doublets between 2.20 and 2.35 ppm as well as a variety of other smaller upfield peaks. The chemical shift and splitting pattern suggests that these peaks may be due to the lactone of isosaccharinate.[65] The singlet at 1.85 ppm might be 2-hydroxy-2-methylpropanoic acid or acetic acid, or any other degradation product containing an sp_3 hydridized carbon with no vicinal protons. Other peaks in the region between 0.7-1.8 ppm must correspond to protons bound to sp_3 carbons with no electron withdrawing groups, but there is too much overlap between peaks in this region of the spectrum to be able to determine what compounds they might belong to.

The ^{1}H-NMR spectra of the ENPs after being subjected to aq. NaOH (pH 13) at 30, 40 and 60 ^{o}C for 24 h followed by neutralization with aq. HCl and lyophilization are shown in Figure 2.5. Peaks due to degradation of the ENPs were evident. Not suprisingly, the extent of degradation increased with increasing temperature.

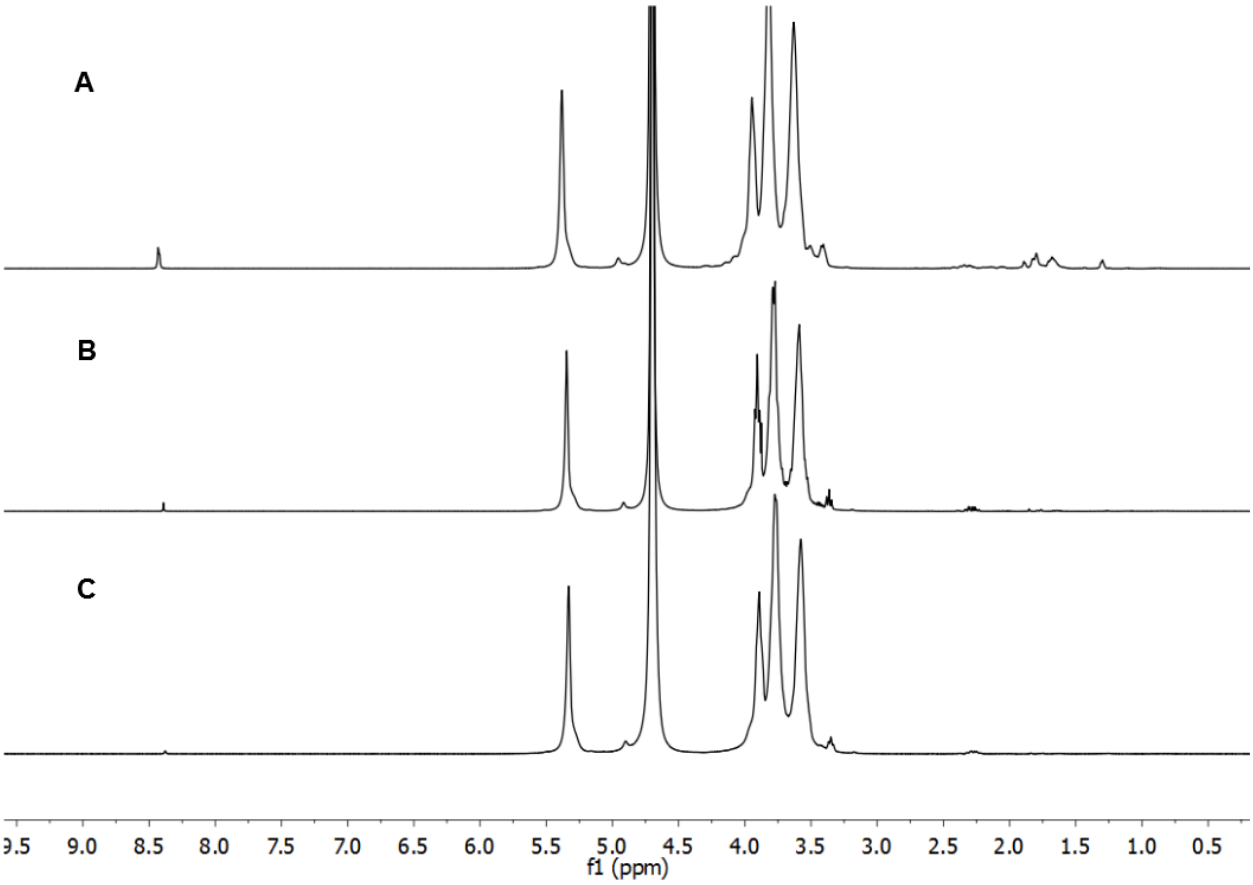


Figure 2.5 ^{1}H-NMR spectra (500 MHz) of ENPs after being subjected to aq. NaOH (pH 13) at 30, 40 and 60 ^{o}C for 24 h (100 mg/mL in D_2O). Spectrum **A**: 60 ^{o}C. Spectrum **B**: 40 ^{o}C. Spectrum **C:** 30 ^{o}C.

The ^{1}H-NMR spectra of the ENPs after being subjected to aq. NaOH (pH 12) at 40-90 ^{o}C for 24 h followed by neutralization with aq. HCl and lyophilization are shown in Figure 2.6. Again peaks due to degradation were evident though less degradation occurred than at pH 13.

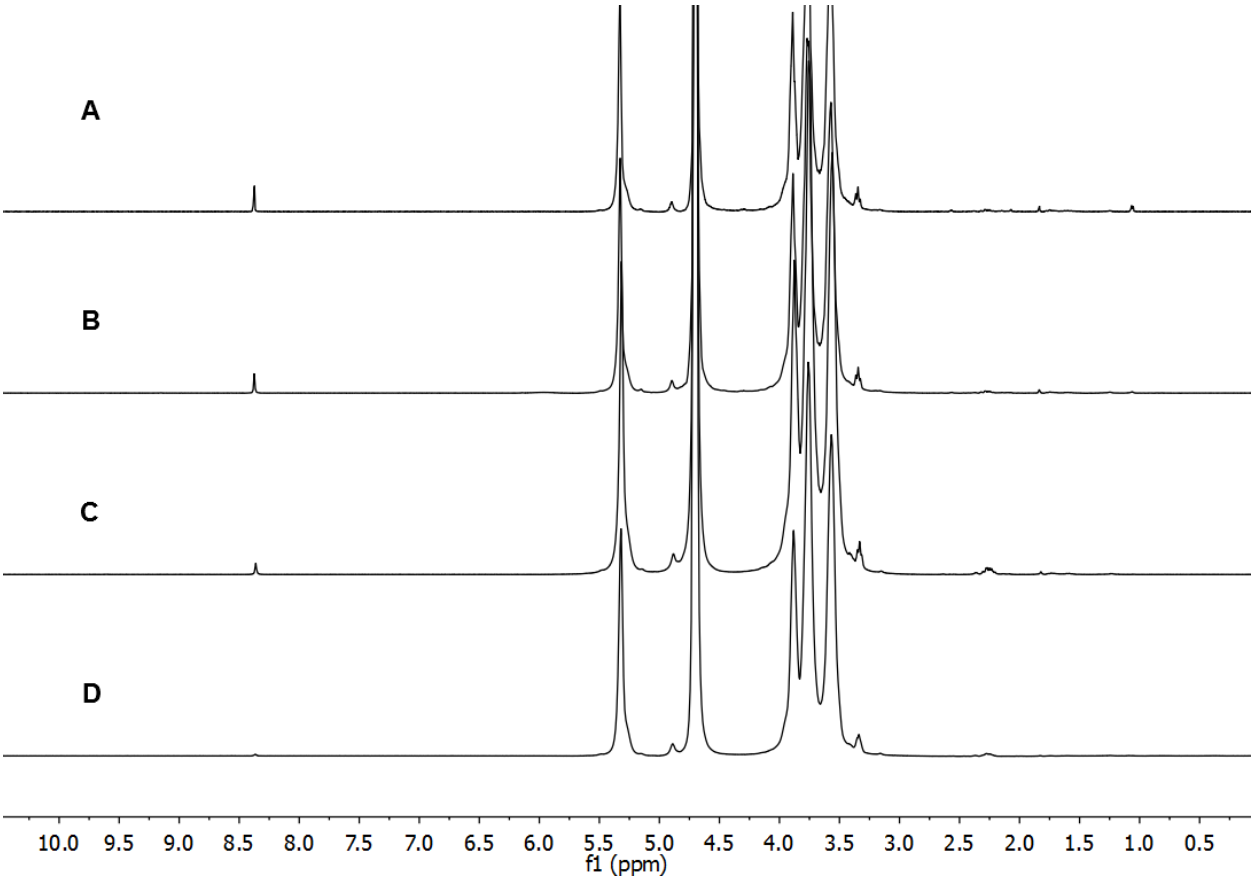


Figure 2.6 ^{1}H-NMR spectra (500 MHz) of ENPs after being subjected to aq. NaOH (pH 12) at 40-90 ^{o}C for 24 h (100 mg/mL in D_2O). Spectrum **A**: 90 ^{o}C. Spectrum **B**: 80 ^{o}C. Spectrum **C:** 60 ^{o}C. Spectrum **D:** 40 ^{o}C

Although we were unable to quantify the extent of degradation, from the ^{1}H-NMR spectra shown above, it appears that the extent of degradation at pH 12 and 13, for temperatures 30 and 40 ^{o}C, was very small. Consequently, it was decided that the hydroxyalkylation of ENPs could be conducted under these conditions without significant loss of the ENPs due to degradation.

2.3 Hydroxypropylation of ENPs

The ENPs were modified with PO in pressure tubes in aq. NaOH at pH 12-13 at rt, 30 °C or 40 °C for 16 or 24 h. After 24 h the reactions were neutralized with aq. HCl then dialyzed to remove unreacted PO or low molecular weight byproducts. After dialysis, the solution was lyophilized and, the NMR spectrum obtained. In terms of temperature and time, these conditions are similar to those used to produce hydroxypropylated starch on an industrial scale.[55]

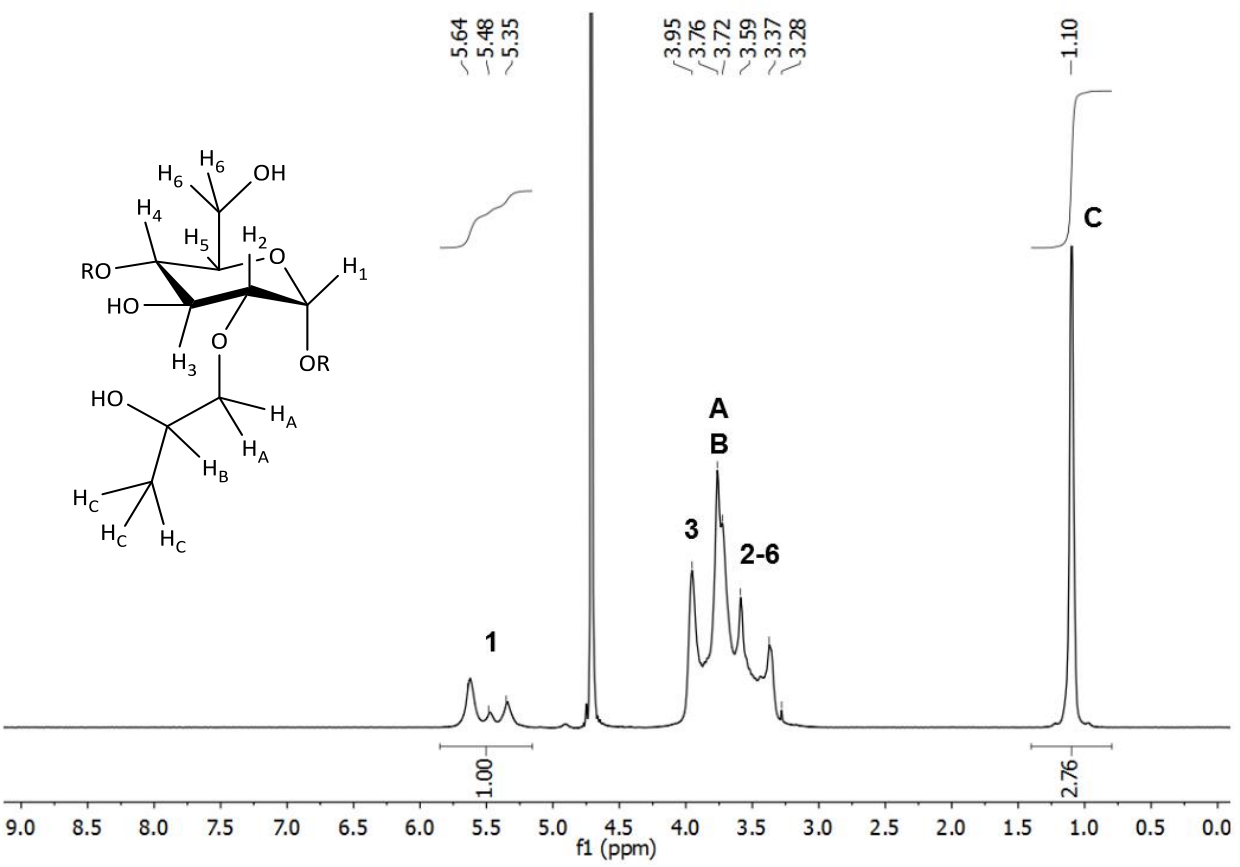


Figure 2.7 ^{1}H-NMR spectra (500 MHz) of ENPs after being subjected to 1.5 molar equiv PO and aq. NaOH (pH 13) at 40 °C for 24 h (100 mg/mL in D_2O) followed by dialysis. MS = 0.92. Substitution is shown at O-2 though modification also occurred at O-3 and, most probably, O-6.

The ^{1}H-NMR spectrum of ENPs after being subjected to 1.5 molar equivalents of PO in aq. NaOH (pH 12) at 40 ^{o}C for 24 h followed by dialysis is shown in Figure 2.7. The methyl group in the hydroxypropyl ENPs (HPENPs) appears at 1.08 ppm. In the spectrum of purified starch and starch degraded under alkaline conditions, the anomeric proton appears at around 5.35 ppm. The NMR spectrum in Figure 2.7 shows a peak at 5.32 ppm which is due to the anomeric proton of the unmodified ENPs. However, it also shows peaks at 5.46 and 5.61 ppm. These peaks are also visible in the spectra of other hydroxypropylated ENPs, as shown in Figure 2.8.

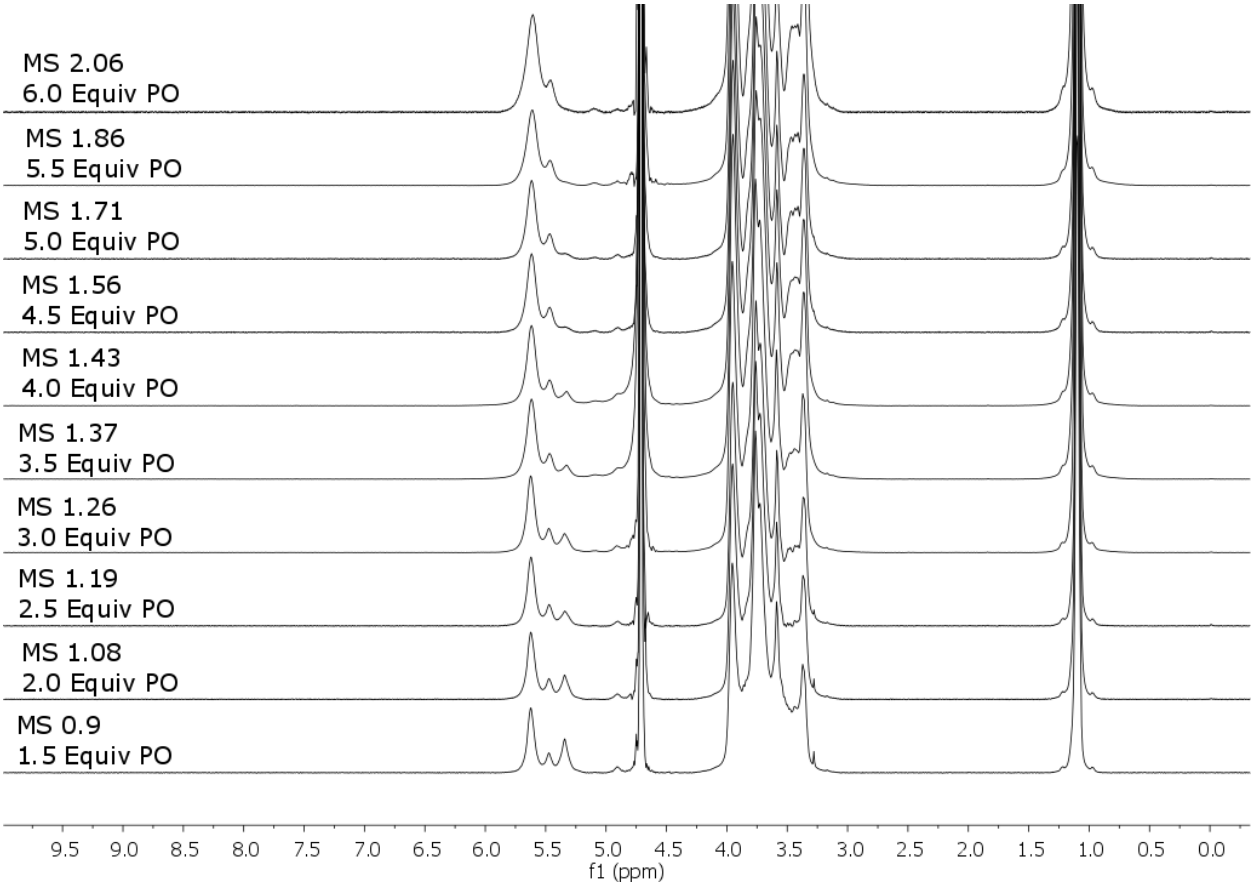


Figure 2.8 ^{1}H-NMR spectra (500 MHz) of the hydroxypropylated ENPs (100 mg/mL) prepared in aq. NaOH (pH 13), 30 ^{o}C for 24 h with varying amounts of PO.

As the molar equivalents of PO used in the hydroxypropylation reactions was increased the peaks at 5.46 and 5.61 ppm also increased in intensity. Figure 2.8 shows the ^{1}H-NMR spectra for a series of hydroxypropylation reactions conducted at 30 ^{o}C in aq. NaOH at pH 13 for 24 h. The signal for the anomeric proton that appears in unmodified ENPs at 5.35 ppm is no longer evident when more than 5.0 eq of PO are used in the reaction (as seen in spectrum 8 and 9 in Figure 2.8).

Changes in chemical shift of the anomeric proton following modification of nearby hydroxyl groups is a known phenomenon. Gidley[64] identified four signals for the anomeric proton in the ^{1}H spectrum of starch: 4.68 ppm (β-form at the reducing end), 5.00 ppm (α-form at branch points), 5.27 ppm (α-form, reducing end), and 5.35 ppm (α-form, internal α-1,4-linked glycosyl). Xu and Sieb identified these signals in α-limit dextrins (branched oligosaccharides produced through hydrolysis of amylopectin by α-amylase) of unmodified and hydroxypropylated starches.[66] However, they also identified two new signals in the anomeric region at 5.52 and 5.67 ppm. ^{1}H-NMR studies of hydroxypropylated α-D-glucose showed that hydroxypropylation at 2-OH resulted in a 0.18 ppm increase in the chemical shift of the anomeric signal.[67] Xu and Sieb inferred that modification at the 3-OH would have a less significant impact on the chemical shift because it is further away from the anomeric proton, and that modification at the 6-OH might have no effect at all. They assigned the shift at 5.52 ppm to the anomeric proton of glycosyl residues hydroxypropylated at the 3-OH. They assigned the shift at 5.67 ppm to the anomeric proton of glycosyl residues hydroxypropylated at the 2-OH. The signal at 5.35 ppm corresponds to modification at the 6-OH or no modification. These studies suggested to us that the peaks at 5.61 and 5.46 ppm in Figure 2.7 are due the anomeric protons of the ENPs hydroxypropylated at

the 2-OH and 3-OH groups respectively, while the peak at 5.35 ppm corresponds to unmodified and/or 6-O-hydroxypropylated ENPs.

To determine the molar substitution of the hydroxypropylated ENPs, the integration value (area) for the peaks at 1.08 ppm was divided by three and this value was divided by the integration value (area) for the anomeric proton peaks appearing between 5.32 and 5.61 ppm.

Figure 2.9 shows the relationship between molar substitution (MS) of HPENPs versus molar equiv of PO for the data shown in Figure 2.8. For these reactions the molar equiv of PO to anhydroglucose units was 1.5-6.0.

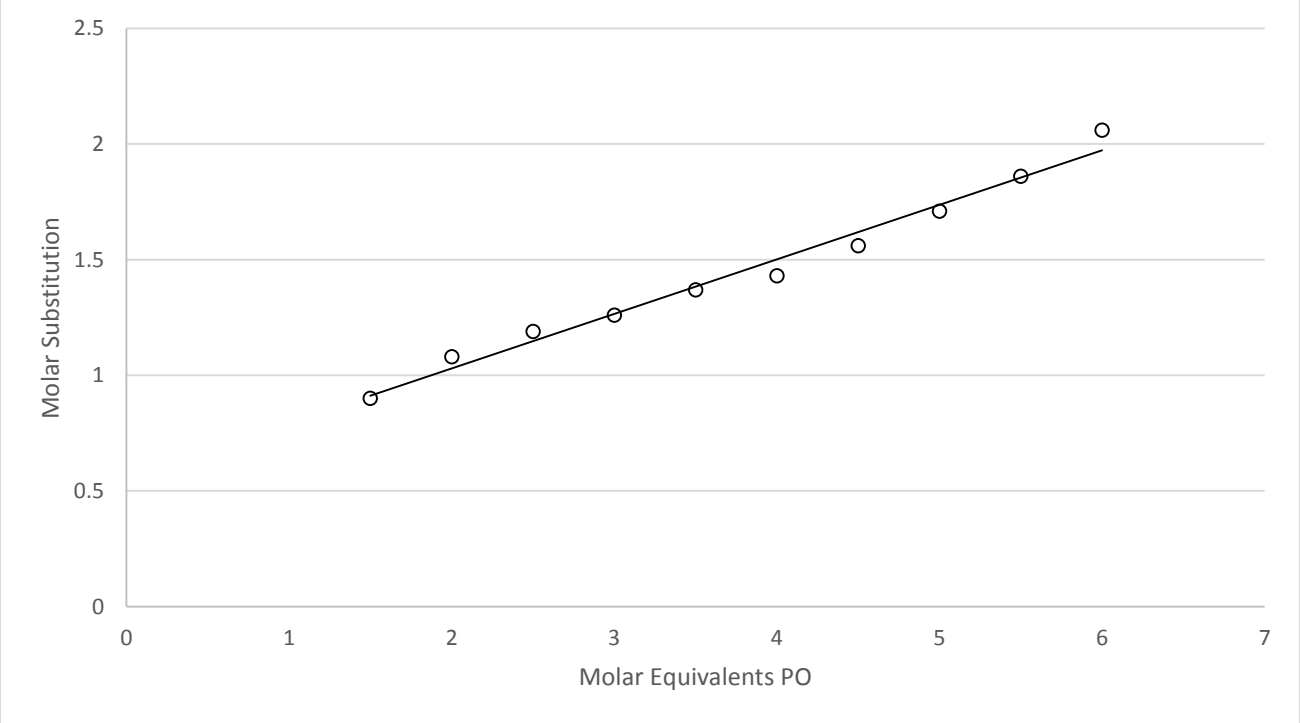


Figure 2.9 Molar substitution (MS) of HPENPs versus molar equiv of PO. The reactions were performed in aq. NaOH (pH 13) at 40 ^{o}C or rt for 24 h.

Figure 2.10 shows the relationship between molar substitution (MS) of HPENPs versus molar equiv of PO used in the reaction when the molar equiv of PO to anhydroglucose units was 0.1-0.5. The pH, temperature, and reaction time are similar to the conditions used when hydroxypropylated starch is prepared on an industrial scale. The MS of the HPENPs obtained for these reactions is typical for commercially available hydroxypropylated starch.

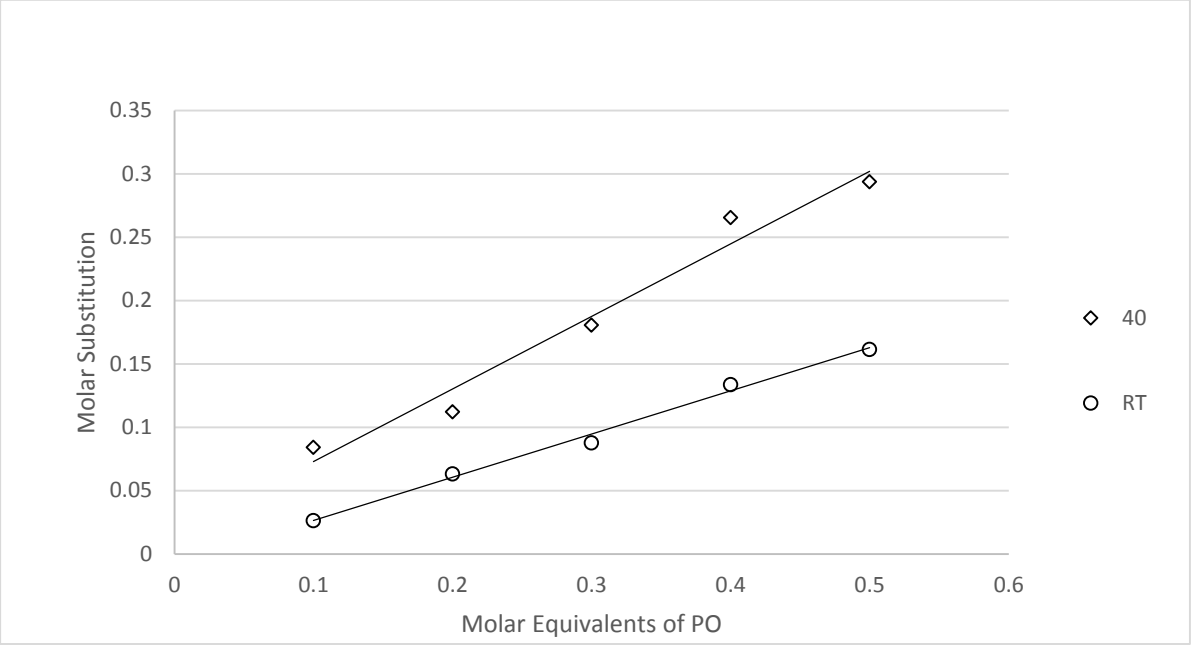


Figure 2.10 Molar substitution (MS) of HPENPs versus molar equiv of PO. The reactions were performed in aq. NaOH (pH 12) at 40 ^{o}C or rt for 24 h.

The reactions were performed in aq. NaOH (pH 12) at 40 ^{o}C or rt for 24 h. The MS increased with temperature and with equivalents of PO. The reaction was more efficient at 40 ^{o}C than rt. At 40 ^{o}C, the efficiency of the reaction was approximately 60% in terms of molar equivalents of PO used versus mols of modified anydroglucose units. None of these HPENPs nor any other HPENPs prepared with a higher MS (Figure 2.8) exhibited thermoresponsive behaviour.

The rate of ENP degradation should have decreased as the hydroxypropylation reaction proceeded since the modification was occurring at the 2-OH. To learn more about the appearance of degradation products during the hydroxypropylation reaction, the reaction was followed by ^{1}H-NMR by dispersing the ENPs in 0.01 M NaOD (10 % dispersion) in an NMR tube at 40-50 ^{o}C followed by the addition of 3 equiv of PO at 4 ^{o}C. The NMR tube was sealed with parafilm and then placed into an NMR machine whose probe had been pre-equilibrated to 40 ^{o}C. The first spectrum, shown in Figure 2.11, was recorded after 30 minutes and subsequent spectra were recorded every 15 min.

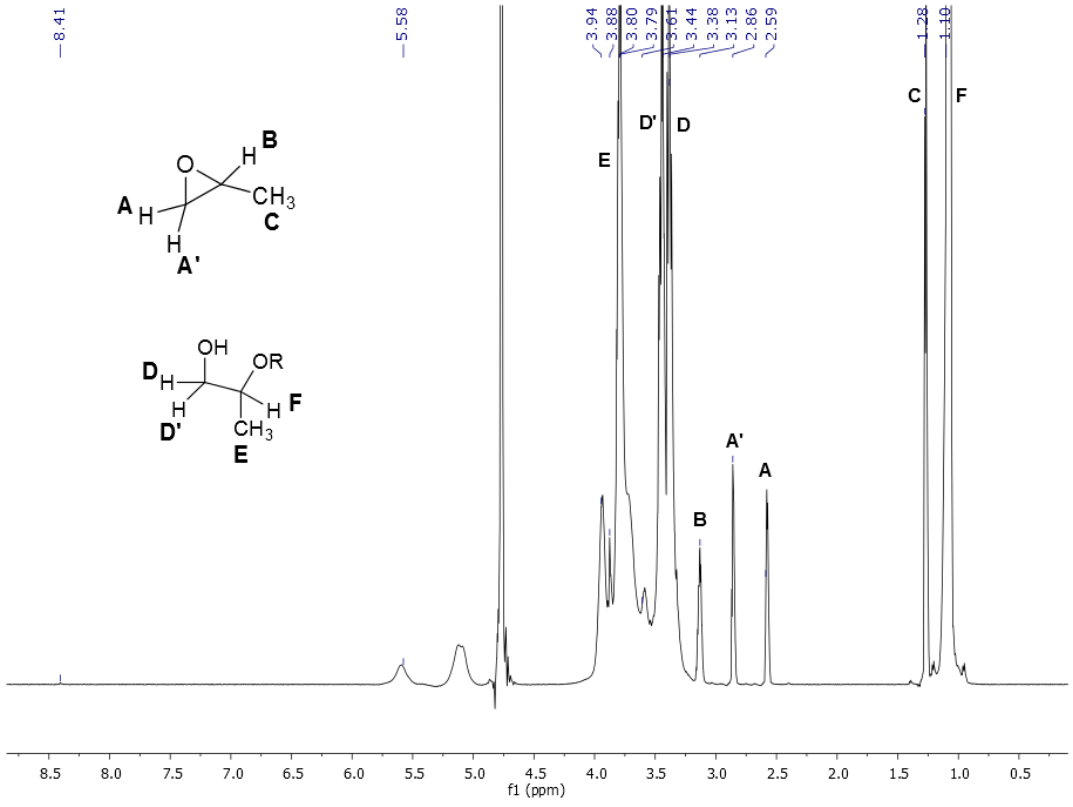


Figure 2.11 ^{1}H-NMR spectra (500 MHz) of the reaction of PO (3 equiv.) with ENPs in aq. NaOD (0.01 M) at 40 ^{o}C (100 mg/mL) after 30 min. R = H, D, or O-starch.

After 30 min, 86% of the PO was consumed. This was determined by integrating over the entire region where the signals for the methyl groups of PO (1.28 ppm) and hydroxypropyl or propylene glycol (1.10 ppm) appear (1.35 to 1.00 ppm) and calibrating this region to 1.0. The integration value from 1.35 to 1.20 ppm gives the fraction of PO (0.14), and the integration values from 1.20 to 1.00 ppm gives the fraction of hydroxypropyl groups or propylene glycol. Other peaks corresponding to PO, a multiplet at 3.13 ppm ('B') and two diastereotopic protons at 2.86 ppm (A') and 2.59 ppm (A), are present in the first spectrum, but quickly disappear, as shown in Figure 2.12.

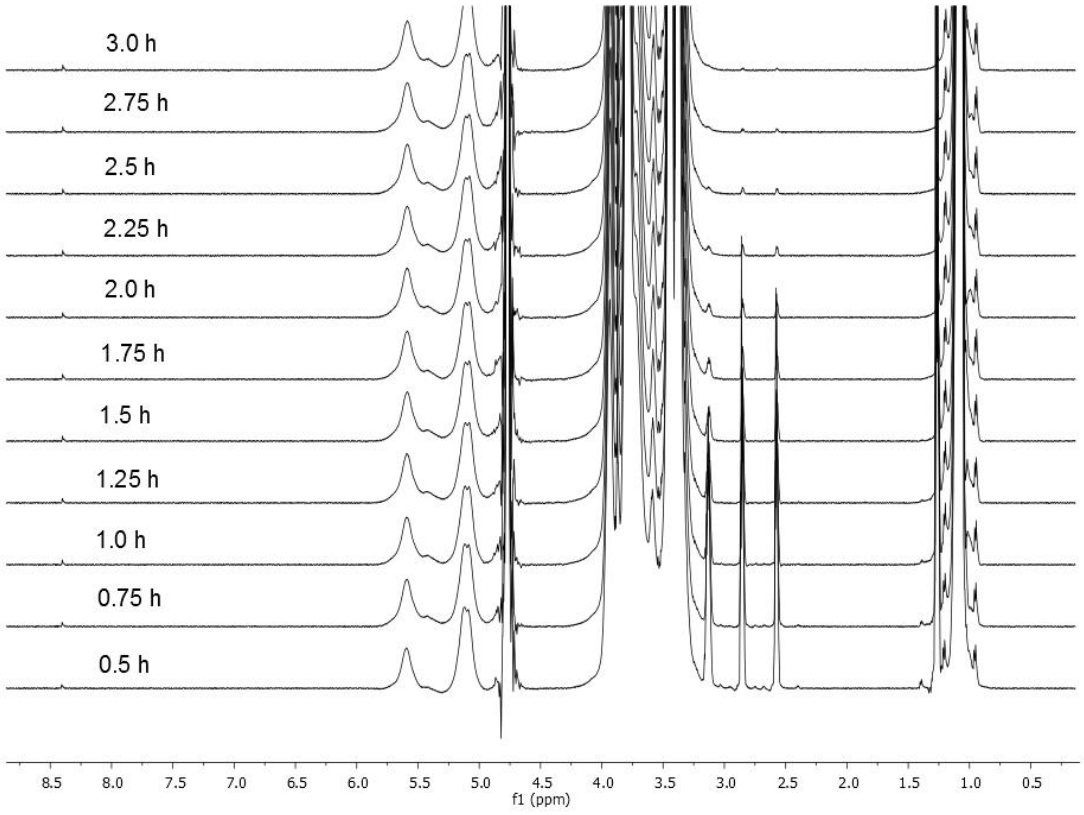


Figure 2.12 ^{1}H-NMR spectra (500 MHz) of the reaction of equiv PO with ENPs in aq. NaOD (0.01 M) at 40 ^{o}C (100 mg/mL). Spectrum # 1 was taken after 30 min. Each subsequent spectrum was obtained in 15 min intervals.

As seen in Figure 2.12, almost all of the PO was consumed after just 3 h as determined by the inability to detect peaks corresponding to protons of PO after this time. Evidence for degradation (peaks appearing at 2.2-2.3 ppm or an increase in the height of the peak at 8.41 ppm) was not evident in any of the NMR spectra in Figure 2.12 or any subsequent spectra taken up to the 18 h mark (spectra not shown). This is in contrast to spectrum D in Figure 2.6 which clearly shows some degradation products appearing at 2.2-2.3 ppm. A small peak at 8.41 ppm is present in all of the spectra in Figure 2.12. We believe this peak corresponds to formic acid. Since this peak does not increase in intensity during the reaction then formic acid is not being produced during the reaction. These results strongly suggest that hydroxypropylation at O-2 (or, most likely, any etherification taking place at O-2) helps protect the ENPs against alkaline degradation. Moreover, this experiment shows that, under these conditions, the PO was consumed relatively quickly. Also, the ratio of the peaks at 5.6 and 5.45 ppm, which correspond to anomeric protons or 2-O or 3-O substituted products, to the peak at 5.15 ppm, which corresponds to the unmodified ENPs or 6-O-modifed ENPs does not change much after 30 min. This indicates that the hydroxypropylation reaction is almost complete after the first 30 min and that reaction time of 24 h that was used to prepare the HPENPs was excessive. If there is a need to perform any future hydroxypropylation reactions on the ENPs, then the reaction time could be scaled back considerably. However, since none of the HPENPs produced were thermosensitive, this modification was no longer pursued.

2.4 Hydroxybutylation of ENPs

Since no TRPs were obtained via hydroxypropylation of ENPs, we then turned to hydroxybutylation of ENPs. Hydroxybutylated ENPs were produced by modifying the ENPs with butene oxide (BO). The ^{1}H spectrum of hydroxybutylated ENPs (HBENPs) is shown in Figure 2.13.

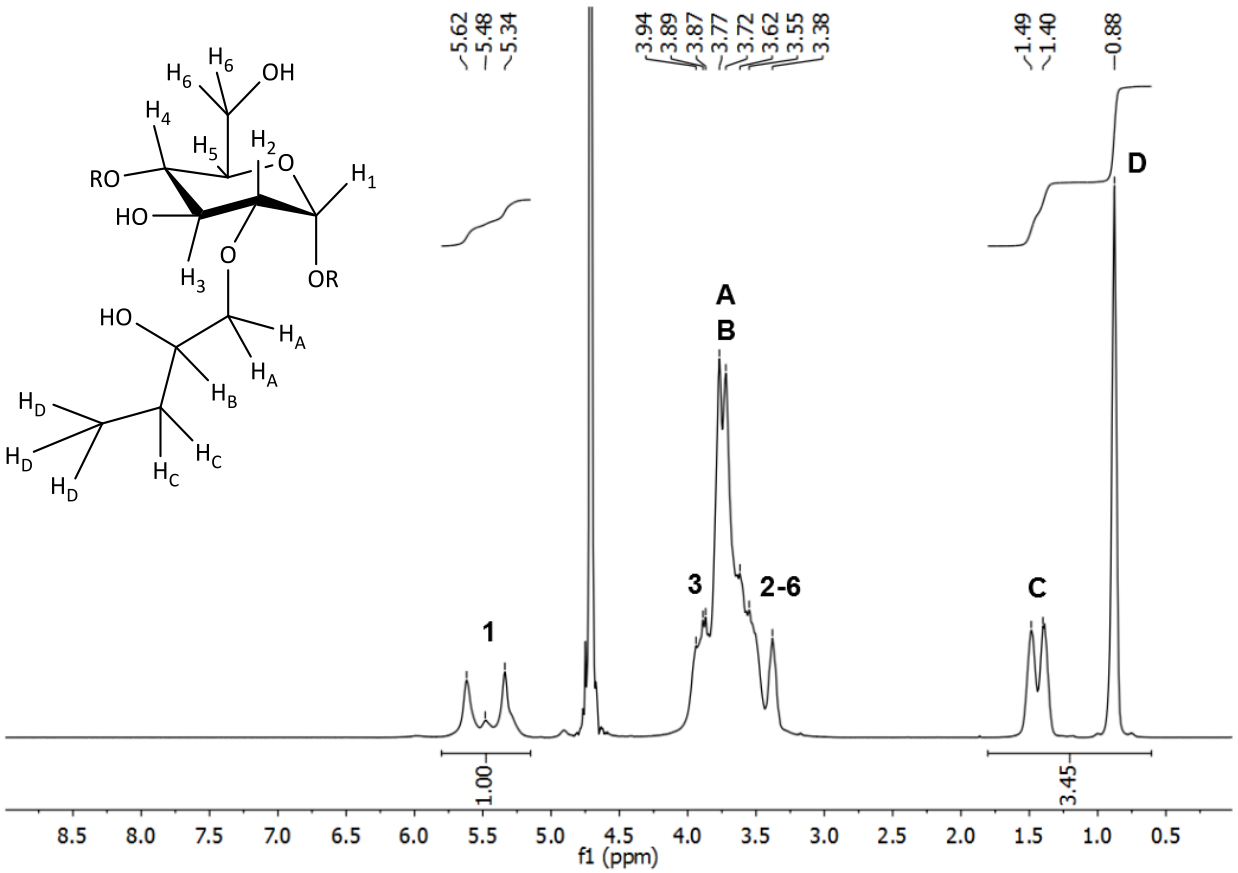


Figure 2.13 ^{1}H-NMR spectra (500 MHz) of hydroxybutylated ENPs prepared using 1.5 equiv BO in 0.1 M NaOH at 40 °C. The MS was 0.84. The hydroxybutyl group is shown attached to O-2. Hydroxybutylation has also occurred at O-3 and O-6.

Hydroxybutylation of the ENPs was initially carried out in 0.1 M NaOH (pH app. 13) at 40 °C. As with hydroxypropylation, hydroxybutylation of the ENPs occurred at O-2 and O-3 as evidenced by the appearance of peaks 5.48 and 5.61 ppm. 2-O-Hydroxybutylated ENPs were the dominant product. 6-O-hydroxybutylated ENPs were probably also produced; however, the

anomeric proton for this product has a chemical shift that is the same as the anomeric proton of the unmodified ENPs (5.33 ppm).

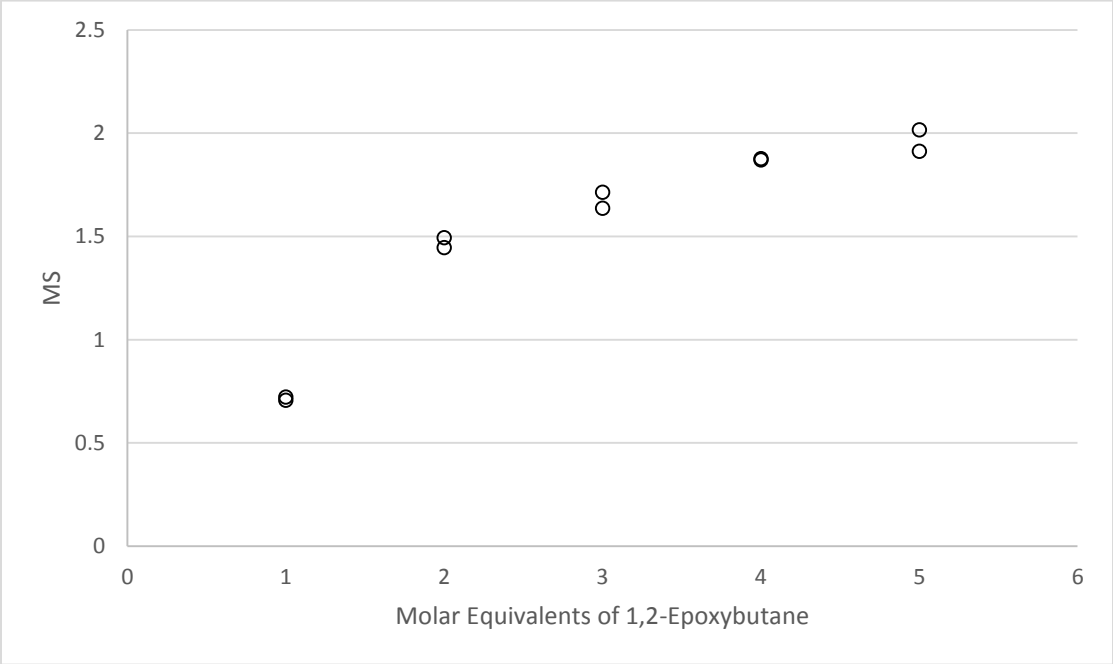


Figure 2.14 Molar substitution (MS) of HBENPs versus molar equiv of BO. The reactions were performed in 0.1 M NaOH at 40 ^{o}C for 24 h.

A plot of the MS of the HBENPs versus molar equivalents of BO, when the reactions were performed in 0.1 M NaOH at 40 ^{o}C for 24 h and using 1, 2, 3, 4 and 5 equiv BO, is shown in Figure 2.14. These reactions were done in duplicate to determine the reproducibility of the procedure. The reactions were quite reproducible in terms of MS obtained. The MS increases significantly when going from 1-3 molar equiv of BO. Beyond 3 equivalents of BO the MS does not increase substantially and levels off with an MS of about 2. BO is not highly soluble in water and the reactions were initially biphasic but became homogeneous after 24 h reaction time. This indicated that most or all of the BO was consumed over the 24 h reaction period. However, BO's low solubility in water cannot explain why the MS does not increase substantially when going

from 3 to 5 equiv of BO. As the BO is consumed, more BO should enter the aq. phase and so the aq. layer should always be saturated with BO. We noticed that, as the reactions proceeded, the HBENPs precipitated out of the mixture. This was especially the case for those reactions where using larger amounts of BO were used. Precipitation of the HBENPs out of the reaction mixture might have limited accessibility of the polymer to reagents and so prevent or slow the reactions down.

ENPs were reacted with 1.5, 2, 2.5, and 3 equiv of BO in aq. NaOH, pH 13 for 16 or 24 h at 40 ^{o}C. The MS of the resulting HBENPs was determined. The reaction run for 24 h produced HBENPs with MSs slightly greater than reactions run for 16 h. The reaction efficiency decreased as the equiv of BO increased (Table 2.1).

Table 2.1. Preparation and Characterization of HBENPs

Reaction Time (h)	Equiv BO [a]	MS of HBENPs [b]	Reaction Efficiency (%)[c]	LCST (^{o}C)[d]
16	1.5	1.10	73.3	ND[e]
16	2.0	1.32	66.0	46.8
16	2.5	1.52	60.8	43.5
16	3.0	1.56	52.0	39.8
24	1.5	1.12	74.7	41.8
24	2.0	1.46	73.0	40.5
24	2.5	1.56	62.4	39.8
24	3.0	1.66	55.3	37.8

[a]Ratio of the mols BO to mols of anhydroglucose units (AGU).
[b]MS determined through ^{1}H NMR
[c]Reaction efficiency was determined by dividing MS by the mols of BO.
[d]LCST determined through UV-Vis Spectroscopy
[e]ND = Not determined

The HBENPs prepared in Table 2.1 were examined for thermal responsive behaviour. The results of these studies for the HBENPs prepared are shown in Figures 2.15 and 2.16. All of these HBENPs showed thermoresponsive behaviour.

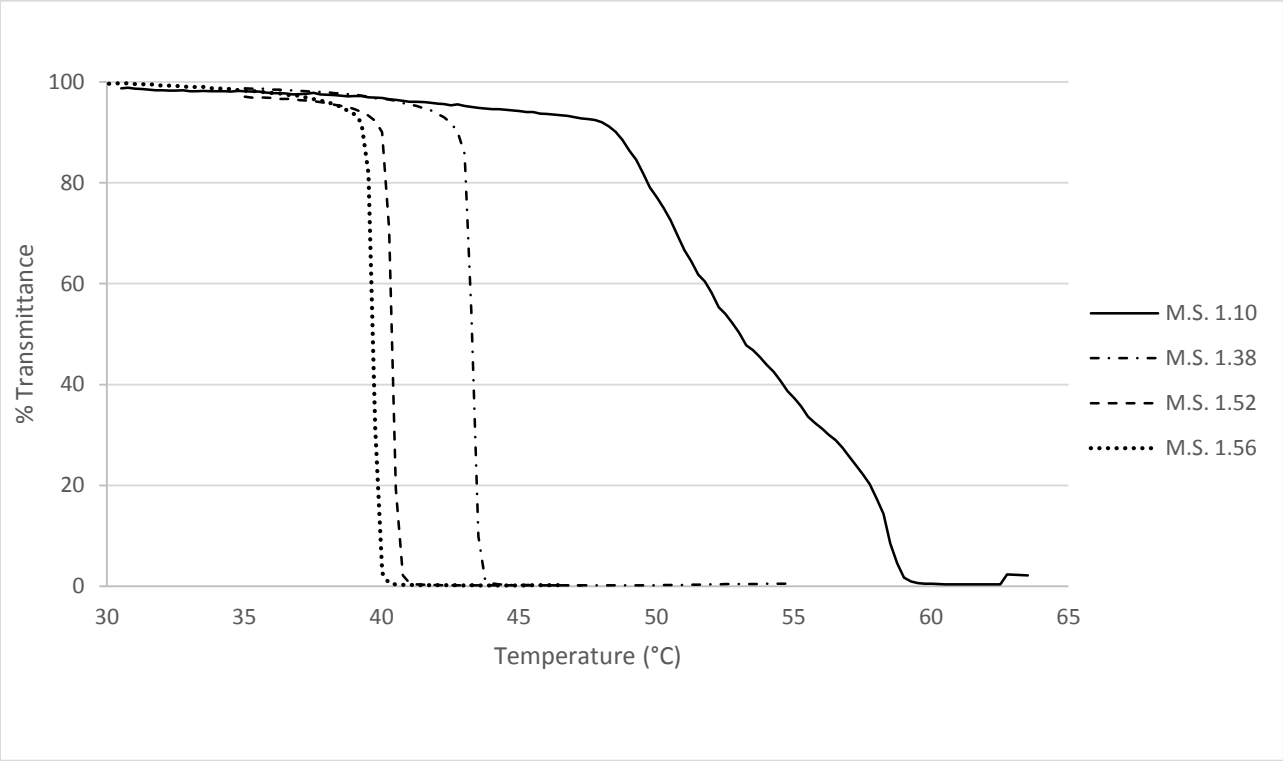


Figure 2.15 Transmittance changes for 10 mg/mL aqueous solutions of HBENPs, prepared at pH 13 at 40 ^{o}C for 16 h, with different MS.

The LCSTs decreased with increasing MS (see Table 2.1). HBENPs with MSs > 1.12 exhibited relatively sharp, cooperative thermal transitions. It is interesting that the HBENP with a MS of 1.12 exhibited a single, relatively sharp thermal transition while the HBENP with an MS of 1.10 (a difference in MS of just 0.02) exhibited what appears to be two transition points, one between 52-53 ^{o}C (which is very broad) and a second at ~58.5 ^{o}C (which is sharper).

There have been reports in the literature of thermoresponsive hydroxypropyl methylcellulose with two thermal transitions.[68-71] Two-stage thermal transitions are common for hydrophobically-modified cellulose derivatives. The first thermal transition is thought to occur as a result of chain clustering due to hydrophobic association. Residual crystallinity of the cellulose derivative restricts chain mobility and consequently the ability of hydrophobic clusters to associate. This crystallinity gradually disappears as the temperature increases, allowing for more clustering of hydrophobically modified segments. This process continues until there is enough hydrophobic clustering to induce the second thermal transition, exclusion of water from the polymer matrix, or phase separation.[68,70] Considering that the ENPs have been previously determined to be amorphous (REF), the above explanation is unlikely to apply to the TRENPs. A more plausible explanation might involve inhomogeneous modification of the ENPs. After a starch fragment has been hydroxybutylated, that fragment becomes more hydrophobic than unmodified starch. As long as the MS is low, butylene oxide might be more likely to go to ENPs that have more hydrophobic character. This might cause the number of hydroxybutyl groups on each particle to vary considerably. The broadness and 'unevenness' of the transmittance curves of inhomogeneously modified nanoparticles As the temperature increases, particles with more hydroxyalkyl groups could collapse before particles with fewer hydroxyalkyl groups, resulting in a broad and 'uneven' transmittance curve.

For HBENPs with MS values of 1.16 and greater, the LCSTs decreased linearly with increasing MS, as shown in Figure 2.16.

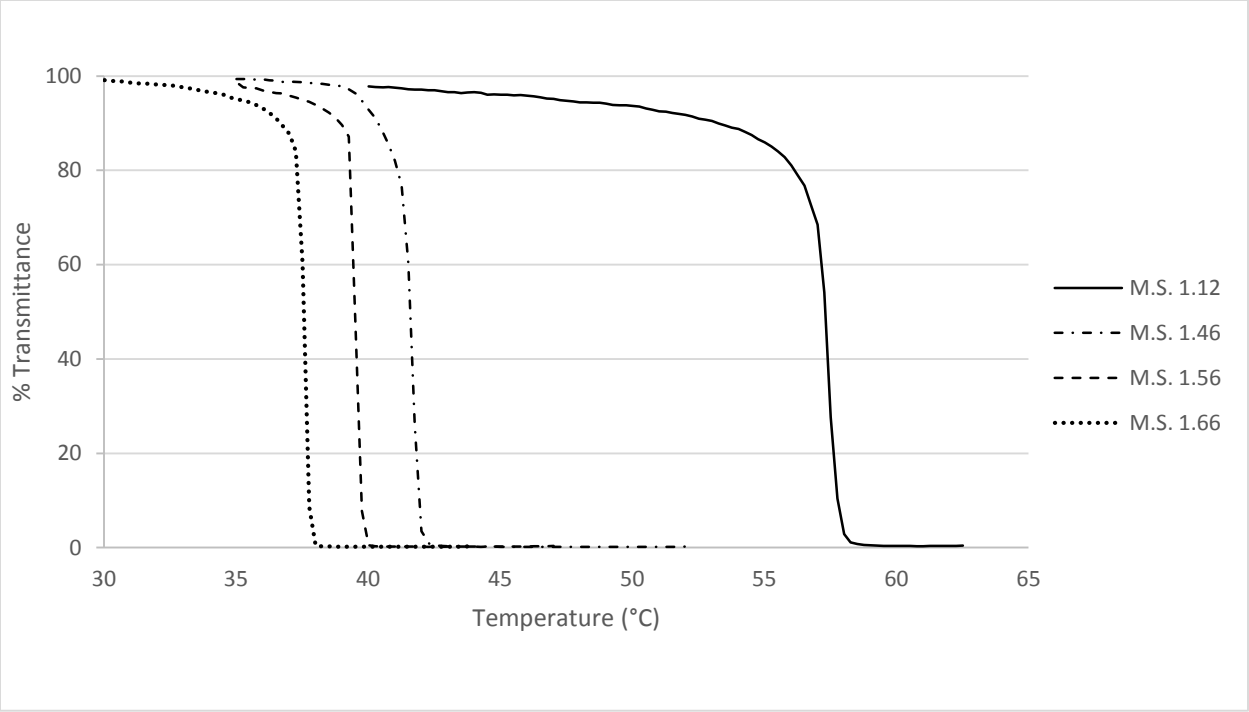


Figure 2.16 Transmittance changes for 10 mg/mL aqueous solutions of HBENPs (prepared at pH 13 at 40 ^{o}C for 24 h) with different MS

Any HBENP with an MS between 1.0-1.16 deviated from this trend and did not exhibit sharp thermal transitions. It was found that HBENPs with an MS less than 1 did not show thermal responsive behaviour. The MS values used to generate the plot in Figure 2.17 were obtained from HBENPs prepared using different reaction temperatures and times (HBENPs from Figures 2.15, 2.16). Hence, neither of these variables (temperature or time) seemed to affect the linear relationship between MS and LCST.

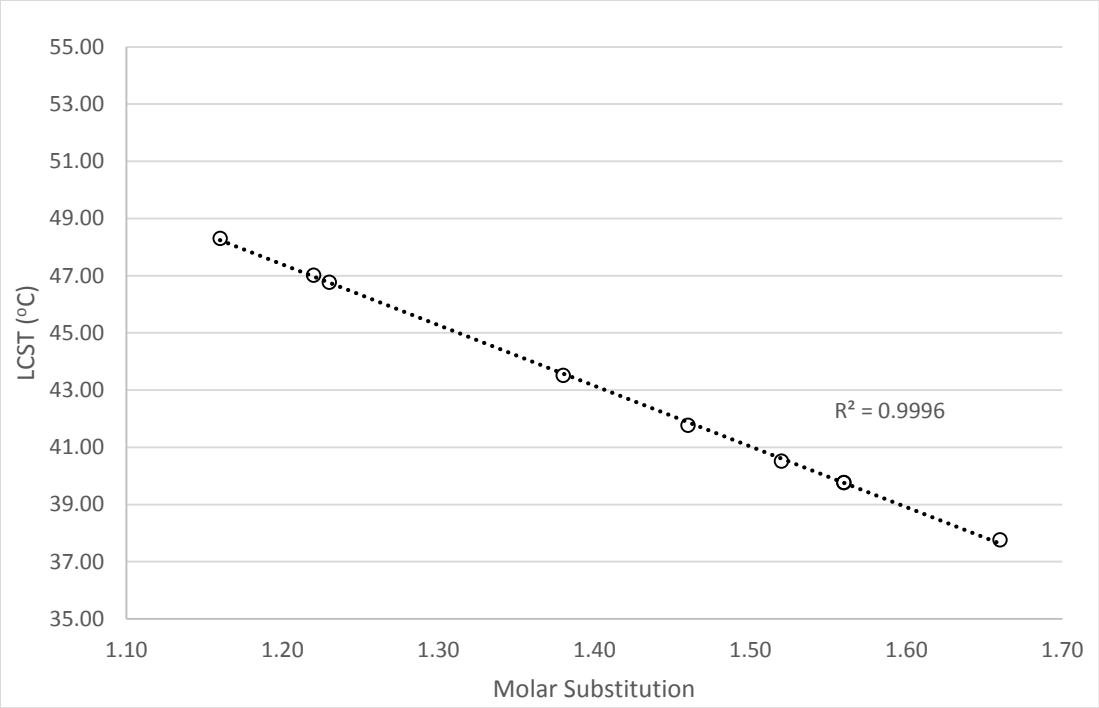


Figure 2.17 Relationship between MS and LCST of HBENPs (1 mg/mL) in water.

2.5 Effect of HBENP Concentration, NaCl and Alcohols on the LCSTs of HBENPs

The effects of HBENP concentration and additives, such as salts and cosolvents, on the LCST of the HBENPs was examined. The information obtained from these studies will help identify the application in which the HBENPs could be used. Different applications might require different concentrations of HBENPs. The thermal responsive ENPs might have to work in the presence of salts or other additives. In any *in vivo* biomedical application the TENPs will be exposed to many different types of solutes that might affect the thermal transition. In oil extraction or column chromatography, the HBENPs would have to function in the presence of co-solvents.

2.5.1 The Effect of HBENP Concentration on LCST

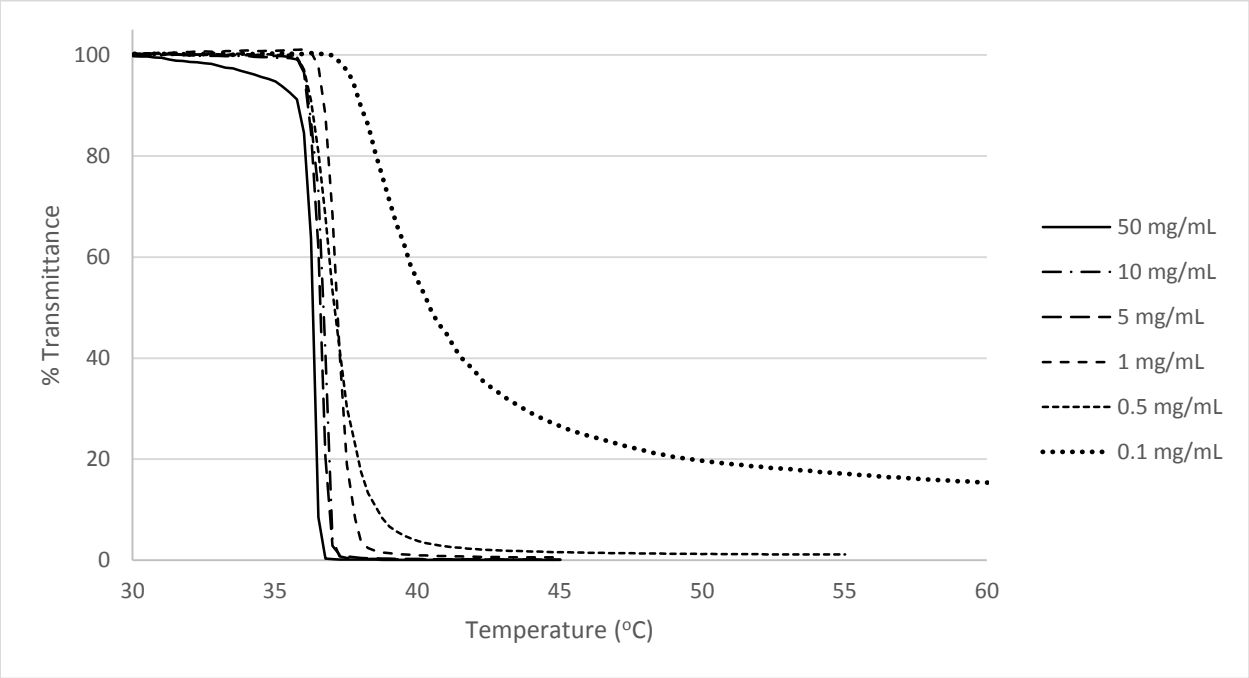


Figure 2.18 Effect of HBENP (MS = 1.68) concentration on the LCST in water.

The effect of HBENP concentration on the LCST is shown in Figure 2.18 and Table 2.2. From 50 mg/mL to 5 mg/mL, the thermal transitions are very similar in terms of the cooperativity of the transition and LCST. A small shift from 36.8°C to 37.3°C occurs when the concentration is decreased from 5 mg/mL to 1 mg/mL. From 1 mg/mL to 0.5 mg/mL there is no change in the value of the thermal transition, but it does become broader or less cooperative. At 1 mg/mL, it takes 1.5 °C for the transmittance to go from 50% to 2%. At 0.5 mg/mL, it takes 5.5°C. As the concentration decreases to 0.1 mg/mL, the thermal transition becomes very broad. A possible reason that the broadness of the thermal transition increases as the concentration decreases might be that with lower concentrations, it takes longer for particles to aggregate, and for the aggregates to become large enough to precipitate out of solution.

Table 2.2. Change in LCST with HBENP concentration

Concentration (mg/mL)	LCST (°C)[a]
0.1	39.3
0.5	37.3
1.0	37.3
5.0	36.8
10.0	36.8
50.0	36.5

[a] LCSTs were determined from the data shown in Figure 2.18.

2.5.2 The Effect of NaCl Concentration on the LCST of HBENPs

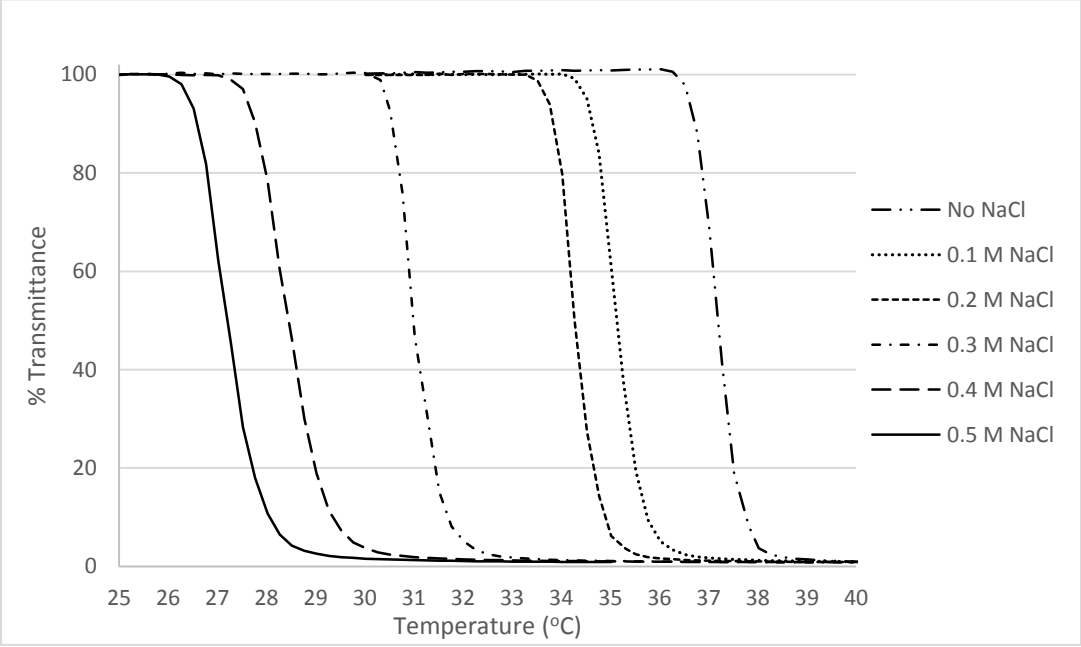


Figure 2.19 The effect of NaCl on the thermal transitions of aqueous dispersions (1 mg/mL) of a HBENP (MS = 1.68).

As discussed in Chapter 1, the presence of added salts often affects the LCSTs of TRPs. The effect of sodium chloride, a cosmotropic salt, on the LCST of a HBENP is shown in Figure 2.19. As expected, the LCST decreases as the concentration of NaCl increases. Explanations for this behaviour (the decrease in LCST with increasing concentration of cosmotropic salts) were presented in detail in Chapter 1. In the concentration range studied (0-0.5 M NaCl), there is a linear relationship between the concentration of sodium chloride and the value of the thermal transition (Figure 2.20).

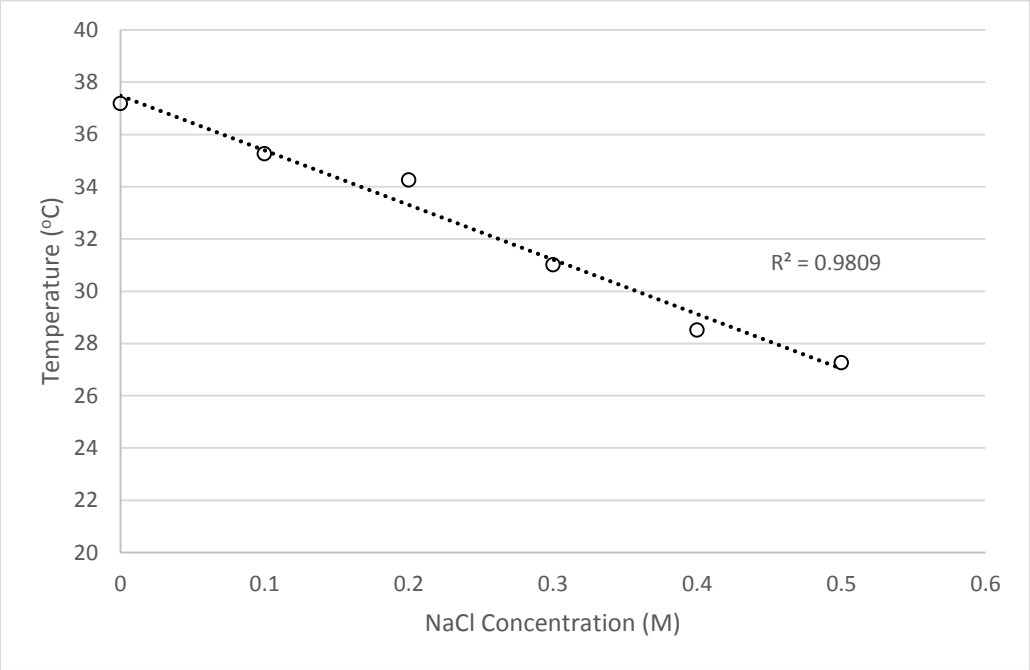


Figure 2.20 The effect of NaCl concentration on the LCST of a HBENP (MS 1.68, 1 mg/mL).

2.5.3 Effect of alcohols on the LCST of HBENPs

As mentioned previously, for applications such as column chromatography, sensor technology, and oil extraction, the thermosensitive HBENPs might be exposed to solvent mixtures. The presence of added solvents, such as alcohols, often effects the LCSTs of TRPs (discussed in Chapter 1). In the present study, the effect of added alcohols on the LCSTs of HBENPs was assessed. The alcohols chosen to study the phenomenon of co-nonsolvency in aqueous dispersions of an HBENP were methanol, ethanol, propanol, and butanol. These differ from each other only in terms of the size of the alkyl group.

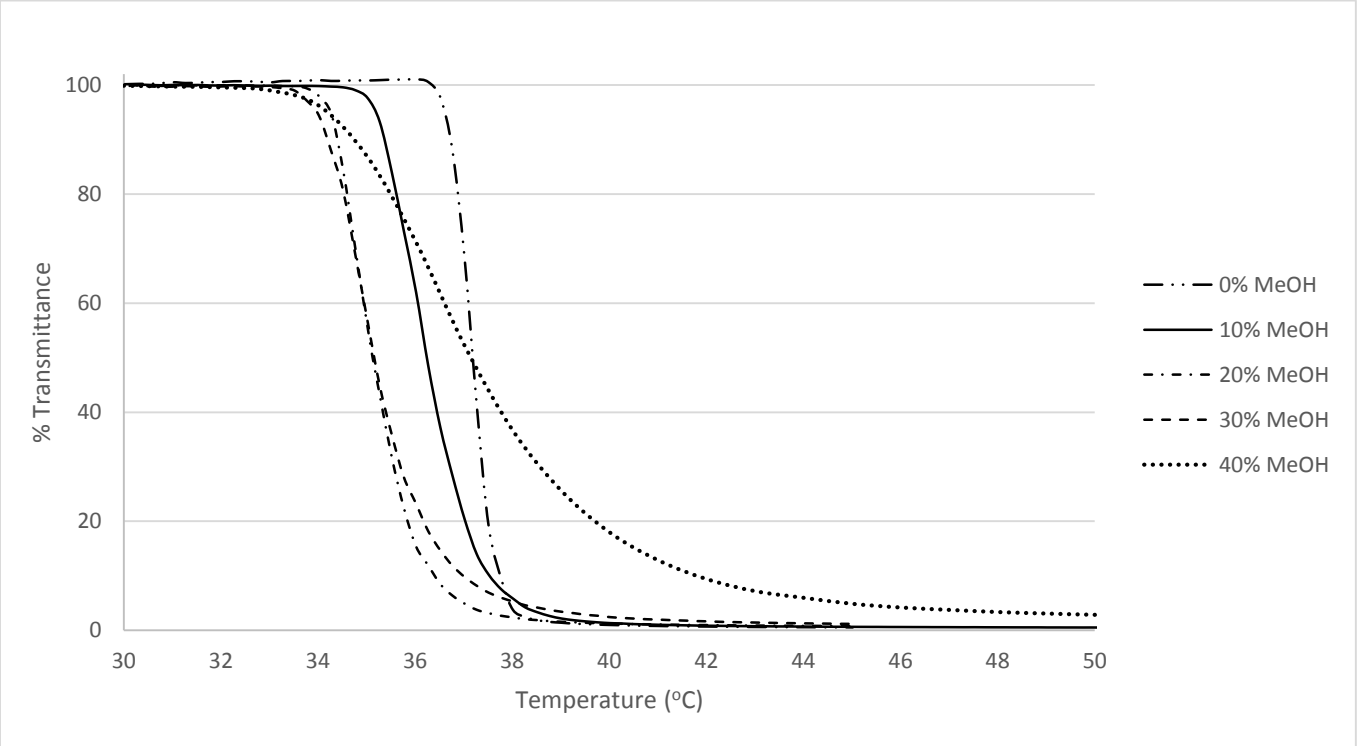


Figure 2.21 The effect of MeOH on the thermal transitions of aqueous dispersions (1 mg/mL) of a HBENP (MS = 1.68).

The effect of MeOH on the LCST of a HBENP is shown in Figure 2.21. This HBENP was readily dispersible in 100% MeOH at 1 mg/mL. The addition of up to 30 % MeOH resulted in a decrease in LCST from 37.3 ^{o}C (no methanol) to 35.3 ^{o}C (20 and 30% methanol). The small decrease in LCST suggests that the effect of methanol on hydrogen bonding between water and the polymer is small. Between 20 and 30% the LCST does not change, but the transition is slightly broader at 30% methanol. At 40% MeOH, the transition is even broader than at the lower MeOH concentrations and the LCST has increased compared to when no MeOH is present. At 50% methanol, the thermal transition disappeared entirely. At high methanol concentrations, all of the water molecules present are involved in MeOH-water complexes and no "free" H_2O is available to bind to the polymer. However, some methanol will remain free in solution which binds to and solubilizes the HBENP at any temperature.

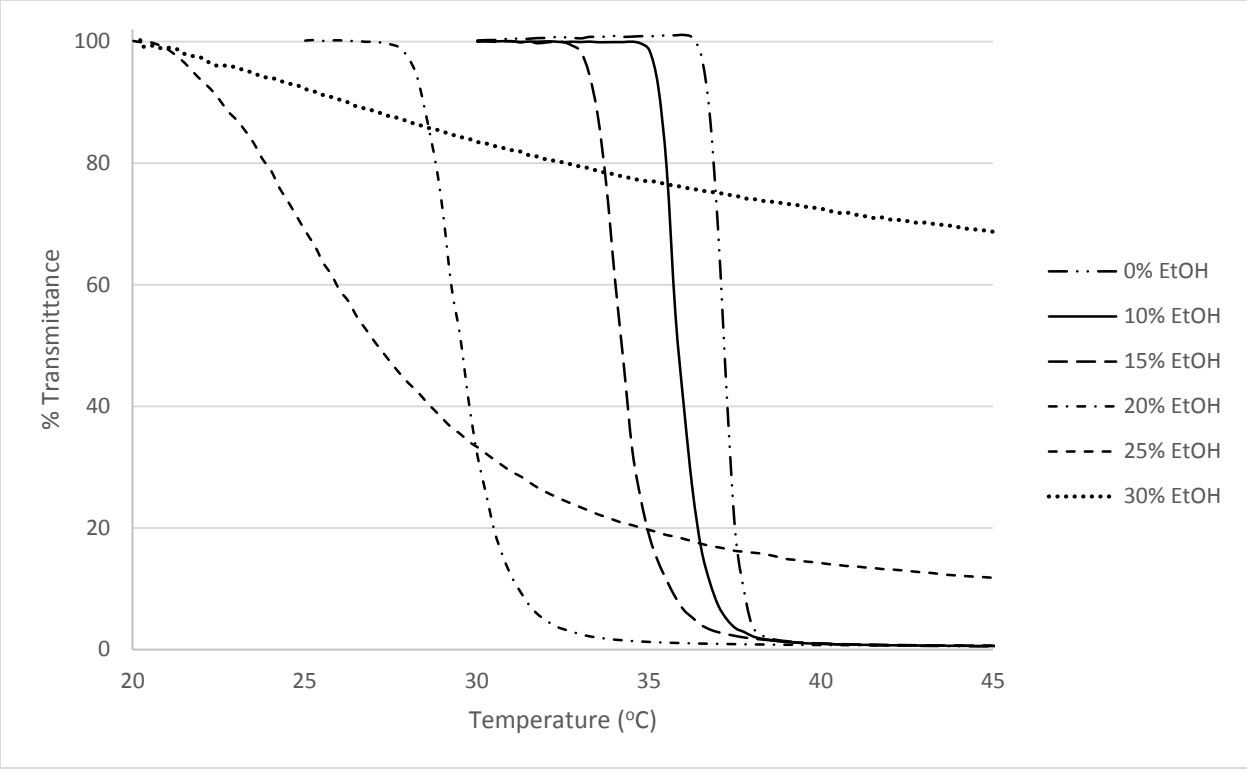


Figure 2.22 The effect of EtOH on the thermal transitions of aqueous dispersions (1 mg/mL) of a HBENP (MS = 1.68).

The effect of EtOH on the LCST of a HBENP is shown in Figure 2.22. This HBENP was readily dispersible in 100% EtOH at 1 mg/mL. The LCST decreases up to 25 % EtOH. In comparison to the effect of added MeOH, the decrease in LCST with EtOH occurs at a lower concentrations than MeOH and the magnitude of the decrease in LCST is greater from 37.3 °C (with no added solvent), to 25.0 °C (at 25% ethanol). Up to 20% ethanol, the cooperativity (sharpness) of the transition is not affected. With an additional 5% ethanol (25 % EtOH), the LCST continues to decrease. The cooperativity of the thermal transition decreases. At 30 % EtOH, the HBENP exhibits little the thermal responsive behaviour in that there is only a 26 % decrease in transmittance between 20-45 °C. This might mean that, at 25 and 30 % EtOH, some sections of the HBENPs are solvated by

ethanol. As chain fragments solvated mostly by water dehydrate, fragments that contain ethanol will not collapse to the same extent. The fact that the LCST decreases further at 25% ethanol might indicate that ethanol is disrupting solvation shells around hydrophobic groups in the polymer, inducing dehydration of the polymer at a lower temperature.

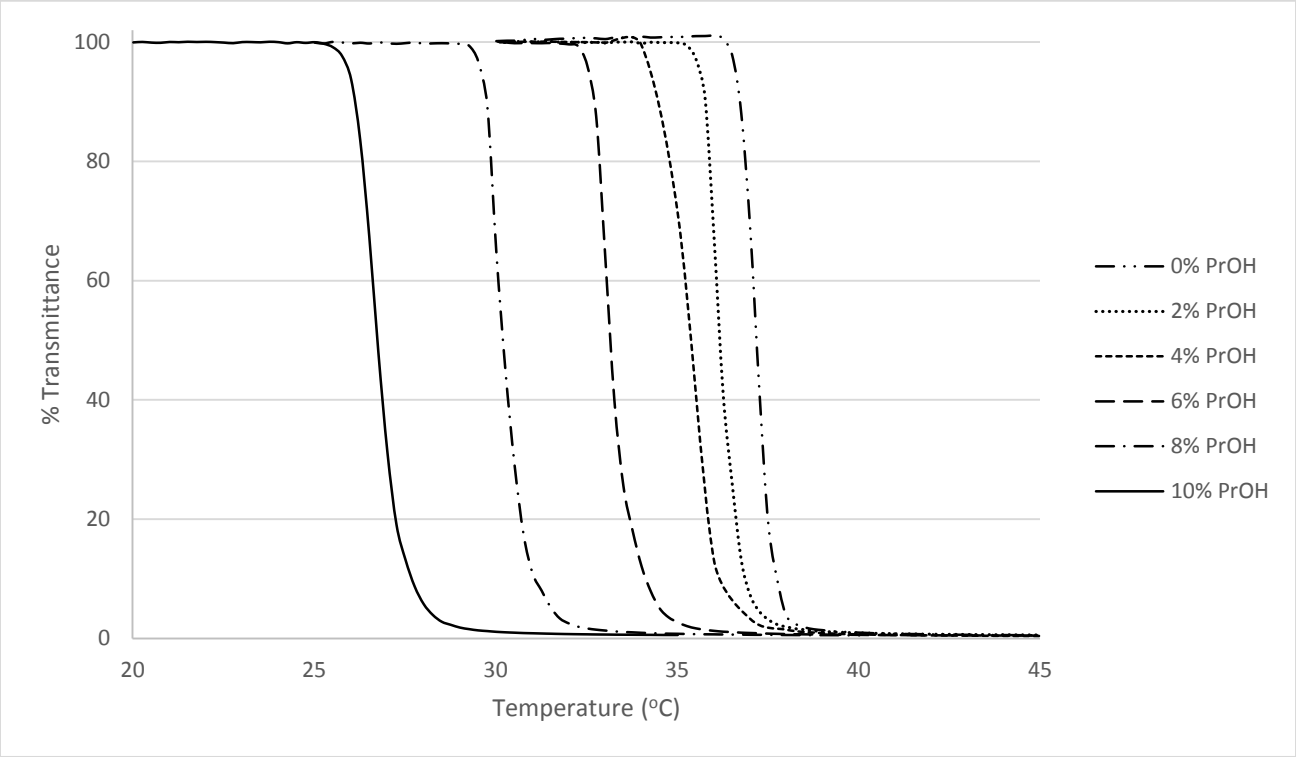


Figure 2.23 The effect of n-PrOH on the thermal transitions of aqueous dispersions (1 mg/mL) of a HBENP (MS = 1.68).

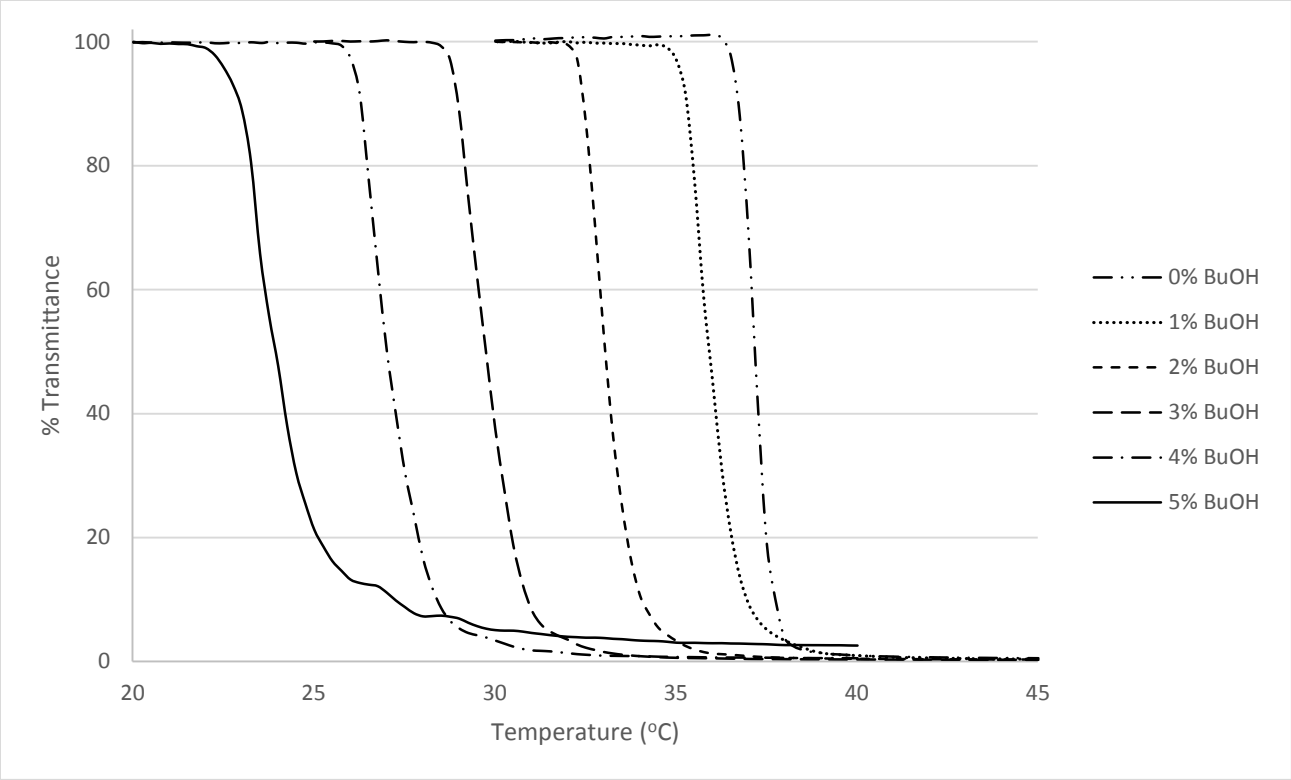


Figure 2.24 The effect of n-BuOH on the thermal transitions of aqueous dispersions (1 mg/mL) of a HBENP (MS = 1.68).

The effect of n-PrOH and n-BuOH on the LCST of a HBENP is shown in Figures 2.23 and 2.24. These HBENPs were not dispersible in pure n-PrOH or n-BuOH at 1 mg/mL. We were unable to examine n-PrOH concentrations greater than 10% and n-BuOH concentrations greater than 5 % because of difficulties in dispersing the HBENP at rt. The LCST decreases as the amount of n-PrOH or n-BuOH increases.

As the hydrophobicity of the alcohol increased (from MeOH to n-BuOH) it took less alcohol to cause a significant decrease in LCST. Explanations for this behaviour (the decrease in LCST with increasing hydrophobicity of the alcohol) were presented in detail in Chapter 1.

A feature of the thermal transition of the 5% n-BuOH data is the unevenness of the transition at the lower temperatures. This might be due to a delay between collapse of the polymer chain and aggregation. The initial sharp decrease in transmittance might be a result of a coil-to-globule transition. The dispersion of collapsed HBENPs is briefly stable. The collapsed ENPs aggregate, some fraction of them precipitate out of solution, and the dispersion is again briefly stable as indicated by a "levelling off" of the transmittance. It is known that the more hydrophobic an alcohol, the greater its effect on water structure. It is possible that butanol changed the hydrogen bonding structure of water in a way that hindered the process of aggregation. It might be the case that the collapsed SNPs might move more slowly where the hydrogen bonding network is more rigidly ordered, as it would be around molecules of butanol.

2.6 Hydroxyhexylation and Hydroxypentylation

While we were able to obtain thermoresponsive ENPs through hydroxybutylation, the MS required for thermoresponsive behaviour was quite high (MS > 1.00). We hoped that by modifying the ENPs with more hydrophobic epoxyalkanes, such as pentene oxide and hexene oxide, we might be able to obtain thermoresponsive ENPs with MS values lower than 1. Our preliminary results of these studies are presented below.

To our knowledge there are no reports of hydroxypentylation of starch outside the patent literature. Previous reports describing synthesis of hydroxyhexyl starch reported highest reaction efficiencies at high temperatures and high pH.[58,59] Funke and Lindenauer reported reaction efficiencies for hydroxyhexylation of starch granules (pH 13.8) of 23% at 120 ^{o}C, 60% at 140 ^{o}C,

and 43% at 160 °C.[58] Values of pH lower than 13.8 was not considered and an increase in pH decreased the reaction efficiency for hydroxyhexylation. It had been previously determined that the ENPs would not be able to withstand these conditions, so the ENPs were subjected to 4 equiv of pentene or hexene oxide at 40 °C in 0.1 M NaOH for 24 h. The reactions were worked up in the same manner as the reactions conducted with PO and BO. The ^{1}H-NMR spectra of the hydroxypentylated ENPs and hydroxyhexylated ENPs are shown in Figure 2.25 and Figure 2.26. Hydroxyalkylation occurred on O-2 and O-3 and, most likely, also occurred on O-6. Under these conditions the MS for the pentene oxide reaction was determined by ^{1}H-NMR to be 0.54 and the MS for the hexene oxide reaction was determined by ^{1}H-NMR to be 0.14

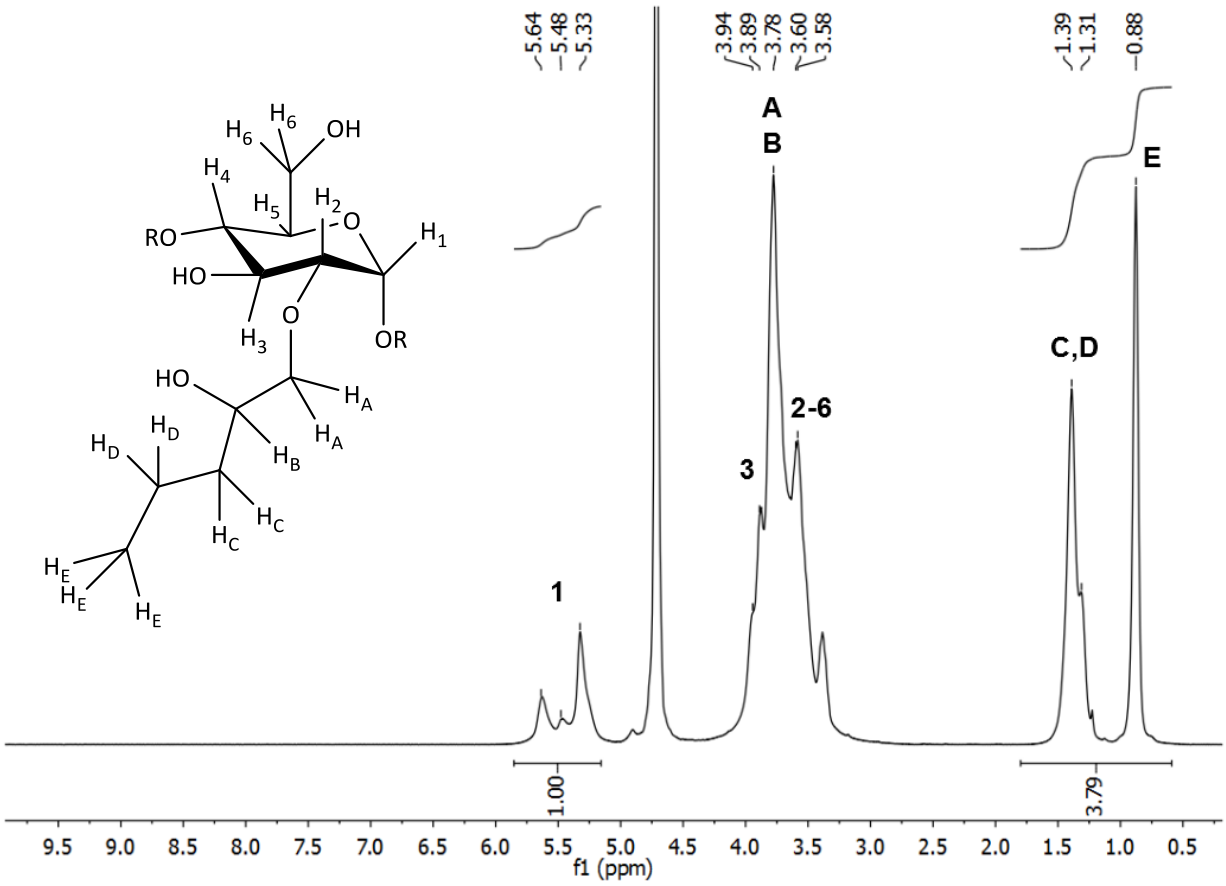


Figure 2.25 ^{1}H-NMR spectra (500 MHz) of ENPs (100 mgs/mL, D_2O) subjected to 4 molar equiv hexene oxide in 0.1 M aq. NaOH at 40 °C for 24 h (MS = 0.54).

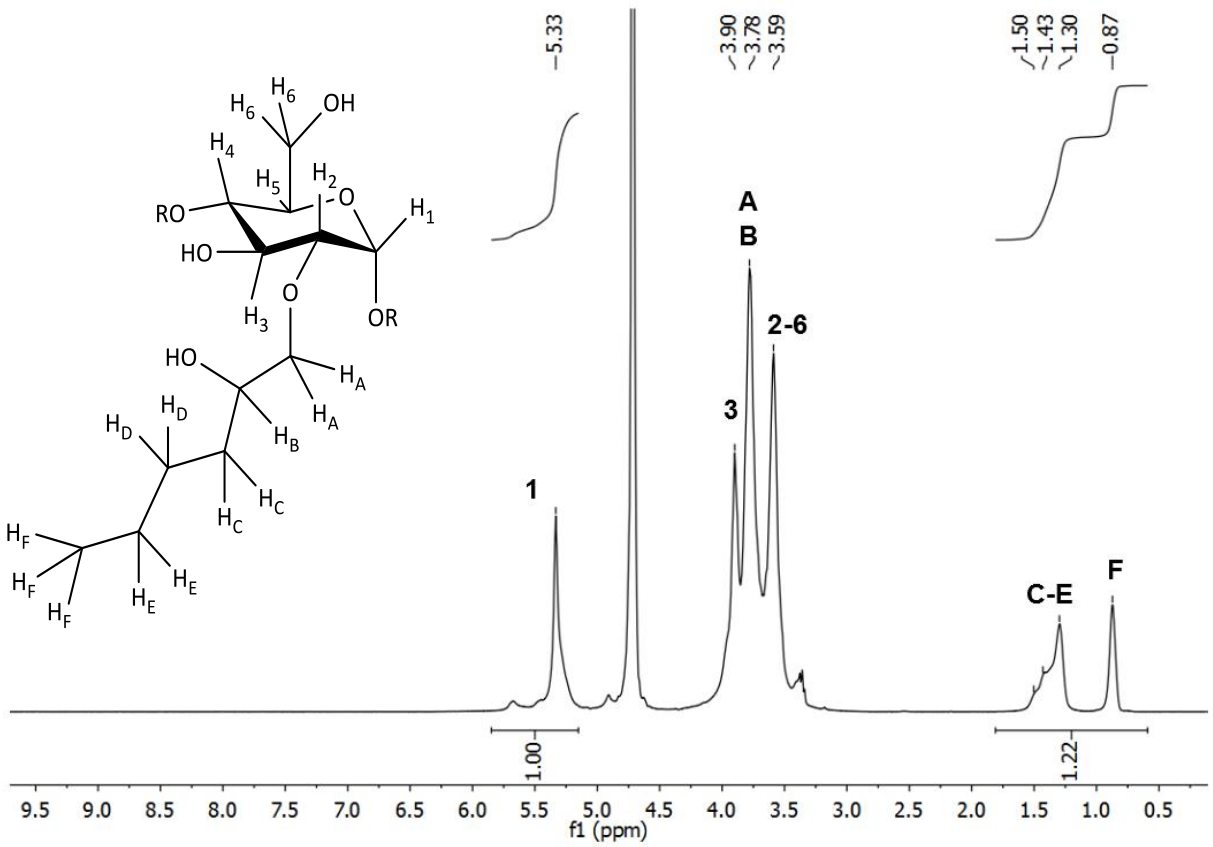


Figure 2.26 ^{1}H-NMR spectra (500 MHz) of ENPs (100 mgs/mL, D_2O) after being subjected to 4 molar equiv pentene oxide in 0.1 M aq. NaOH at 40 ^{o}C for 24 h. (MS = 0.14).

Only the hydroxypentylated ENPs were thermoresponsive (Figure 2.26). The critical temperature was 52.8 ^{o}C for this sample. This demonstrates that, by using pentene oxide, it is possible to obtain a thermoresponsive ENP with an MS of well under 1 (0.54 in this case). In comparison, an HBENP with an MS of 1.12 (Figure 2.15) had a thermal transition of 57.5 ^{o}C.

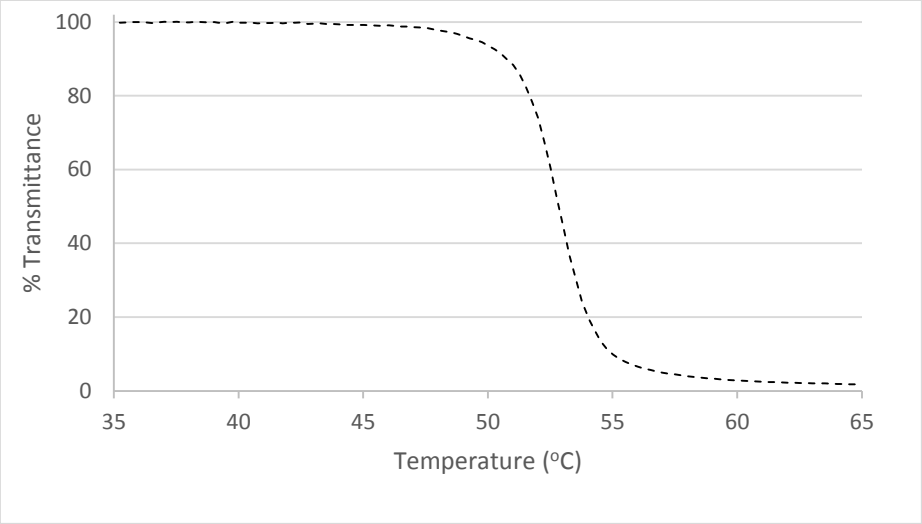


Figure 2.27. Thermal transition of a hydroxypentylated ENP (prepared at 40 °C in 0.1 M NaOH for 24 h) in water (1 mg/mL).

2.7 Summary and Future work

Dynamic light scattering (DLS) could be used to characterise the thermal transition of the TRENPs described here and future TRENPs. During DLS measurements, the sample is irradiated with monochromatic light and the intensity of light scattered by the sample is measured over a short time (less than 1 ms). Analysis of the change in intensity of scattered light over time reveals information about the sample, such as the distribution of hydrodynamic radii (R_h, the radius of a polymer particle dispersed in a solvent in which it is miscible) of the particles in the sample. When this technique is used to study the thermal transition of a TRP with a LCST, the R_h is first measured below the LCST, where little aggregation is expected. As the temperature increases above the LCST, aggregates are observed as indicated by a dramatic increase in the particle size. When Ju

et al.[46] measured the Z-average particle size[1] of a thermoresponsive 2-hydroxy-3-butoxypropyl starch (MS 0.32, LCST 32.5 ^{o}C) was 35 nm. Measurements taken at 40 ^{o}C detected aggregates as indicated by the increase in Z-average particle size to 856 nm.

Although TRENPs were obtained via hydroxybutylation of ENPs, several issues have to be dealt with before the TRENPs can be considered for most practical applications (especially high volume applications). The MS of the HBENPs had to be greater than 1 in order for them to exhibit thermosensitivity in pure water. Moreover, the reaction efficiencies of the hydroxybutylation reactions were not high. In order for TRENPs to have practical value then it will be necessary, for reasons of cost, to obtain TRENPs with a lower MS and, the modification reaction will have to proceed with greater efficiency. Although hydroxypentylated ENPs could be produced with an MS less than 1, the reaction efficiency was also poor with pentene oxide. Moreover, the cost of pentene oxide (10 mL = $173.00) is very high thus making this an impractical reagent for making TRENPs.

One possible way to improve the reaction efficiency might be through the use of a phase transfer agent. These reagents interact with molecules in a biphasic mixture and transport them to a phase where they are otherwise poorly soluble.[72] There have been reports of starch hydroxyalkylation and other etherifications with more than three carbons using quaternary ammonium salts as phase transfer agents.[55] Such quaternary ammonium salts might be effective as phase transfer catalysts for the hydroxyalkylation of ENPs. The ammonium salt would initially be present in the aqueous phase. Molecules of the ammonium catalyst could coordinate with

[1] The Z-average particle size is the particle size obtained through standard DLS analysis. Other average sizes (i.e. intensity, volume, number) are derived from the Z-average. Explanation of how this is derived is outside the scope of this work.

transient alkoxides in the ENPs, neutralising their charges thus facilitating their entry into the organic (alkene oxide) phase. There, an alkoxide might be able to react with alkene oxide more quickly. Once the reaction is complete, the ammonium catalyst would return to the aqueous phase.

Thermoresponsive cellulose derivatives (i.e. EHEC, HPMC) with two types of modifying groups have been reported in the literature, as discussed in Chapter 1. Disubstituted ENPs could also be produced to see what the effect might be on the LCST (if these derivatives are thermoresponsive).

It is well known that the rate at which phase separation occurs is often much higher than the rate at which the polymer resolubilizes. This is known as hysteresis,[50] and it must be understood if the TRENPs are to be used for any application where the temperature cycles above and below the LCST (as it would in the oil extraction protocol, if the TRENPs are to be reused). While determining LCSTs, it was noticed that the level of hysteresis increases with any factor that contributes to the hydrophobic effect. Any condition that caused the LCST to decrease also increased amount of time required for a turbid dispersion to become clear again. This effect was especially pronounced for TRENP dispersions with added propanol or butanol. For a 4% butanol dispersion, at room temperature over an hour was required to redisperse the TENPs. When the temperature was reduced to 4 ^{o}C, the sample redispersed in under 10 min. This phenomenon was observed and noted, not measured, so a rigorous experimental treatment of this subject remains future work.

2.8 Experimental

2.8.1 General

EcoSphere™ starch nanoparticles (ENPs) were commercial grade and were provided by EcoSynthetix Inc. (Burlington, Ontario, Canada). All other reagents and solvents were commercially available and used without further purification. The number of moles of anydroglucose units per gram of purified ENPs was taken to be the same as native starch (6.17×10^{-3} mol/g or 162 g/mol [molar mass of an anhydroglucose unit]). Dialysis tubing (Spectra/Por 6®, regenerated cellulose, 1000 MWCO) was purchased from Spectrum Laboratories Inc. ^{1}H-NMR spectra were obtained on a Bruker Avance 500 MHz NMR spectrometer (Bruker Inc., Billercia, MA, USA). The NMR data were processed using MestreNova 9.1.0. Baseline correction algorithm used was the Whittaker Smoother. All NMR spectra were obtained in D_2O and referenced to the HDO peak at 4.68 ppm. The MS of the hydroxyalkylated particles was determined as shown below,

$$M.S. = \frac{I_{alkyl}/p_{alkyl}}{I_{anomeric}}$$

Here, I_{alkyl} is the integration value for the alkyl protons (0.80-1.40 ppm for HPENPs, 0.60-1.80 ppm for all others), p_{alkyl} is the number of alkyl protons, and $I_{anomeric}$ is the integration value for the anomeric region (5.15-5.85 ppm).

2.8.2 Purification of ENPs

ENPs were dispersed in water (0.25 g/mL) in an Erlenmeyer flask and heated gently for 1h until dispersed. The ENP dispersions were dialyzed against deionised water (ratio of sample to

water ~1:10) at room temperature for 24 h. The water was changed 3 times. The dialysed ENPs were frozen and lyophilised. The purified ENPs were analyzed by NMR to ensure complete removal of glycerol and glyoxal.

2.8.3 Alkaline Degradation of ENPs

For degradation studies performed at pH 12, ENPs (1 g) were dispersed in 2 mL of deionised water in a vial equipped with a stir bar and heated gently until fully dispersed. The pH of the dispersion was adjusted to pH 12 using aq. 1 M NaOH. For degradation studies performed at pH 13, the ENPs (1 g) were dispersed in 2 mL of 1 M NaOH. The pH of this dispersion was found to be 13. For degradation studies performed at pH 14, the ENPs (1 g) were dispersed in 2 mL of 1 M NaOH. The pH of the dispersion was adjusted to pH 14 using aq. 1 M NaOH. The reaction vials were capped and parafilmed and placed in a water bath that was pre-heated to the desired temperature. The mixtures were stirred for 24 h. After 24 h, the reaction vessel was removed from the water bath. The mixture was neutralized with dilute HCl then dialyzed against water at room temperature for 24 h. The water was changed 3 times. The dialysed ENPs were frozen and lyophilised. The ENPs were dispersed in D_2O and analyzed by ^{1}H-NMR.

2.8.4 Hydroxypropylation

All hydroxypropylation reactions were performed in pressure tubes. For degradation studies performed at pH 12, ENPs (1 g) were dispersed in 2 mL of deionised water with a stir bar and heated gently until fully dispersed. The pH of the dispersion was adjusted to pH 12 using aq. 1 M NaOH. For degradation studies performed at pH 13, the ENPs (1 g) were dispersed in 2 mL of 1 M NaOH. The pH of this dispersion was found to be 13. The tubes were cooled in an ice bath

and the appropriate amount of PO was added. The pressure tubes were capped and parafilmed, placed in a water bath that had been pre-heated to the desired temperature. The reaction mixture was stirred for 24 h. After 24 h, the tubes were removed from the water bath, the mixture was neutralized with dilute HCl and dialyzed against water at room temperature for 24 h. The water was changed 3 times. The dialysed ENPs were frozen and lyophilised. The HPENPs were dispersed in D_2O and analyzed by ^{1}H-NMR.

2.8.5 Hydroxybutylation, Hydroxypentylation, and Hydroxyhexylation of ENPs

The ENPs (1 g) were dispersed in 2 mL 1 M NaOH in a vial. The pH of this dispersion was found to be 13. The vial was equipped with a stir bar and the ENPs were gently heated until dispersed. The alkene oxide was added was added at rt and the vials were capped and parafilmed. The vials were placed in a water bath that had been pre-heated to 40 ^{o}C. The reaction mixture was stirred for 24 h. After 24 h, the vial was removed from the water bath and the mixture was neutralized with dilute HCl and dialyzed against water at room temperature for 24 h. The water was changed 3 times. The dialysed ENPs were frozen and lyophilized. The hydroxyalkylated ENPs were dispersed in D_2O and analyzed by ^{1}H-NMR.

2.8.6 Determination of LCSTs

The LCSTs of TRENPs were determined using a Cary 100 Bio UV-Vis spectrophotometer equipped with a Cary temperature controller. The transmittance of the TRENPs in aqueous solution was measured at 500 nm under heating at a rate of 1 °C/min. For LCST determinations in aq. NaCl, a 10% aqueous dispersion of HBENPs was diluted 10-fold into aq. NaCl solutions. For LCST determinations in the presence of alcohols, the samples were prepared in the following

manner. 0.5 mL of a 10% aqueous dispersion of HBENPs was added to water and a pre-determined amount of alcohol was added. For example, for the study with 10% ethanol, 0.5 mL of the HBENP dispersion was added to 4 mL water, followed by the addition of 0.5 mL EtOH. The final volume for all stock solutions was always 5 mL. Some of the alcohol-water HBENP dispersions became cloudy during the addition of the alcohol but became clear when cooled to 4 ^{o}C for 5-15 min. The amount of time required for the solutions to become clear increased as the % alcohol increased, the hydrophobicity of the alcohol increased. The LCSTs was defined as the temperature at the inflection point of the transmittance curve.[69-71]

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