Controlled synthesis of starch-based branched polymers

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Chapter 1

Modification of starch: The application of “green” solvents and controlled functionalization

Abstract

Starch is a polysaccharide widely present in nature. The application fields of starch have been extended to areas such as oil exploration, pharmaceutical, tissue engineering and so on. Such a wide range of applications implies the necessity for various novel properties with respect to those of native starch. This is often achieved via chemical modification routes and strategies. Many work has been done following the conventional ways (e.g. the use of organic solvents etc.) and reviews on the progress of these researches are available in recent years. On the other hand, during the last decades products with new or enhanced properties were prepared from starch because of the adoption of some “green” solvents (ionic liquids and supercritical CO$_2$) and several new techniques (regioselective derivatization, atom transfer radical polymerization etc.) that characterized by controlled modification. In this article, the application of ionic liquids and supercritical CO$_2$ in the modification and processing of starch is summarized. The development of regioselective derivatization and controlled grafting of starch are also introduced in the second part of this article.

This chapter is based on Yifei Fan, and Francesco Picchioni, Modification of starch: the application of green solvents and controlled functionalization (2018). To be submitted
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1.1. Introduction

Starch, as the most abundant natural occurrence of polysaccharides that is just second to cellulose, widely exists in the roots, seeds and leaves of various plants and also some algae. Typically, starch granules are composed of two main fractions: amylose, a kind of linear polymer of glucose unit with $\alpha-(1\rightarrow4)$ linkage and amylopectin, which is highly branched with lots of short chains that linked through $\alpha-(1\rightarrow6)$ linkage to the linear parts of the macromolecule (see Scheme 1-1). The difference in structure renders these two components differ significantly with each other in both physical and chemical properties. For example, amylopectin is easier to dissolve in hot water than amylose. Another significant difference is that only amylose could complex with iodine and make their solution appear blue-black color.

Scheme 1-1. Illustration of the structure of amylose and amylopectin

People have made use of starches from various botanical resources for thousands of years and its application in modern industrial products has also been developed for many years. This is often related to its characteristic properties such as biodegradability, renewability and cheapness. For instance, varieties of derivatives have been made from starch to oil drilling additives, for coatings/films, adhesives, tissue engineering and drug carriers, biofuels and so on.
Because of the technology developments, the application field of starch is broadened year by year and different techniques have been developed for the modification of starch to overcome its shortcomings. These include poor processability and solubility in common organic solvents, retrogradation and syneresis, low shear stress resistance and thermal decomposition. Generally, there are four categories of methods for the modification of starch: physical, chemical, enzymatic and genetic engineering respectively. While the main purpose of genetic engineering is to modify the ratio of amylopectin to amylose and also their structures for specific applications, physical modifications are employed to improve the granule size and solubility of starch in water. The applications of physical and biotechnological methods in the modification of starches have been kindly reviewed by Ashogbon et al. and Halley et al. respectively.

Compared with physical and genetic engineering modification, chemical methods provide more option for the functionalization of starch and therefore broaden the application field significantly, especially with the help of new emerging chemical techniques. As is known, there are three hydroxyl groups adjacent to the carbon atoms at 2, 3 and 6 positions in each anhydroglucose unit (AGU), which enable the modification of the starch could be easily achieved through chemical reaction with various functional groups. Traditional chemical modifications, including esterification and etherification, cationization, oxidation as well as crosslinking, are usually realized through the reaction of starch with small molecules. These derivatives are widely used in the food industry and as additives for medicine, cosmetics, coatings, oil exploiting fluids and so on. The synthesis and applications of these kinds of derivatives have been reviewed in many papers published in recent years. In the present work, we mainly focus on the new emerging solvents for the chemical modification of starch and the progress of controlled modification of starch.

1.2. “Green” solvents for starch modification

Because of the intra-/inter- hydrogen bonds between the hydroxyl groups along the backbone of the molecules, starch has a poor solubility in water and most common organic solvents. The modification of starch in aqueous solutions is actually carried out in a heterogeneous way, which, together with many other side reactions, limits the degree of substitution (DS) and the further application of these derivatives. In order to get a higher DS, starch is usually dissolved and modified in some organic solvents with the help of some catalysts. Solvents and catalysts such as dimethyl sulfoxide (DMSO) and pyridine, however, are usually flammable, toxic and therefore hazardous towards both human and environment.
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With more and more attention being paid to health, safety and environment (HSE) protection, chemist are trying to find new solvents to displace traditional organic solvents for dissolving natural macromolecules and subsequent chemical reactions. In the past decades, more and more attention has been mainly paid to two kinds of “green” solvents, which are ionic liquids and supercritical carbon dioxide respectively, for the dissolution and modification of natural polysaccharides such as starch, cellulose and chitin. The applications of these two solvents for starch are summarized below.

1.2.1. Ionic liquids

Ionic liquids (ILs) are usually composed of a combination of organic cations and smaller organic or inorganic anions. With properties such as negligible volatility, non-flammability, thermal and chemical stability, tunable polarity and so on, ILs attracted the attention of scientists who work on polymer science. Many reports on the successful application of various ionic liquids for different polymerization techniques, such as free radical and ionic polymerizations as well as controlled polymerizations, have been made and reviews on this fields are also available in recent years. In this present work, the applications as well as limitations of ionic liquids as solvent and/or catalysts in the modification of starch will be reviewed briefly.

Various derivatization of starches with ILs as solvents have been studied in recent years. For example, cationic corn starch was successfully synthesized by Wang and Xie et al. with 1-butyl-3-methyl-imidazolium chloride ([BMIM]Cl) as solvent. Glycidyltrimethylammonium chloride (GTAC) was used in their reaction for the derivatization of starch and a maximum DS of 0.99 was obtained. [BMIM]Cl was also used as solvent for the esterification and carboxymethylation of starches. In the experiment carried out by Xie et al., corn starch was esterified with succinic anhydride and acetic anhydride with pyridine as catalyst in [BMIM]Cl. Succinylated starch with DS values that varied from 0.03 to 0.93 and acetylated starch with DS values ranged from 0.37 to 2.35 were successfully synthesized respectively. In another report, the carboxymethylation of corn starch in [BMIM]Cl was investigated and a maximum DS of 0.76 was obtained. Desalegn et al. also reported the synthesis of cassava starch epoxy fatty acid esters (starch vernolates) in which [BMIM]Cl was used as solvent and pyridine as catalyst. A derivative with a DS that up to 1.03 was obtained within 24 h in their experiment. All these modifications were carried out in homogeneous conditions, thus had a good control over the DS and the distribution of functional groups. Despite all these successes, however, systematic comparisons on the effectiveness and efficiency of starch derivatization in ILs and other organic solvents (DMSO,
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N,N-dimethylacetamide (DMAc)/LiCl) are still not available. In addition to the research mentioned above, the grafting of corn starch with L-Lactide by ring-opening graft polymerization (ROP) was also studied. In the report by Xu and Wang et al., 1-allyl-3-methylimidazolium chloride ([AMIM]Cl) was used as reaction medium for the grafting of starch and the grafting efficiency of poly(L-lactide) (PLLA) was calculated to reach 30% according to the standard curve based on FT-IR method. Recently, Wang et al. also reported the synthesis of starch-based macroinitiator in [AMIM]Cl for the ATRP grafting of polystyrene (PS) and poly(methyl methacrylate) (PMMA). Corn starch was esterified with 2-bromoisobutyryl bromide in [AMIM]Cl at room temperature without using any additional catalysts. Compared with reactions carried out under heterogeneous conditions, the graft density and ratio were significantly improved with [AMIM]Cl as solvent. Besides the application as solvent, several ILs, mainly 1-N-alkyl-3-methylimidazolium chlorides could also act as catalysts for the esterification of high-amylose maize starch. In the research of Lehmann and Volkert, different carboxylic anhydrides were used as both reactants and solvents for the esterification of maize starch, while the amount of ILs was just kept to a low value. Imidazolium-based ILs with halogenides as counter ions were proved to be good catalysts for this reaction. They also found that the degradation of maize starch could be suppressed by the addition of pyridine or 1-methylimidazole as base and the average molar mass of starch acetate could be adjusted through varying the amount of pyridine added.

![Figure 1-1. ILs studied for the dissolution and depolymerization of barley starch](image)
To successfully achieve the modification of starch in ILs, it is of great importance to get a deep knowledge of the interactions between starch molecules and ILs. Lappalainen et al. studied the dissolution and depolymerization of barley starch in ten ILs (Figure 1-1) with p-TsOH as catalyst and microwave as heating method. It was found that barley starch could be depolymerized into 1000 - 2000 Da-sized, water-soluble oligomers by dialkylimidazolium halides that analyzed. Both cation and anion of the ILs were found to have significant influences on this process, to which a smaller cation or a more nucleophilic anion was proved to be more beneficial. This was related to the steric hindrance caused by the dialkylimidazolium cation and the ability of anion to break intra- and intermolecular hydrogen bonds of starch. In their research, [NH$_3$CH$_2$CH$_2$OH][HCOO] and [EMIM][Me$_2$PO$_4$] were proved to be the most suitable solvent among those ten ILs for dissolving starch with a notable decreasing in depolymerization. Besides the influence of composition, the presence of water was also revealed to play a special role in dissolving starches. Liu and Budtova found that the dissolution of waxy corn starch in 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) could be accelerated with a proper amount of water (≤ 50 wt.%) and no gelatinization was observed in this process. Moreover, a decrease of about 20 °C in the beginning and final temperature of dissolution was noticed. A possible explanation for this is that the presence of water could induce the swell of starch granules which is favorable to the diffusion of [EMIM][OAc]. Mateyawa and Xie et al. also studied the influence of water on the phase transition of maize starch (waxy and regular) in [EMIM][OAc]. They concluded that a higher water content (> 25%, mol ratio) could strengthen the interactions between [EMIM][OAc] and water which reduced the available [EMIM][OAc] for starch molecules, and thus made gelatinization dominates the phase transition process of starch. With the decreasing of water content, more [EMIM][OAc] became available and resulted in a conversion from gelatinization to dissolution for the phase transition process. However, a proper ratio (7.2 mol/mol) of water to [EMIM][OAc] is required for the effective disruption of starch granules at a relatively lower temperature, which is in accordance with the results from other reports.

In addition to its use as solvents and catalysts for the chemical modification of starch, the applications of ILs as plasticizer for the processing of starches have also been investigated. Sankri et al. verified the feasibility of plasticizing maize starch with [BMIM]Cl and a kind of electrically conductive thermoplastic starch (TPS) was obtained. Compared with samples plasticized by glycerol, TPS modified by [BMIM]Cl showed a lower water uptake and higher elongation at break in its rubbery state. The results from a control experiment in their research showed that the structure of ILs and processing conditions probably have significant influences.
on the properties of the final product, but these still await deep studies. [BMIM]Cl was also used as plasticizer for the processing of starch, zein and their blends with glycerol plasticized samples as control groups. According to the report by Leroy et al., [BMIM]Cl is a better plasticizer for maize starch than glycerol but has no obvious effects on the plasticization of zein 35. However, in the blends of starch and zein, [BMIM]Cl was proved to be a good compatibilizer for the two components. This makes it possible for the production of biocomposites from raw natural polymers and have a potential application in food packaging and other areas.

To be successfully applied for the production of biocompatible materials, there are still some problems to be overcome for ILs. For example, ILs are challenged in terms of “green” because of their poor performance in biodegradability, biocompatibility, and sustainability 36. In recent years, a new generation of environment-friendly ILs was introduced as deep eutectic solvents (DES) 37. Leroy et al. reported the plasticization of corn starch/zein blends with two DES formed by urea-choline chloride and glycerol-choline chloride 38. Their research showed that DES based on choline chloride could be used as an efficient functional plasticizer for TPS. Besides plasticization, DES was also proved to be able to reduce the water sensitivity of TPS and act as a good compatibilizer for the blend of corn starch and zein. Natural deep eutectic solvents (NDES) were also developed in recent years with all the components from natural products, and thus should be more “green” compared with other solvents 36,39. However, so far as we know, the application of NDES in the processing of starch has not been reported.

1.2.2. Supercritical CO₂

Supercritical fluids are defined as substances that are held at or above their critical point with a certain temperature and pressure, which makes the distinct interface between liquid and gas disappear. This kind of fluid possesses both the properties of gas and liquid-like low viscosity, high diffusion coefficient (Table 1-1) and good permeability and dissolving capacity. All the properties make the mass transfer process easier for chemical reaction or abstraction, which usually means a higher reaction rate or a faster abstraction than that in traditional solvents. Furthermore, there is a temperature and pressure zone near the critical point within which a slight change in temperature and (or) pressure will result in a significant change in density and many other properties, which enables the fluids to be tunable for different solutes. For all the reasons mentioned above, the applications of supercritical fluids have become a hot point for scientific research in recent decades. Among all the supercritical fluids that have been reported, water and
supercritical CO\textsubscript{2} (scCO\textsubscript{2}) are the most commonly used and commercially available fluids.

**Table 1-1. Typical values of physical properties of gases, supercritical fluids and liquids**  \(^{40}\)

<table>
<thead>
<tr>
<th>Properties</th>
<th>Gas</th>
<th>Supercritical fluid</th>
<th>liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\rho) (kg/m(^3))</td>
<td>1</td>
<td>100 - 800</td>
<td>1000</td>
</tr>
<tr>
<td>(\eta) (Pa·s)</td>
<td>(1\times10^{-5})</td>
<td>(1\times10^{-5} - 1\times10^{-4})</td>
<td>0.001 - 0.1</td>
</tr>
<tr>
<td>(D) (m(^2)s(^{-1}))</td>
<td>(1\times10^{-5})</td>
<td>(1\times10^{-7})</td>
<td>(1\times10^{-9})</td>
</tr>
</tbody>
</table>

* \(\rho\), \(\eta\) and \(D\) stands for density, viscosity and diffusivity, respectively.

By comparing the data in Table 1-2 and given the reasons such as safety (non-toxic and non-flammable), it is clear that the use of scCO\textsubscript{2} as solvent has many advantages over other supercritical fluids. For instance, its supercritical conditions are relatively easy to achieve and the mild processing conditions, together with the chemical inertness of CO\textsubscript{2} makes scCO\textsubscript{2} an ideal solvent for many compounds that are easy to be damaged \(^{41}\). Besides, the separation of scCO\textsubscript{2} by simply depressurization enables it to be more convenient and thus more economy than using other solvents. To the best of our knowledge, as a kind of green solvents, scCO\textsubscript{2} has been successfully used in many areas such as the extraction of caffeine from coffee beans and capsaicin from peppers, for dry cleaning and paint spraying \(^{42-45}\). In this part of the introduction, the application of scCO\textsubscript{2} as solvent for polymer synthesis and processing was summarized first with the hope that new ideas could be enlightened for the modification of starch.

**Table 1-2. Critical properties of various solvents**  \(^{46}\)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Molecular weight (g/mol)</th>
<th>Critical temperature (K)</th>
<th>Critical pressure (MPa atm)</th>
<th>Critical density (g/cm(^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon dioxide</td>
<td>44.010</td>
<td>304.12</td>
<td>7.37 (72.74)</td>
<td>0.468</td>
</tr>
<tr>
<td>Water</td>
<td>18.015</td>
<td>647.14</td>
<td>22.06 (217.72)</td>
<td>0.322</td>
</tr>
<tr>
<td>Methane</td>
<td>16.043</td>
<td>190.56</td>
<td>4.60 (45.40)</td>
<td>0.163</td>
</tr>
<tr>
<td>Ethane</td>
<td>30.070</td>
<td>305.32</td>
<td>4.87 (48.06)</td>
<td>0.207</td>
</tr>
<tr>
<td>Methanol</td>
<td>32.042</td>
<td>512.64</td>
<td>8.10 (79.94)</td>
<td>0.272</td>
</tr>
<tr>
<td>Ethanol</td>
<td>46.069</td>
<td>513.92</td>
<td>6.15 (60.70)</td>
<td>0.276</td>
</tr>
<tr>
<td>Acetone</td>
<td>58.080</td>
<td>508.10</td>
<td>4.70 (46.38)</td>
<td>0.278</td>
</tr>
</tbody>
</table>
1.2.2.1. The application of scCO$_2$ in the modification of starches

1.2.2.1.1. Physical modification of starch with scCO$_2$

Normally, starches exist in nature in the form of granules with various diameters, depending on their botanical sources. Polarizing microscope observations and X-ray diffraction experiments have proved the existence of crystalline structures in starch granules. The crystalline zones have a significant influence on the processing of starches because of their obstruction to the accessibility of amorphous zones. Thus, in order to get a deep knowledge about the structural change of starch macromolecules after modification and also final products with better properties, there have been many reports on the study of the gelatinization of starches which related to the disruption and breakdown of crystalline structures in granules. Most of these experiments were carried out at various temperatures with water as medium and sometimes high pressure was applied. With a diffusivity that is two orders of magnitude larger than that of liquid solvents at relatively mild conditions as indicated in Table 1-1 and Table 1-2, scCO$_2$ is believed to be a more effective solvent for the gelatinization of starches. To the best of our knowledge, Francisco and Sivik first reported the gelatinization of cassava, potato and wheat starch (72% wt./wt. water content) with the assistance of CO$_2$ under different temperatures (50 - 70 °C) and pressures (up to 30 MPa). An increase in the degree of gelatinization (DG) was found when the pressure was kept below 8 MPa, higher than which decreases in DG were noticed. They concluded that the DG was positively related to the plasticizing effect of scCO$_2$, but negatively related to pressure. Muljana and Picchioni et al. also studied the effect of scCO$_2$ on the gelatinization of potato starch with various water content (16.2 - 40% wt./wt.) In their experiments, various temperatures (50 - 90 °C) and pressures (up to 25 MPa) were applied and a maximum DG of about 14% (quantified by DSC) was observed at the pressure of 25 MPa and temperature of 90 °C. Contrary to the conclusion made by Francisco and Sivik with higher water content starch, the DG of lower water content starch was found to increase with increasing temperature and pressure. Except for these two papers, the influence of scCO$_2$ on the gelatinization temperature of different starch blends was also studied. In the research of Zaidul and Noda et al., different starches such as potato starch and cassava starch as well as their blends with wheat starch (70% wt./wt. water content) at a series of ratios were gelatinized by scCO$_2$ treatment and decreases of 10 °C to 18 °C in the gelatinization temperature for all the samples were found. They attributed the reduction of gelatinization temperature to the pH changes caused by the contact of water with CO$_2$ and the plasticization of starch granules caused by scCO$_2$. 

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In addition to researches on scCO\(_2\) induced gelatinization, many other studies on the application of scCO\(_2\) in the processing of starches such as supercritical fluid extrusion (SCFX) and SCFX-based crosslinking have also been carried out. Chen and Rizvi et al. studied the relationships between the DG and rheology as well as expansion properties of wheat starch-water mixtures under SCFX processing conditions \(^{56}\). A reduction of 14% in the apparent viscosity (measured between 100 - 200 s\(^{-1}\)) was found with the injection of 0.45 g scCO\(_2\)/100 g sample, regardless of the DG of mixtures. Their experiments also indicated a minimum DG of 80% was required for the starch-water mixture in order to get a suitable gas-holding rheology for the production of extrudate with nonporous skin and desirable cellular morphology. In their later research, Rizvi and Ayoub’s group reported the production of SCFX-based cross-linked starch microcellular foam (XL-SMCF) using wheat starch with epichlorohydrin (EPI) and sodium trimetaphosphate (STMP) as cross-linker respectively \(^{57,58}\). The results showed that using scCO\(_2\) for SCFX process could achieve a good control over the expansion of extrudates as well as their microstructures by adjusting the injection rate of scCO\(_2\). XL-SMCF from this experiment showed good water-resistance, which is favorable for its future application. With respect to the water resistance property, a dual-modification of crosslinking and acetylation of corn starch was carried out in continuous supercritical fluid extrusion and a more hydrophobic material was successfully achieved \(^{59}\). A combination of SCFX and the subsequent solvent exchange process was also studied in an attempt to get a deeper understanding of the influence of processing conditions on foam microstructure and macro-properties \(^{60}\). The solvent exchange was proved, in this experiment, to be more important than other factors like crosslinking and existence of CO\(_2\) for the formation of microcellular structures which have a great contribution to the brightness of material. Moreover, the brightness was also found to be linearly related with the density of the foams. In brief, starch foams produced by all these methods based on SCFX, with properties like low density, non-porous skin and water resistance, have potential use as biodegradable materials.

In addition to its usage in SCFX, the application of scCO\(_2\) for tissue engineering, pharmaceutical processing and the production of antimicrobial materials have been explored in recent years. For instance, supercritical immersion precipitation technique was used by Duarte et al. to evaluate the feasibility of using scCO\(_2\) as non-solvent for preparing starch-based scaffold for tissue engineering \(^{61}\). A matrix with high porosity and interconnected microstructure was obtained by supercritical assisted phase-inversion. Pressure was proved to play a more important role than temperature in controlling the morphology of the scaffold. Besides this research, pressure was also found to play a significant role in other studies. Varona et al.
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Impregnated lavandin oil into waxy maize starch modified with n-octenyl succinate and the influences of different operational conditions like lavandin oil to starch mass ratio, pressure as well as temperature were analyzed. Higher impregnation loads were observed at a relatively low concentration of scCO$_2$ fluids (low pressure) and high depressurization rate. Similar observations were reported by Comin et al., in the research of whose oleic and flax oil were impregnated in pregelatinized corn starch. It is believed that a lower pressure (15 MPa) could weaken the interactions between scCO$_2$ and solutes in it, which is favorable to strengthen the interactions between solutes and starch. High depressurization rate, on the other hand, could facilitate the condensation of lavandin oil. The droplets formed in this process deposited in starch particles and thus increased the impregnation load. Another important factor that influences the efficiency of impregnation is solubility and diffusion of solutes in scCO$_2$. For example, Comin et al. found that simple lipids like oleic acid had a higher load on starch than flax oil which is a more complex lipid mixture. They attributed this to the higher solubility and diffusion coefficient of oleic acid in scCO$_2$ than that of flax oil. In the search carried out by Souza et al., a kind of antimicrobial film was successfully developed by impregnating cinnamaldehyde into cassava starch-based film. The highest impregnation load was achieved at higher pressure (25 MPa) which is opposite to that mentioned by Varona et al. and Comin et al. A conclusion was drawn that the solubility of cinnamaldehyde in scCO$_2$ played a vital role in the impregnation process.

1.2.2.1.2. Chemical modification of starch in scCO$_2$

Although there are many advantages to use scCO$_2$ as solvent for chemical reaction and many reports on this kind of application have been published, papers on chemical modification of starches in scCO$_2$ are still very few (summarized in Table 1-3).

Muljana et al. reported the synthesis of fatty acid-starch esters in scCO$_2$ with different vinyl, methyl esters as esterification agents. The highest degree of substitution (DS = 0.31, 150 °C and 8 MPa) was obtained with the combination of vinyl laurate and K$_2$CO$_3$. In their later work, the acetylation of potato starch with acetic anhydride (Ac$_2$O) was studied in sub- and supercritical CO$_2$ medium. The DS was evaluated within a broad range of pressure (8 - 25 MPa) at 90 °C and the highest DS of 0.29 and 0.62 (corresponding to 1 h and 24 h reaction time respectively) were obtained near the critical point of the mixture (15 MPa). Besides, the DS was found to relate with the size of starch granules, which means the mass transfer inside the granule plays a more important role than intrinsic reaction...
kinetics in controlling the overall conversion rates. In addition to the esterification and acetylation, the grafting of starch was also studied with scCO\textsubscript{2} as solvent. Salimi et al. reported that lactic acid was successfully grafted onto pregelatinized corn starch by controlled polymerization with stannous 2-ethyl hexanoate (Sn(Oct)\textsubscript{2}) as a catalyst in scCO\textsubscript{2}\textsuperscript{66}. Higher pressure, temperature and flow rate of scCO\textsubscript{2} were observed unfavorable to the chain growth. However, a relatively higher temperature was proved to be acceptable for chain growth when the flow rate of scCO\textsubscript{2} was maintained at a relatively lower value.

Table 1-3. The application of scCO\textsubscript{2} in the modification of starches

<table>
<thead>
<tr>
<th>Method</th>
<th>Application</th>
<th>Starch source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical</td>
<td>Gelatinization</td>
<td>Cassava, potato and wheat starch \textsuperscript{55}</td>
</tr>
<tr>
<td></td>
<td>SCFX</td>
<td>Wheat starch \textsuperscript{58}</td>
</tr>
<tr>
<td></td>
<td>Phase-inversion</td>
<td>- \textsuperscript{61}</td>
</tr>
<tr>
<td></td>
<td>Impregnation</td>
<td>Waxy maize, corn and cassava starch \textsuperscript{64}</td>
</tr>
<tr>
<td>Chemical</td>
<td>Esterification and acetylation</td>
<td>Potato starch \textsuperscript{65}</td>
</tr>
<tr>
<td></td>
<td>Grafting</td>
<td>Corn starch \textsuperscript{66}</td>
</tr>
</tbody>
</table>

1.3. Controlled modification of starch

Chemical modification, which could expand the application of starch to areas beyond imagination (as can be seen in Figure 1-2), has been under investigation for a long time. As is known, the application fields of materials are determined by their intrinsic properties which are actually closely related to their microstructures. In the case of polymers, their microstructures are influenced by the composition and structure of both backbones and side chains or groups. According to this principle, varieties of starch derivatives with different properties could be obtained by:

- substituting hydroxyl groups with other functional groups;
- grafting side chains with different composition, length and structure;
- varying the distribution of groups or chains along the backbone.

As a matter of fact, lots of the work that has been done was carried out following the three mentioned guidelines. Compared with traditional chemical methods, from our point of view, the controlled modification of starch is of great interest as it could provide us with functional products that have tailor-made properties for specific applications. Another advantage of controlled modification is, as will be illustrated in the following part, that some properties could even be achieved or
enhanced just by the switching of reaction sites. For this reason, many researches have been done on the controlled modification of starches during the last decades. In this part of the introduction, attention was paid to the progress on the controlled modification of starches with the hope that more efficient work could be done in future.

1.3.1. Regioselective functionalization of OH-groups

For polysaccharides like starch, the existence of three hydroxyl groups with different chemical environments in the AGU provides us the possibilities to achieve derivatives that have the same side groups/chains but different properties. For example, after preserved at room temperature for a hundred days a significant degradation could be observed in the starch acetate prepared according to the conventional procedure (acetylation of starch in aqueous medium with acetic anhydride as reagent). On the contrary, regioselective synthesized 2-O-acetyl starch could be stored in sterilized distilled water for over 200 days without significant change in viscosity and only a slight decrease in the DS was observed. Significant increases in the viscosity (400% higher), glass transition temperature and degradation temperature (34 °C and 36 °C higher respectively) were also observed in the 2-O-acetyl starch when compared with conventional acetylated starch that has the same DS. A possible explanation for this is the more uniform structure resulted from regioselective derivatization enhances intra- and intermolecular interactions (hydrogen bonds or hydrophobic interactions) and thus improves the macro properties of the products. Besides, 2-O-acetyl starch could only swell in water while conventional starch acetate was soluble with similar DS. Another interesting report was that 2-O/3-O- acetyl starch had a higher
biodegradation rate than 6-O-acetyl starch when exposed to α-amylase. Clearly, the regioselective functionalization could endow starch derivatives with novel properties with currently available reagents and thus expand the application fields of starches, especially in the areas like biomaterials.

As far as we know, the regioselective functionalization of polysaccharide has been studied for over 50 years. Generally, there are three approaches available now for the effective regioselective functionalization of polysaccharides (see below):

- protection and deprotection approach in which bulky protecting groups such as silyl and trityl groups are employed for the preparation of 6-O or 2,6-O protected intermediates;
- catalyzed selective functionalization of OH groups without using protecting groups (enzymes, inorganic salts, etc.);
- regioselective replacement of OH groups with other functional groups through reactions controlled by stereochemical mechanism (SN2 reaction etc.).

1.3.1.1. Protection and deprotection approach

Two different regioselective approaches, in which tosyl and trityl groups were employed as leaving/protecting groups for the introduction of 3,6-anhydro rings into amylose, were reported by Whistler et al. Results showed that trityl group had a better regioselectivity on the derivatization of primary hydroxyl groups than tosyl group. However, the detritylation process carried out in the acid medium may cause the breakdown of polysaccharide chains. To overcome this problem, Horton et al. developed a new approach for the selective protection of primary hydroxyl groups in amylose. In this new approach simple carboxylic ester groups were used as protecting groups and 6-O-acetyl amylose was prepared by the reaction of per(trimethylsilylated) amyllose with the mixture of acetic anhydride/pyridine/acetic acid. The deprotection could be easily realized through the base-catalyzed hydrolysis which avoids the degradation of amylose. Besides groups mentioned above, the silyl group has also been used for the regioselective functionalization of starches. For instance, a highly regioselective approach that uses thexyldimethylchlorosilane (TDSCI) as the protecting reagent was developed by Petzold et al. for the protection of primary hydroxyl groups in potato starch. The silylation of potato starch was carried out in the mixture of N-methylpyrrolidone (NMP) and ammonium at -20 °C. Different from their another research in which the silylation was carried out in the mixture of DMSO and pyridine, 6-O-TDS starch ether could be obtained exclusively by this approach with a DS up to 1 even when the TDSCI was excessive. The introduced protecting group could be easily removed by the treatment with tetrabutylammonium fluoride.
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solution in THF\textsuperscript{73}.

According to the aforementioned researches, not only the structure of the protecting reagent affects the selective protection of hydroxyl groups, but the reaction medium also has a significant influence on the regioselectivity. Besides the selective silylation of starch, another example is the tosylation of starch. In the researches carried out by Whistler et al. and Clode et al., pyridine was used as the reaction medium for the tosylation of amylose and results shown that this process mainly occurred at the C-6 position\textsuperscript{77,80}. However, after switching the reaction medium to the mixture of DMAc and LiCl the tosylation at the C-2 position was found to be more preferable than that occurred at C-6 and C-3 position\textsuperscript{84}. The influence of solvent on the regioselectivity of protecting groups was related to the supramolecular structure of polymer formed in specific conditions, but no evidence is available now and further studies are needed to support this hypothesis.

1.3.1.2. Catalyzed regioselective functionalization

Besides the utilization of protecting groups, regioselective derivatization of polysaccharides could also be achieved directly by using enzymes, catalytic active salts and heterocyclic compounds such as pyridine to promote the reaction. As for the enzyme-catalyzed site-selective functionalization, one problem faced is that the use of polar aprotic solvents, which are used to dissolve polysaccharides, may decrease the activity of enzymes\textsuperscript{85}. To overcome this problem, an approach developed by Paradkar and Dordick was employed by Bruno et al. to incorporate Subtilisin Carlsberg into surfactant micelles for the regioselective acylation of amylose film\textsuperscript{86,87}. Although this research was carried out successfully, only the surface of the amylose film was accessible for the enzyme and thus the efficiency of functionalization was not satisfactory. In the research carried out by Chakraborty et al., a new approach was proposed to solve this problem\textsuperscript{79}. Starch nanoparticles were incorporated into Aerosol-OT (AOT, bis(2-ethylhexyl)sodium sulfosuccinate) micelles to improve its accessibility to immobilized Candida antarctica Lipase B (CAL-B). As a comparison, free CAL-B was also incorporated into AOT micelles together with starch nanoparticles in the control experiments. The esterification of AOT-coated starch nanoparticles was carried out with toluene as solvent in which vinyl stearate, ε-caprolactone and maleic anhydride were dissolved respectively as esterification reagent. Starch esters were obtained with DS values ranging from 0.8 to 0.4 after reaction at 48 °C for two days. Results showed that the catalyzed esterification process, whether the CAL-B was immobilized or not, was regioselective for the primary hydroxyl groups in the AGU. However, from the point of view of post-purification, immobilized CAL-B is preferable for this
Chapter 1

approach. An alternative to the aforementioned approach was reported by Klohr et al., by whom protease was employed to achieve the regioselective functionalization of C-2 position. In their reported approach, protease was first activated in phosphate buffer solution (pH = 7.8, c = 0.15 M) and then lyophilized before added to the starch/DMSO solution. Various esterification reagents, such as vinyl acetate, acetic anhydride or propiolactone could be used for the regioselective substitution of starch in this approach. One thing should be noted is that chemical esterification may occur besides the enzyme-catalyzed reaction when this reaction is carried out at 40°C. To substantially suppress the chemical reaction, the esterification procedure could be carried out at a lower temperature (20 - 25°C) or conducted in an almost anhydrous system (water content < 0.01%).

In addition to enzyme, a number of salts and heterocyclic compounds were also found to be catalytically active for the regioselective functionalization of starches. For instance, ten different salts (Table 1-4) were tested by Dicke for the catalyzed regioselective acetylation of starches in DMSO and their performances in regioselectivity were compared with that of 4-(dimethylamino)pyridine (DMAP)/pyridine. These salts, according to their site-selectivity and the pH values of their aqueous solutions, were divided into three types. A high regioselectivity at the C-2 position was shown when using neutral, weak acid or alkaline salts as catalysts, while further acetylation would occur to some extent at C-6 and C-3 position when alkaline salts were employed. No catalysis was shown when acid salt was employed as catalyst. The regioselectivity in DMSO was related to the formation of intramolecular hydrogen bonds between the C-2 hydroxyl groups and that in the C-3 position of the neighboring AGU (Figure 1-3). The formation of these hydrogen bonds, together with other factors such as solvent and ring oxygen, were believed to make the protons of C-2 hydroxyl groups have a stronger acidic character than other two protons. However, it should be kept in mind that there is still no evidence to prove this hypothesis. Besides the type of salt, regioselectivity was also influenced by the amount of salts used as well as the type of acetylation reagent (Table 1-4). The latter factor was attributed to their differences in reactivity. The regioselective substitution of C-2 hydroxyl groups was also achieved by Liebert et al., in the study of whose starches were acetylated homogeneously by acetic acid/N,N’-carbonyldiimidazole (CDI) and acetic anhydride/imidazole respectively with DMSO as solvent. Starch acetates with DS values ranging from 0.4 to 1.2 could be obtained after a reaction at 60°C for two hours and then kept at room temperature for another 16 h. In both cases a high regioselectivity at the C-2 position could be detected and this was contributed to the formation of reactive intermediate (acetic acid imidazolide) and the possible...
solution state of starch in DMSO.

![Figure 1-3. Solution state of starch (amylose) in DMSO](image)

**Table 1-4.** The influence of catalysts, catalyst concentration and acetylation agents on total and partial DS of starch acetate

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>c(cat) /wt. %</th>
<th>Acetylation reagent</th>
<th>DS&lt;sub&gt;total&lt;/sub&gt;</th>
<th>DS&lt;sub&gt;C2&lt;/sub&gt;</th>
<th>DS&lt;sub&gt;C6&lt;/sub&gt;</th>
<th>DS&lt;sub&gt;C3&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMAP/pyridine</td>
<td>2</td>
<td>Acetic anhydride</td>
<td>1.00</td>
<td>0.37</td>
<td>0.50</td>
<td>0.13</td>
</tr>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;HPO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>2</td>
<td>Acetic anhydride</td>
<td>1.00</td>
<td>0.69</td>
<td>0.20</td>
<td>0.11</td>
</tr>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;HPO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>2</td>
<td>Vinyl acetate</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>NH&lt;sub&gt;4&lt;/sub&gt;Cl</td>
<td>2</td>
<td>Vinyl acetate</td>
<td>0.95</td>
<td>0.95</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>NaCl</td>
<td>2</td>
<td>Vinyl acetate</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Na-citrate</td>
<td>2</td>
<td>Vinyl acetate</td>
<td>0.80</td>
<td>0.80</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>2</td>
<td>Vinyl acetate</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>5</td>
<td>Vinyl acetate</td>
<td>1.82</td>
<td>1.00</td>
<td>0.55</td>
<td>0.27</td>
</tr>
<tr>
<td>K&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>2</td>
<td>Vinyl acetate</td>
<td>2.18</td>
<td>1.00</td>
<td>0.72</td>
<td>0.46</td>
</tr>
<tr>
<td>K&lt;sub&gt;3&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>2</td>
<td>Vinyl acetate</td>
<td>1.03</td>
<td>1.00</td>
<td>0.00</td>
<td>0.03</td>
</tr>
<tr>
<td>Mg-acetate</td>
<td>2</td>
<td>Vinyl acetate</td>
<td>1.15</td>
<td>1.00</td>
<td>0.05</td>
<td>0.10</td>
</tr>
<tr>
<td>Na-acetate</td>
<td>2</td>
<td>Vinyl acetate</td>
<td>1.82</td>
<td>0.91</td>
<td>0.37</td>
<td>0.54</td>
</tr>
</tbody>
</table>

* Reaction conditions: 40 °C, 70 h, 2.3 mol acetylation agent/mol AGU
The same approach was also employed by Hampe et al. to study the influences of solvent and many other factors (such as molecular weight and the concentration of starch) on the substitution patterns of starch acetate. Another approach used in their experiment was acetylation of different starches by acetic anhydride with molten imidazole as solvent and the reaction was kept at 100 °C for one hour. When using DMSO as solvent, potato starch was acetylated predominately at the C-2 position and the DS of starch acetate increased (up to 0.71) with a higher molar ratio of acetic anhydride to AGU (reagent/AGU ratio, up to 1.0). However, an even higher reagent/AGU ratio which could result in additional acetylation of the C-6 hydroxyl groups was needed in order to get similar DS at the C-2 position for degraded potato and tapioca starches. One possible explanation proposed for this was that degradation as well as the branching points reduced the amount of 2,3-hydrogen bonds between adjacent AGUs and thus the 2-0 proton had less activity for acetylation. Another interesting found was that, besides the reagent/AGU ratio, increasing the concentration of degraded starches in DMSO could also promote the acetylation of primary hydroxyl groups. When molten imidazole was used as solvent, contrary to that occurred in DMSO, regioselective acetylation at the C-6 position was found at low reagent/AGU ratio (≤ 0.5 and 0.3 for potato and degraded potato starch respectively). At higher reagent/AGU ratio, hydroxyl groups at C-2/C-3 positions could also be acetylated and resulted in a random distribution of acetyl moieties.

1.3.1.3. Regioselective replacement of OH groups

In addition to regioselective esterification, etherification and so on, the hydroxyl groups could also be selectively replaced by various nucleophiles (such as oxygen, nitrogen and sulfur nucleophiles) through S_N2 reaction (as seen in Figure 1-4). To achieve this purpose, a good leaving group is needed to make the target carbon atom more susceptible to the attack of nucleophiles. Commonly used leaving groups are sulfonates (RS(O)2O-) and halides.

In the research carried out by Whistler et al., the tosylate group was employed for the introduction of 3,6-anhydro rings into amylose. According to the reported procedure, amylose was first tosylated at the C-6 position and then the introduction of 3,6-anhydro rings was realized through the substitution of C-6 tosylate group by the oxygen at the C-3 position under alkaline conditions. Another example for the application of tosylate group was reported by Clode and Horton, in whose research both potato amylose and whole starch were tosylated in pyridine and then converted to corresponding 6-azido-6-deoxy derivatives (DS 0.62 and 0.92 respectively) with DMSO as solvent. 6-aldehydo-amylose (DS 0.48) and 6-aldehydo-starch (DS 0.57) could be achieved through the photolysis and successive
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mild hydrolysis of corresponding 6-azido-6-deoxy derivatives.

Figure 1-4. Reaction scheme for the regioselective replacement of starch

In addition to tosylate groups, halides were also proved to be good leaving groups for the regioselective derivatization of starches. In the research carried out by Cimecioglu et al., 6-halogen-6-deoxyamylose with DS values up to 1 was synthesized by the reaction of amylose with Ph$_3$P/N-bromosuccinimide and methanesulfonyl chloride respectively with dimethylformamide (DMF)/LiBr (LiCl) as solvent$^{81}$. The halides were replaced by azide and then Staudinger reaction was employed to reduce azide to amine. 6-amino-6-deoxyamylose with DS values up to 1 could be achieved according to this approach. Although the halogenation procedure was proved to be high regioselective, degradation was reported when the similar approach was employed for the chlorination of chitin with excess N-chlorosuccinimide at relatively higher temperature$^{90}$. Besides, the described procedure for the preparation of 6-azido-6-deoxyamylose was also time-consuming and not economical. In order to overcome these problems, a more direct and mild approach was introduced by Cimecioglu et al. for the azidation of amylose$^{82}$. Amylose was dissolved in the mixture of DMF and LiN$_3$ and then Ph$_3$P as well as freshly prepared CBr$_4$/DMF solution were added successively. This homogeneous reaction system was kept at room temperature for about 18 h with the protection of nitrogen. According to this approach, 6-azido-6-deoxyamylose could be achieved with nearly all the primary hydroxyl groups substituted. Given the high price of amylose, this one-pot approach has been extended to the azidation of different starches in recent years. Lithium azide, because of its high hygroscopicity and the resulting adverse effect on azidation, was replaced with sodium azide by Shey et al.
Not only DMF, but DMAc was also used as solvent in their experiments. In both cases, pregelatinized starches could be derivatized with high regioselectivity at the C-6 position while no desirable result could get from those untreated starches. This was attributed to the low solubility of untreated starch granules.

Table 1-5. Summary of the regioselectivity of different methods that carried out for various purpose

<table>
<thead>
<tr>
<th>Method</th>
<th>Solvent</th>
<th>Protecting group/ Catalyst</th>
<th>Regio-Selectivity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Purpose of functionalization</th>
<th>DS&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMP/ammonia</td>
<td>Silyl group</td>
<td>6-&lt;i&gt;O&lt;/i&gt;</td>
<td>Acetylation/etherification</td>
<td>1&lt;sup&gt;74&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>DMSO/pyridine</td>
<td>Silyl group</td>
<td>6-&lt;i&gt;O&lt;/i&gt;</td>
<td>Acetylation</td>
<td>1&lt;sup&gt;75&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Pyridine</td>
<td>Trityl</td>
<td>6-&lt;i&gt;O&lt;/i&gt;</td>
<td>2-&lt;i&gt;O&lt;/i&gt; amination</td>
<td>1&lt;sup&gt;91&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Protection-deprotection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyridine</td>
<td>Trityl</td>
<td>6-&lt;i&gt;O&lt;/i&gt;</td>
<td>Introduction of 3,6-Anhydro Rings</td>
<td>1~0.76&lt;sup&gt;77&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Pyridine</td>
<td>Tosyl</td>
<td>6-&lt;i&gt;O&lt;/i&gt;</td>
<td>—</td>
<td>~0.73&lt;sup&gt;84&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>DMAc/LiCl</td>
<td>Tosyl</td>
<td>2-&lt;i&gt;O&lt;/i&gt;</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyridine</td>
<td>Acetyl group</td>
<td>6-&lt;i&gt;O&lt;/i&gt;</td>
<td>—</td>
<td>1&lt;sup&gt;76&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Toluene</td>
<td>&lt;i&gt;CAL-B&lt;/i&gt;</td>
<td>6-&lt;i&gt;O&lt;/i&gt;</td>
<td>Esterification</td>
<td>0.8~0.4&lt;sup&gt;79&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>Protease</td>
<td>2-&lt;i&gt;O&lt;/i&gt;</td>
<td>Esterification</td>
<td>1&lt;sup&gt;88&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Catalyzed derivatization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>Mainly sodium salts</td>
<td>2-&lt;i&gt;O&lt;/i&gt;</td>
<td>Acetylation</td>
<td>0.69~1&lt;sup&gt;69&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>CDI (Imidazole)</td>
<td>2-&lt;i&gt;O&lt;/i&gt;</td>
<td>Acetylation</td>
<td>0.4~1&lt;sup&gt;68&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Molten imidazole</td>
<td>Imidazole</td>
<td>6-&lt;i&gt;O&lt;/i&gt;</td>
<td>Acetylation</td>
<td>0.34~0.47&lt;sup&gt;78&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The most preferable reaction site; <sup>b</sup> The largest partial degree of substitution could be got before other secondary hydroxyl groups are substituted.
Continuing

<table>
<thead>
<tr>
<th>Method</th>
<th>Solvent</th>
<th>Leaving group/ Catalyst</th>
<th>Regio-selectivity $^a$</th>
<th>Purpose of functionalization</th>
<th>DS $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridine</td>
<td>Tosylate</td>
<td>C-6</td>
<td>Introduction of 3,6-Anhydro Rings</td>
<td>0.76 $^77$</td>
<td></td>
</tr>
<tr>
<td>Pyridine</td>
<td>Tosylate</td>
<td>C-6</td>
<td>Azidation</td>
<td>0.62-0.92 $^80$</td>
<td></td>
</tr>
<tr>
<td>DMAc/LiBr (LiCl)</td>
<td>Br (Cl)</td>
<td>C-6</td>
<td>Azidation</td>
<td>~1 $^81$</td>
<td></td>
</tr>
<tr>
<td>DMF/LiN$_3$</td>
<td>Br</td>
<td>C-6</td>
<td>Azidation</td>
<td>~1 $^82$</td>
<td></td>
</tr>
<tr>
<td>DMF/NaN$_3$ (DMAc/NaN$_3$)</td>
<td>Br</td>
<td>C-6</td>
<td>Azidation</td>
<td>~ $^83$</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>TEMPO/ NaBr/NaClO</td>
<td>C-6</td>
<td>Oxidization</td>
<td>~ $^92$</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>TEMPO/ NaClO</td>
<td>C-6</td>
<td>Oxidization</td>
<td>~ $^95$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ The most preferable reaction site; $^b$ The largest partial degree of substitution could be got before other secondary hydroxyl groups are substituted.

Besides S$_N$2 substitution, another reported stereochemically controlled reaction is 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-mediated oxidation of starch. One example is the successful oxidation of various polysaccharides (starch included) reported by Chang et al. $^92$. Results showed that polysaccharides, whether water-soluble or not, could be oxidized with high regioselectivity at the C-6 position by using TEMPO with sodium bromide/sodium hypochlorite as co-catalyst (pH = 10.8, 0 °C). The high regioselectivity of this reaction was related to the steric hindrance caused by the bulky TEMPO ion as well as the conformation of AGU. Despite the high selectivity, the practical application of this technique was hindered by the use of bromide which is considered to be hazardous to both environment and production equipment. To overcome this problem, a bromide-free
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approach in which TEMPO ion could be directly generated by hypochlorite was reported by Bragd et al.\textsuperscript{93}. According to this approach, high regioselectivity could be achieved with a significant suppress on the degradation of molecular chains when the reaction was carried out under suitable conditions (temperature ≤ 20 °C and pH < 9). Considering the comparable reaction rate (20 °C, pH = 8.5) to that of bromide co-catalyzed reaction (2 °C), this approach should be a good alternative for the conversion of primary alcohol groups into aldehyde and carboxyl groups.

For clarity, the reported methods for regioselective modification of starch are summarized in Table 1-5.

1.3.2. Controlled grafting of starch

Compared with starches modified through the substitution of hydroxyl groups with small molecules, such as hydroxyethyl starch and cationic starches, brushed starches have some unique properties which could expand the application of starch-based materials as high value-added products. For example, amphiphilic branched starches have been synthesized as potential drug carriers with the property of self-assemble\textsuperscript{94}. Besides, many other grafted-starches have also been reported for various applications such as biopolymers and enhanced oil recovery (EOR)\textsuperscript{95-98}. As it is known, there are many other factors besides the botanical sources of starches have significant influences on the properties of final branched products. For instance, the intrinsic properties of branch chains as well as their grafting density and length are directly related with the rheological and mechanical properties of modified starches. Clearly, to achieve a successful application of these materials, it is of great importance to have a deep knowledge of the relationship between macroscopic properties and microstructures. Thanks to the development of techniques in controlled/living polymerization, many new approaches have been adopted for the synthesis and analysis of branched starches in recent years. In the following part of this work, the progress and open questions in the controlled modification of starch with techniques like atom transfer radical polymerization (ATRP), reversible addition-fragmentation chain transfer (RAFT), nitroxide-mediated polymerization (NMP), “click” and chemoenzymatic methods are summarized.

1.3.2.1. ATRP

ATRP is one of the most widely used controlled polymerization techniques for the preparation of polymers with defined architectures, through which desirable properties as predesigned could be achieved conveniently. Many works have been done on this subject since Wang et al. reported the first paper in 1995 and the progress of this technique has been kindly reviewed by He et al. and
Matyjaszewski respectively\textsuperscript{99-102}. As a matter of fact, most of the reported controlled grafting of starches were accomplished by ATRP (see example in Figure 1-5).

**Reaction mechanism of ATRP**

\[
\begin{align*}
\text{Chain initiation} & : R \cdot X + Cu^{+}/L \xrightleftharpoons[k_{\text{f}}]{k_{i}} R \cdot + XCu^{2+}/L \\
\text{Chain propagation} & : R \cdot M \cdot X + Cu^{+}/L \xrightleftharpoons[k_{\text{p}}]{k_{i}} RM_{n} \cdot + XCu^{2+}/L \\
\text{Termination} & : RM_{n} \cdot + RM_{m} \rightarrow \text{dead polymer}
\end{align*}
\]

**Example of ATRP grafting**

![ATRP grafting reaction mechanism diagram](image)

**Figure 1-5.** The reaction mechanism for ATRP and an example of its application in starch modification

Surface-initiated atom transfer radical polymerization (SI-ATRP) was first employed by Liu et al. for the grafting of potato starch granules with the hope that grafted starches could be used for the production of biodegradable plastics\textsuperscript{103}. Bromoacetyl bromide was immobilized onto the surface of starch granules in the first step and then poly(n-butyl acrylate) was grafted from the surface of macroinitiator with CuBr/1,10-phenanthroline as catalyst in toluene. A conversion of 9.9% for the monomer was achieved after 5 h. Similar method was also used by
Moghaddam et al. in the modification of starch surfaces with polystyrene (PS) and polyacrylamide (PAM) for the purpose of developing biodegradable thermoplastic copolymers. In another research reported by their group, both free radical polymerization and ATRP were used for the synthesis of starch-g-polyacrylamide and starch-g-polyhydroxyethylacrylate and the performance of copolymers in controlled release of cephalixin antibiotic were compared. Results showed that it would take a longer time for copolymers obtained from the ATRP method to release all the loaded drugs. It was attributed to the more orderly chain length and larger graft densities (2.51 vs. 1.89) in these polymers. In order to get a high graft density, Wang et al. prepared starch-based macroinitiator in ionic liquid ([AMIM]Cl) and then grafted that with PS and PMMA respectively. Compared with products from SI-ATRP, it was reported that both the graft density and ratio were significantly improved because of the homogeneous reaction condition in preparing macroinitiators.

Besides starches, their derivatives were also used for grafting in a homogeneous way. Nurmi et al. reported the ATRP grafting of acetylated potato starch oligomer with MMA. In their experiment, acetylated potato starch was first functionalized with 2-bromoisobutryl bromide as macroinitiator and then controlled polymerization of MMA was carried out with CuBr/2,2'-bipyridine as catalyst. The graft densities were controlled through varying the DS of macroinitiator (0.02 - 0.76) while the branch chain length was controlled by quenching the living polymers at certain time intervals. As for this paper and also for the sake of future research, one problem that should be taken into account is that the polymerization has to be terminated at low conversions because of the so-called intermolecular termination which was also mentioned in some other reports. The addition of CuBr₂ as deactivator and decreasing the concentration of catalyst were shown to be able to suppress the intermolecular termination but further studies are still needed.

As mentioned earlier, starches that modified by the ATRP method are potential candidates for drug carriers and so on. However, the biotoxicity of the commonly used copper catalyst is one of the problems that hinder their practical applications. Several techniques have been developed for decreasing the concentration of Cu(I) used for polymerization as well as the residues in final products. Among them, two reports seem to have significant meaning for eliminating the influence of monovalent copper ions. One was reported by Wang et al., in the research of whose MMA was polymerized with FeBr₂ as catalyst and no ligand was needed. In contrast with traditional ATRP, their experiments were successfully carried out in several polar solvents (N-methyl-2-pyrrolidone, DMF and acetonitrile), which were
believed to act as ligands for the dissolution of FeBr$_2$ and thus the polymerization could be controlled. One should note that, however, a too strong coordination between solvent and Fe could lead to failure in controlling the polymerization. In another research reported by Aggarwal, the technique named as supported aqueous-phase catalysis (SAPC) for activator generated by electron transfer (AGET) ATRP was developed $^{109}$. $N,N',N''$-pentamethyldiethylenetriamine (PMDETA) was used as ligand and its complex with CuBr$_2$ was loaded onto various inorganic (for example Na-Clay) and organic materials (starch and cellulose). The performance of SAPC AGET ATRP in polymerization and its ability in controlling the monovalent copper ions in final products were tested by the synthesis of linear poly(benzyl methacrylate) (PBnMA) and PMMA. Catalyst-free polymers were obtained with narrow PDI in the research, which could be taken as effective proofs for the success of the technique. What’s more, branched/star PBnMA was also successfully synthesized in his later studies, which reflected the development potential of SAPC AGET ATRP in wide areas.

1.3.2.2. RAFT and NMP

Although RAFT-mediated polymerization has advantages in preparing metal-free polymers with tailor-made structures and many researches have been done on its application in the modification of cellulose and chitosan $^{110-113}$, only a handful of papers were found to be related to the modification of starch by this technique (see example in Figure 1-6). This may be attributed to the following two reasons: the procedure is more complex than ATRP, most of the chain transfer agents are unavailable as commercial products.

In the research carried out by Lu et al., starch-based CTA was synthesized for the controlled grafting of poly(vinyl acetate) (starch-g-PVAc) with AIBN as initiator and starch-g-poly(vinyl alcohol) (starch-g-PVA) was also prepared as derivative $^{114,115}$. It’s interesting to notice that starch-g-PVAc showed the ability to self-assemble into micelles when mixed with water, while starch-g-PVA could physically crosslink into hydrogel. All these properties were found to depend on the length of grafted chains which could be well controlled with a narrow PDI (around 1.2).

Compared with RAFT, there is even no report on the application of NMP in the modification of starch. Only a few researches have been done on the NMP grafting of polysaccharides such as cellulose and guar gum $^{116,117}$. 
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Figure 1-6. The reaction mechanism for RAFT and an example of its application in starch modification

1.3.2.3. Click

The introduction of “click” chemistry to polymer science opened up a new way to synthesize various polymers with well-defined structures, especially when this was combined with controlled/living radical polymerization techniques like ATRP and so on. Different techniques that use “click” reaction to prepare polymers and various clickable initiators, monomers, and polymers were kindly reviewed by Mansfeld et al. The application of the most widely used click reaction, which is known as copper-catalyzed [3+2] Huisgen cycloaddition reaction (CuAAC), together with other more environmental friendly metal-free reactions such as thiol-yne radical addition, was also reviewed in plenty of works.
When used for the modification of starch, the commonly employed “click” reaction is also CuAAC (Figure 1-7). Reports on the combination of CuAAC with ATRP, RAFT and enzymatic method in the grafting of starches are available in recent years. In the research carried out by Borsacchi et al., amylose-graft-poly(n-butyl methacrylate) (Am-g-PBMA) was successfully synthesized through the combination of ATRP and CuAAC. Subsequent electron paramagnetic resonance (EPR) analysis revealed the presence of residual Cu(II) after extensive purification process. The total amount of residual Cu(II) which existed in three different forms (coordinate with ligands, coordinate with triazole nitrogens and Cu(II) cluster) was calculated to be about 7% of that introduced as catalyst. The last two forms of Cu(II) were believed to be difficult to remove through prolonged dialysis. This may hinder the practical application of CuAAC in the production of biocompatible materials.

Besides the application in starch grafting, “click” reaction was also used to crosslink starch with other polysaccharides such as cellulose in the hope that a new kind of biocomposite materials could be achieved by this method. One example is the research carried out by Elchinger et al., in the experiments of whose possibility was shown to employ CuAAC for the crosslinking of starch with microcrystalline cellulose but further studies are still needed.
1.3.2.4. Chemo-enzymatic modification

Various enzymes have been discovered and employed for the processing of starches in the past few decades. For example, amylases are used to hydrolyze starches into small molecules like maltose and glucose, while α-glucanotransferases (AGTases) are useful for remodeling the molecular structures of starches. The properties and applications of microbial AGTases in the modification of starches have been kindly reviewed by van der Maarel et al. and as illustrated in their paper various derivatives could be achieved by this treatment $^{127}$.

Although amylose, which has helical conformation and thus is expected to act as a good host molecule for the preparation of functional materials, could also be prepared by AGTases, one of the most effective ways to get pure product is considered to be α-glucan phosphorylase (GP) catalyzed α-D-glucose 1-phosphate (G-1-P) polymerization $^{128}$. Maltooligosaccharides, the DS of which should be large enough to be recognized by GP, are needed as a primer to initiate the polymerization. Take potato GP catalyzed polymerization as an example, maltotetraose is usually employed as primer and the chain will propagate at the nonreducing end of the primer in a step-growth manner. Hence, this process could be taken as a living polymerization and the molecular weight of resultant amylose can be tailored by varying the molar ratio of G-1-P to primer while the PDI could be controlled within 1.2 $^{128,129}$. The application of this technique in the preparation of functional polymers could be seen in the review composed by Kadokawa $^{128}$. As implied by this review, amylose has been successfully grafted to many other polysaccharides like chitosan and cellulose by combining GP catalyzed polymerization with certain chemical reactions (chemoenzymatic approach) $^{130,131}$. Besides, functional supramolecular materials with amylose as host molecules have also been prepared through this approach by so-called vine-twining polymerization $^{132,133}$.

In addition to the materials mentioned above, this chemoenzymatic approach has also been employed to modify the surfaces of different materials in order to prevent biofouling. For instance, potato phosphorylase was tested by van der Vlist et al. for the grafting of amylose brushes from the surface of silicon and Deinococcus geothermalis branching enzyme was then employed for the preparation of hyperbranched polysaccharide surface coating $^{134}$. Results from X-ray photoelectron spectroscopy (XPS) and spectroscopic ellipsometry revealed the successful grafting of both linear and hyperbranched brushes. A similar method was also used by Mazzocchetti et al. to graft amylose brushes from the surfaces of Si and Au, in which experiment 3-aminopropylmethoxyethoxysilane (APDMES)
and cystamine were used respectively for the amination of the two different surfaces and then maltoheptaoses were immobilized onto the surfaces as primers to initiate polymerization. Phosphorylases from rabbit muscles and potatoes were studied for the catalytic polymerization of G-1-P and it was reported that amylose brushes synthesized by rabbit muscle phosphorylases showed a better performance in preventing protein adsorption.

**Figure 1-8.** One-pot enzymatic synthesis of amylose from sucrose

Despite all the advantages brought by GP catalyzed polymerization, there are still some problems faced by the industrialization of this approach. For example, the monomer used in this approach, G-1-P, is too expensive and the commonly used potato phosphorylase is not thermostable enough for practical application. Several researches have been done to overcome these problems and these could be seen in the review that composed by Ohdan. According to this article, the most suitable method available by now for the synthesis of amylose is the combining use of sucrose phosphorylase (SP) with GP in the presence of inorganic phosphate with sucrose as substrate. G-1-P was produced in this process as the result of the SP catalyzed phosphorolysis of sucrose and then used as the substrate for GP catalyzed polymerization (Figure 1-8). Results showed that amylose could be achieved with a higher yield than directly synthesized from G-1-P. The molecular weight of
amylose from this method could also be controlled by varying the molar ratio of sucrose to primer and the PDI was found to be about 1. However, no report has been found by now on the application of this method in the preparing of functional materials as that synthesized from GP catalyzed polymerization of G-1-P.

1.4. Conclusions

With the development of technology and society, more and more attention is paid to the utilization of polysaccharides such as starch in order to reduce the reliance on fossil fuel which is nonrenewable. Starch, as a kind of widely existed renewable polysaccharide, has been modified for the applications in various areas which are now being extended day by day with the development of polymer science and technology. However, although megatons of starch derivatives are produced and consumed every year, the conventional ways in which they are modified are still not eco-friendly enough to reduce the environmental impact (wastewater, toxic organic solvent). What’s more, most of the starch products are low value-added and their performance is far from excellent for application, which means in some cases large amounts of them have to be used to achieve desirable properties. Thus, nowadays more and more attention is being paid to the development of new techniques that could produce “high-performance” starch derivatives in a more “green” way. In the past decades, one interesting progress in the modification of starch is the introduction of “green” solvents such as ionic liquids (ILs) and supercritical CO$_2$ (scCO$_2$) for the modification of starch. Not only could ILs serve as the reaction medium, in some cases they could also catalyze the chemical reaction or act as better plasticizers than traditional ones such as glycerol. Success was also obtained when using scCO$_2$ as solvent for the modification of starch, in both physical (gelatinization, extrusion, foaming and impregnation) and chemical (esterification and grafting) ways.

While the introduction of “green” solvents provides us the possibilities to prepare desirable products without significant environmental impact that caused by the use of organic solvents in traditional methods, controlled modification (regioselective functionalization of hydroxyl groups and controlled grafting) of starch makes it possible to achieve starch derivatives with tailor-made properties. Generally, regioselective functionalization could be achieved by three different methods which are protection-deprotection approach, catalyzed functionalization and site-selective replacement of OH-groups respectively. In these methods, regioselectivity is related to steric hindrance or supramolecular structure formed in specific solvents. As for controlled grafting, methods like ATRP, RAFT, “click” reaction and chemo-enzymatic approach are already available.
1.5. Aim and scope of the research

According to previous research in our group, (co)polymers with branched structure may display better viscoelasticity, especially in saline water and (or) high temperature, than corresponding linear ones for potential applications like enhanced oil recovery (EOR)\textsuperscript{138-141}. In contrast with these reports in which the “core” of the branched structure is of synthetic nature, in the present research, efforts were made to homogeneously synthesize waxy potato starch-based copolymers, this aiming at achieving branched copolymers in a more efficient economic way. The composition of the grafted copolymers was also varied in attempt to obtain satisfactory viscosity and (or) thermo-response property.

Instead of conventional free radical polymerization (e.g. initiated by Ce\textsuperscript{4+}) used for grafting, controlled polymerization was employed to avoid the generation of relatively large amounts of homopolymers. More importantly, this also makes the comparison of rheology properties between different polymers feasible. Compared with RAFT, “click” reaction and chemo-enzymatic method, Cu\textsuperscript{0}-mediated living radical polymerization (Cu\textsuperscript{0}-mediated LRP, different with conventional ATRP only in the catalyst) was chosen for the controlled grafting of starch due to advantages like ease in the synthesis of macroinitiator (see illustrations shown in Figure 1-5 and Figure 1-6), high efficiency in grafting and purification (compared with “click” and chemo-enzymatic method), and better controllability on the polymerization than that of ATRP in aqueous solution.

To take advantage of the highly branched structure of waxy potato starch, the synthesis of macroinitiator need to be carried out homogeneously. While scCO\textsubscript{2} was not chosen because of the heterogeneous reaction conditions (and thus kinetics), ionic liquids (ILs) were not used as solvent in this work mainly due to their high price. Although the new generation of ILs, known as deep eutectic solvents (DES) and natural deep eutectic solvents (NDES), is much cheaper than conventional ILs, the range suitable for the synthesis of macroinitiator (anhydrous reaction) is still limited\textsuperscript{142}. Consequently, in this research anhydrous mixture of dimethylacetamide/lithium chloride (DMAc/LiCl) was used as solvent for the homogeneous functionalization of waxy potato starch.

In chapter 2, optimum conditions for the synthesis of waxy potato starch-based macroinitiator were explored. Polyacrylamide was then grafted on the starch backbone homogeneously via Cu\textsuperscript{0}-mediated LRP and the factors that affect the control of the polymerization were discussed. A primarily rheological characterization was also presented in this chapter.
In chapter 3, the conditions for the controlled synthesis of zwitterionic polymer in water were studied. We then described the grafting of a zwitterionic polymer on waxy potato starch via Cu\textsuperscript{0}-mediated LRP aiming at obtaining a final product with thermally responsive behavior.

Finally, in both chapter 4 and chapter 5, polyacrylamide copolymers with larger chain length compared with that in the former chapter were grafted on waxy potato starch via improved Cu\textsuperscript{0}-mediated LRP. The influences of chain composition on the rheological and thermo-responsive properties of copolymers were discussed.

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