

# Modification of Starch: Structural and Antimicrobial Properties

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**Abstract:** - In this study, starch chloroacetate (St.ClAc) as a macro-initiator was firstly prepared by the reaction of chloroacetyl chloride with primary OH groups on powder starch. Cu(I)Cl and 2,2'-bipyridine were used as a transition-metal compound and as a ligand, respectively. The graft copolymerization of starch was made with our own synthesized N-(4-nitrophenyl)acrylamide (NPA) monomer by method of atom transfer radical polymerization. St.ClAc macro-initiator and its graft copolymer (St.ClAc-g-NPA) were characterized by FT-IR spectra and elemental analysis. Starch, synthesized St.ClAc and its graft copolymer with NPA were tested for its antimicrobial activity against bacteria and yeast.

**Key-Words:** - Starch, starch chloroacetate, macro-initiator, antimicrobial activity, atom transfer radical polymerization

## 1 Introduction

Starch is a renewable, natural and readily available polysaccharide, which turns it into an important carbohydrate. It is also a good source of energy for human beings and animals. Starch and cellulose are used widely as raw materials in many industries such as paper, paint, textile, food and pharmaceuticals. These polymers can be utilized more widely because of being non-toxic, renewable, biodegradable and modifiable [1]. Renewable resources are more important than ever because of scarce resources and environmental concerns, which have yielded to intensified researches in starch and cellulose as basic materials. Physical, chemical, enzymatic or genetic modification is often necessary for functionality of different starch and cellulose products [2].

Modification changes the properties and behavior of the polymer, making it possible to get rid of unwanted characteristics while promoting desired ones [3]. By chemical modification, the substitution of free hydroxyl groups in the polymer with functional groups, yields various starch or cellulose derivatives. Some factors affecting the properties of a modified polysaccharide could be counted as the modification reaction, the nature of the substitution group, the degree of substitution and the distribution of the substitution groups [4]. In order to direct a modification reaction towards a certain product with the desired properties, correlations between the modification process, chemical structure and functional properties of the final product must be known [2].

Although biodegradability of starch has made it popular in the scientific and industrial fields, raw starch has some defects which limit its industrial applications. This constraint dictates modifications. Grafting is an important way to modify properties of

polymers. Chemical modification of starch via graft copolymerization makes starch and synthetic polymer bind together rather than existing merely as a physical mixture [5]. In order to extend their applications, functional groups can be introduced into starches by using various methods of chemical or physical modifications, which may hopefully result in improved or specific properties of the starches. So that the starch can be converted into a natural polymer semi-synthetic polymer. A step in chemical modification of starch is the reaction of the hydroxyl groups and producing various starch derivatives [6,7].

Similar to the other cellulose and starch grafting study by the author and team, this study is about macro-initiator of starch and its antimicrobial activity properties [7,8]. There are many reports upon the synthesis, characterization, and properties of starch graft copolymers; but less attention has been paid to the biological behavior onto modified starch. Thus, in present study describes the esterification of a part of OH starch groups with chloroacetyl chloride; and thus a new atom transfer radical polymerization (ATRP) macro-initiator has been synthesized. The graft copolymerization of starch was made with our own synthesized NPA monomer [9]. In addition to characterizing macro-initiator and graft copolymerization, their antimicrobial activities were investigated.

## 2 Experimental

### 2.1 Materials and Instrumental Measurements

In this study, powder potato starch obtained from Sigma-Aldrich that has contained about 78% amylopectin/ 22% amylose was used. 4-Nitroaniline, Triethylamine [(Et)<sub>3</sub>N], Acryloyl chloride, Potassium tert-butoxide ((CH<sub>3</sub>)<sub>3</sub>CO<sup>-</sup>K<sup>+</sup>),

Chloroacetyl chloride, Cuprous(I)chloride, 2,2'-used as received. N, N-dimethylformamide, acetonitrile, chloroform, ethanol, diethyl ether were used as absolute solvents.

The IR spectra of all samples were performed with a Perkin Elmer Spectrum One FT-IR spectrometer in the range of 4000-450  $\text{cm}^{-1}$ . Leco CHNS-932 for elemental analysis measurements were used.

## 2.2 Preparation of Starch-macroinitiator and its Graft Copolymer by Atom Transfer Radical Polymerization

To obtain starch graft copolymer, starch chloroacetate was synthesized, primarily. Starch chloroacetate (St.ClAc) and its graft copolymer was synthesized from the literature [7-11]. Raw starch was allowed to swell in the acetonitrile solvent at room temperature, and then  $(\text{CH}_3)_3\text{CO}\cdot\text{K}^+$  dissolved in acetonitrile was added, and thus stirred for 24 h. This mixture was added dropwise with chloroacetyl chloride dissolved in acetonitrile. The reaction mixture was refluxed for 32 hours in a controlled manner and then filtered by cooling. So St.ClAc macro-initiator was synthesized. St.ClAc was washed away to remove salt and impurities; with acetonitrile, water, ethanol, and diethyl ether. The synthesis reaction of the St.ClAc is shown in Figure 1.

N-(4-nitrophenyl)acrylamide (NPA) was synthesized by a method adapted from literature [9]. 2,2'-bipyridine (ligand) and  $\text{Cu(I)Cl}$  by N,N-dimethylformamide solvent were added to a polymerization tube and stirred under the inert gas. St.ClAc as macro-initiator and NPA monomer were added into it, and argon was passed through. The polymerization tube was allowed the monomer to do grafting onto starch at  $130^\circ\text{C}$  for 24 h. St.ClAc,  $\text{Cu(I)Cl}$ , 2,2'-bipyridine catalytic system, and the monomer were used at a mole ratio of 1:1:3:100, respectively. After the mixture was cooled and filtered, it was washed with N,N-dimethylformamide, acetonitrile, chloroform, a mixture of water-ethanol-HCl, water, acetone and diethyl ether to eliminate impurities that can form in the reaction [8-10]. The synthesis reaction of the grafting of St.ClAc with NPA monomer is shown in Figure 2.

## 2.3 In vitro Antimicrobial Activity Study

Four bacteria and a yeast were used as test organisms that these *Staphylococcus aureus* (S.a) ATCC 6538 (Gr+), *Bacillus subtilis* (B.s) ATCC

bipyridine (Aldrich) were 6633 (Gr+), *Echerichia coli* (E.c) ATTC 25922 (Gr-), *Enterobacter aeruginosa* (E.a) CCM 2531 (Gr-) as bacteria, and *Candida tropicalis* (C.t) ATCC 13802 as yeast. The polymers and standard antibiotic were homogenized in dimethyl sulfoxide at a sample concentration of 100  $\mu\text{g}/\text{ml}$  by using the antibiotic disk assay [12,13]. The resulting inhibition zones on the plates were measured.

## 3 Results and Discussions

### 3.1 Grafting of Starch and Characterization

The primary OH groups on starch react with  $((\text{CH}_3)_3\text{CO}\cdot\text{K}^+)$ , which is a basic compound, to form starch alkoxide. In another reaction, the starch alkoxide reacts with chloroacetyl chloride to form starch chloroacetate (St.ClAc). Then, a series of graft study was carried with St.ClAc by using NPA monomer (Fig 1 and 2). The NPA monomer used here was synthesized in our own laboratory [9].

It is known that chloroacetyl group in any ester is an effective initiator for atom transfer radical polymerization [8,14]. In order to use this fact in the grafting of starch, as mentioned also in experimental section, starch chloroacetate was first obtained, and then a series of grafting studies onto starch were carried out using  $\text{Cu(I)}/2,2'$ -bipyridine complex as a catalyst of atom transfer radical graft copolymerization. Chloroacetate groups here act as a macro-initiator.

The FT-IR technique is the first spectroscopic method used to identify functional groups within a molecule. FT-IR spectrum of St.ClAc macro-initiator and St.ClAc-g-NPA graft copolymer are shown in Fig 3. Arising of new band at  $\sim 1740\text{ cm}^{-1}$  (C=O stretching) in the spectrum of St.ClAc, which there is no band in that of starch, indicates that chloroacetyl group has attached to starch. The presence of band at  $\sim 1660\text{ cm}^{-1}$  (C=O in the amide group) St.ClAc-g-NPA is the most important evidences of the grafting. Furthermore, the weight increase in the synthesis of the graft copolymers as a result of the values calculated by gravimetric analysis is also evidence that graft copolymers are formed.

The degree of substitution in the glucose units of starch was 28.28% by mole (y) from and 36.75% by weight (Y) the percentage of carbon [7-11,14-16]. Similarly, according to the calculation made in the literature, weight fraction of monomer in graft copolymer is 0.26. According to this calculation made on the amount of carbon in the elemental

analysis, the graft copolymer was formed with NPA and calculation results of weight fraction of monomer can be seen from Table 1.

### 3.2 Antimicrobial Activity of Polymers Derived From Starch

The antimicrobial activities of the polymers were determined against four bacteria and a yeast as described in the experimental section. The zones of inhibition of the microorganism growth of the standard samples and synthesized polymers were measured by millimeter at the end of the incubation period. Table 2 shows the average data of microorganisms. The results show that the St.ClAc is more antimicrobial properties than St.ClAc graft copolymer. It should be considered that this is caused by chlorine and acetate groups attached to the starch [17]. The macro-initiator is effective in some microorganisms, while the graft copolymer synthesized with the help of macro-initiator is not effective. So, the test results showed that synthesized macro-initiator to be effective on some of the microorganisms. It can be said that this is due to the high graft on the initiator that the monomer is linked.

### 4 Conclusion

In this study, starch chloroacetate (St.ClAc) as a macro-initiator was prepared by esterification 28.28% yield by mole. The monomer own synthesized N-(4-nitrophenyl)acrylamide (NPA) was grafted into the St.ClAc via atom transfer radical polymerization. St.ClAc and its graft copolymer that St.ClAc-g-NPA were characterized by FT-IR and elemental analysis. The synthesized macro-initiator and its graft copolymer were tested for their antimicrobial activity against microorganisms. The macro-initiator was effective in some microorganisms, while the graft copolymer synthesized with the help of macro-initiator was not effective. Since starch is low cost and easily obtainable and the nature of the materials offered to us, the synthesized starch-containing polymers could also have the same properties. The newly synthesized biopolymer-containing graft polymers could increase the application area.

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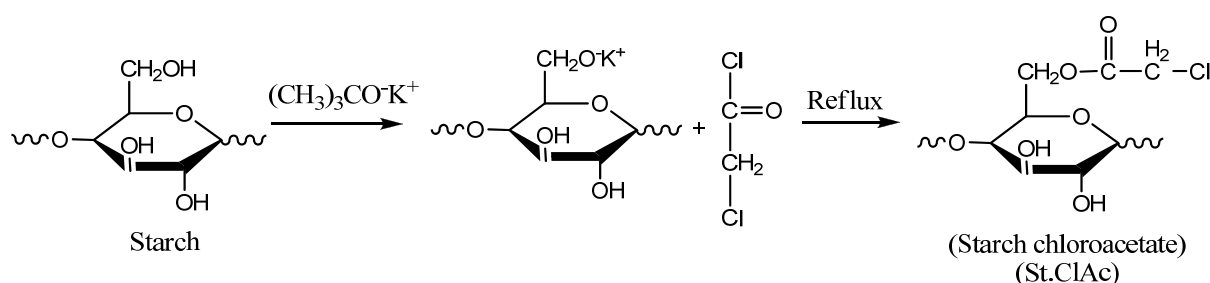
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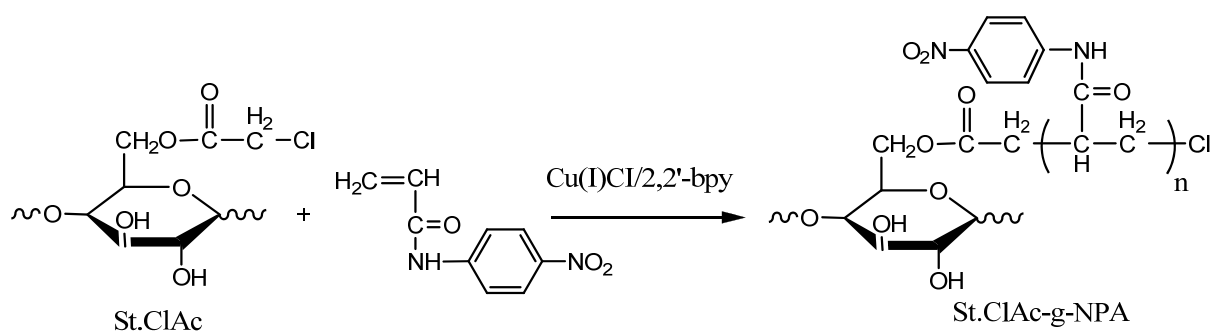
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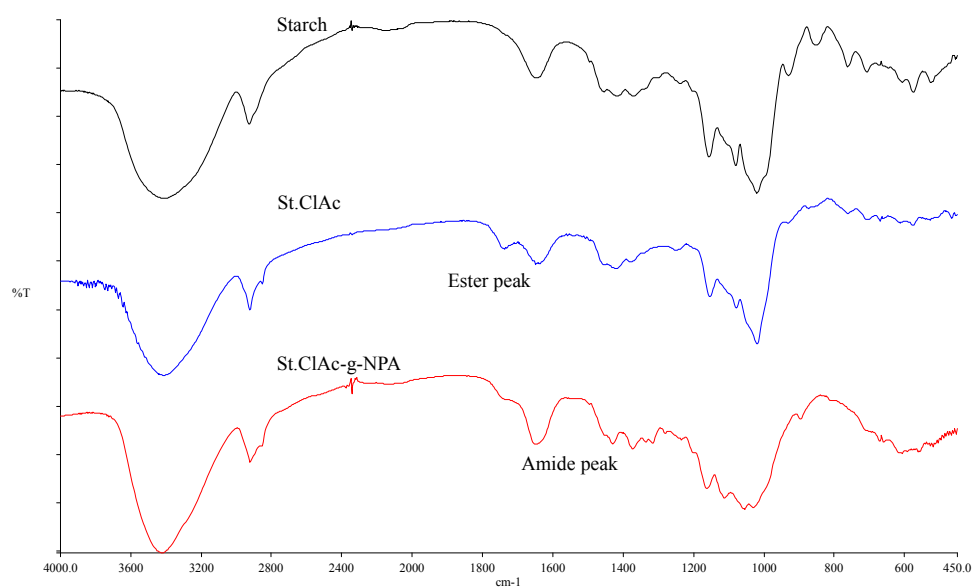
## FIGURES



**Figure 1.** Synthesis of starch chloroacetate as macro-initiator



**Figure 2.** The grafting of starch with NPA monomer



**Figure 3.** FT-IR spectra of the starch, starch chloroacetate and its graft copolymer

## TABLES

**Table 1.** Elemental analysis of starch, starch chloroacetate, and its graft copolymer

Polymer	C (%)	H (%)	N (%)	Weight fraction of monomer
Starch	42.76	5.53	-	-
St.ClAc	41.22	5.22	-	-
St.ClAc-g-NPA	45.15	4.28	3.11	0.26

**Table 2.** Effect of polymers growth of microorganisms

	<i>S.aureus</i>	<i>Bacillus</i>	<i>E.coli</i>	<i>Enterobac</i>	<i>Candida trop</i>
Starch	-	-	-	-	-
St.ClAc	12	12	12	13	8
NPA monomer /homopolymer	-	-	-	-	-
St.ClAc-g-NPA	-	-	-	-	-