

# Development and Evaluation of Mucoadhesive Graft Copolymer of a Natural Polysaccharide Using Microwave Assisted Grafting Technique and Alkaline Hydrolysis

Neha Prajapati

Assistant Professor, Shree Dhanvantary Pharmacy College, Kim, Gujarat, India

**ABSTRACT:** *Grafting of polymers adds numerous beneficial features to a simple polymer. In this study, natural polysaccharide starch has been grafted with acrylamide monomer by microwave assisted grafting method using ammonium persulfate (APS) as a chemical initiator. Five batches of polyacrylamide grafted copolymers (PAM-g-Starch) i.e. G<sub>0</sub>, G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub> and G<sub>4</sub> were prepared by modulating only the microwave radiation time (40s, 60s, 80s, 100s and 120s respectively), keeping all other factors constant. Further to increase the functionality and mucoadhesive property of each graft copolymer, alkaline hydrolysis was carried at 50°C for 1h. Effects of duration of radiation application on the properties of modified polymers were estimated by various evaluation parameters. Grafted polymers were characterized by % grafting, % grafting efficiency, Fourier transform infrared spectroscopy, differential scanning calorimetry, intrinsic viscosity study, elemental analysis, neutralization equivalent and mucoadhesive test. Results show that among all grafted products, batch G<sub>1</sub> showed the highest % grafting, % grafting efficiency and NE values (349.5%, 81.95% and 485.6 respectively).*

**Key words:** *Grafting, High performance biopolymer, Mucoadhesive graft copolymer.*

## I. Introduction

Number of conventional formulations with systemic polymers is available in market with the disadvantages of high dose frequency, allergic reactions, dose dumping, tedious applications patient in compliance etc. Controlled/sustained release formulations such as bio/muco- adhesive systems, prepared using various natural polysaccharides have gained an immense attention due to their sustainability, biodegradability, economy and safety over comparable synthetic materials.

This study includes modification of natural polysaccharide starch to make it sustain release polymer with the grafting technique with further inclusion of mucoadhesive property through alkaline hydrolysis. Starch was selected as it is easily available, cost effective, containing –OH group required for grafting and can be easily grafted without extra need of mechanical force. Techniques of grafting include conventional method using chemical initiator and microwave method using dry or wet medium. As conventional methods have number of demerits like time consumption, need of inert atmosphere, less reproducibility, uneven heat distribution, toxic vapor generation etc. make it less efficient method as compared to microwave method which is more productive and reproducible. Furthermore, in microwave method, microwave assisted method was chosen as compared to microwave initiated method as in assisted method, use of chemical initiator which produces ions enhancing the ability of the aqueous reaction mixture to convert the microwave energy to heat energy making it more reproducible and productive.<sup>1,2</sup>

## II. MATERIALS AND METHODS

### A. Materials

Polysaccharide Maize Starch, monomer Acrylamide and Chemical initiator Ammonium persulfate (APS) were purchased from Astrone Pharma. All the other ingredients used were of analytical grade, and were used as procured.

### B. Methods

a. Synthesis of modified polysaccharide by microwave assisted grafting method<sup>3,4</sup>:

Briefly, 2 gm of starch was dispersed into 100 mL

of deionized water to form suspension. Required quantities of acrylamide and APS were dissolved in quantity sufficient deionized water (aprox.10 mL). This solution was then slowly added to the starch suspension and allowed to mix properly on a magnetic stirrer. The prepared reaction mixture was then placed on a turnable of microwave and irradiated for desired time period. Keeping all other parameters constant, only the irradiation time was changed to control the degree of grafting. After completion of reaction grafted products were cooled to room temperature and graft copolymer was precipitated using three volume methanol: water (80:20). Repeated washing was provided twice by using the same solvent mixture to remove the un-reacted monomers and APS. Finally, it was dewatered using acetone and then dried and stored in well closed container.

Grafted Copolymer (code)	Starch (g)	Acrylamide (mole)	Ammonium persulfate (mole)	Duration of microwave radiation (s)
Go	2	0.12	0.002	40
G1	2	0.12	0.002	60
G2	2	0.12	0.002	80
G3	2	0.12	0.002	100
G4	2	0.12	0.002	120

b. Purification of graft copolymer by solvent extraction technique<sup>5</sup>:

The occluded polyacrylamide (homopolymer) was extracted out using Formamide: Glacial acetic acid (1:1) mixture. The well pulverized copolymer was taken in a beaker and 100 ml of solvent mixture was added to it. It was then stirred at minimum speed in shaker at 40°C for 6 hr. After cooling the sample, it was filtered using Buchner funnel. The residue was washed with the same solvent mixture and the filtrate was checked every time by adding acetone for the precipitation of polyacrylamide (PAM). The residue was washed until the filtrate gives precipitation with acetone. Then washing was given with methanol and the residue was kept in acetone overnight. It was dried subsequently at 50°C in oven.

c. Alkaline hydrolysis of graft copolymer (Hydrolyzed copolymer)<sup>6</sup>:

Briefly 2 g of graft copolymer was completely

dissolved in 0.9 N NaOH. After complete dissolution, the solution was heated at 75°C for an hour. The solution was then cooled and precipitated using excess of methanol and washed twice with the same. After dewatering it with acetone, it was dried at 50°C using vacuum drier and stored in well closed container.

d. Characterization of modified polymer Percentage Grafting (%G)<sup>7</sup>:

It can be estimated using following equation:

$$\% \text{grafting}(\%) = (W_1 - W_0/W_0) * 100$$

Where, W<sub>1</sub> = Weight of graft copolymer(g)

W<sub>0</sub> = Initial weight of polysaccharide(g)

Percentage Grafting Efficiency (%GE)<sup>6,8</sup> :

It can be estimated using following equation:

$$\% \text{grafting efficiency}(\%) = (W_1 - W_0/W) * 100$$

Where, W<sub>1</sub> = Weight of graft copolymer(g)

W<sub>0</sub> = Initial weight of polysaccharide(g)

W = Weight of monomer used (g)

Intrinsic Viscosity<sup>9</sup>:

It was measured using Capillary/Ubbelodhe viscometer. Four concentrations i.e. 0.0125%, 0.025, 0.05% and 0.1% of each hydrolyzed copolymer were prepared. The flow time of each from one to the second mark of capillary viscometer was noted. The graph of Concentration vs.  $\eta_{red}$  and  $\eta_{inh}$  was plotted and the values were extrapolated to zero to obtain intrinsic viscosity.

The intrinsic viscosity of polymer is directly related with its molecular weight (M) as per following equation:

$$\eta_{int} = KM^\alpha$$

Where, K and  $\alpha$  are the constants for specific polymer

Neutralization Equivalent<sup>9,10,11</sup>:

The neutralisation equivalent (NE) is the equivalent weight (g/eq) of an acid as determined by titration with standardized NaOH. Briefly 2 g of hydrolyzed copolymer was dissolved completely in 150 ml of deionized water. Then 40 ml of 0.1 N HCl was added into it and stirred for 6 hr on magnetic stirrer at room temperature. Finally, the mixture was titrated by 0.1 N NaOH using phenolphthalein as an indicator until permanent pink colour was obtained. Blank titration was also performed.

$$1 \text{ equivalent acid (g)} = (x \times 1000)/(y \times z)$$

Where, x = weight of polymer (g)

y = ml of NaOH consumed

z = Normality of NaOH

Fourier Transform Infrared Spectroscopy<sup>6,7,9</sup> :

The IR spectra of starch, graft copolymer (PAM-g-Starch) and hydrolyzed copolymer (HPAM-g-Starch) were taken using KBr dispersion method. The samples were crushed with Potassium bromide to make pellets and scanned between 400 cm<sup>-1</sup> to 4000 cm<sup>-1</sup>.

Elemental analysis<sup>7,8</sup>:

The elemental analysis of starch, PAM-g-Starch and hydrolyzed PAM-g-Starch was performed for the estimation of Carbon, Nitrogen and Hydrogen content.

**III. RESULTS AND DISCUSSION**

**A. Modification of polysaccharide:**

The graft copolymers G1 to G4 were successfully synthesized using microwave assisted grafting method. Change in irradiation time while keeping other parameter constant had successfully managed to control the degree of grafting. The purification of grafted polysaccharide ensured the complete removal of homopolymer, which is very useful in synthesis of tailor made molecular matrix structure. The

microwave irradiation together with chemical initiator is capable of grafting the acrylamide chains on polysaccharide backbone. This change in polymeric backbone brings the neutral amide (-CONH<sub>2</sub>) groups, which offers a platform for their conversions into ionic sodium salt of carboxylic acid (-COO<sup>-</sup> Na<sup>+</sup>) by saponification<sup>6,7,8</sup>. This ionic graft copolymer is reported to improve the overall functionality of polymer.

During microwave irradiation the heat is generated (owing to polar groups of solvent and polymer), which provides an energy for activation of chemical initiator to generate a free radicals (SO<sub>4</sub>\*). These free radicals form a chelate complex with C-4 hydroxyl group of polysaccharide. These complexes then disproportionate and create alkoxy radicals of polysaccharide<sup>7</sup>.

The free radical site on monomer is believed to be generated by microwave. The microwave radiation rotates the acrylamide molecules, leading to elongation of its bond. As C=C bond elongates, the pi bond electron cloud splits up into two localized clouds (i.e. free radical sites on the constituent carbon atoms), which further interact with polysaccharide radicals by usual free radical mechanism<sup>7</sup>. Together a chain of reaction begins, leading to the grafting of acrylamide chains on alkoxy radical site followed by chain elongation.

**B. Characterization of modified polymer:**

Table 1. Results of characterization of modified polymer.

Code for graft copolymer	% grafting (%)	% grafting efficiency (%)	Intrinsic viscosity (dl/g)	Neutralization equivalent (g)
G0	No grafting	-	-	-
G1	349.5	81.95568	32.6	485.6255
G2	315.5	73.98288	25.9	511.1847
G3	285.5	66.94806	21.51	647.5006
G4	212	49.71274	19.19	669.8283
Starch	-	-	0.91	-

### C. Percentage grafting and grafting efficiency:

It was observed that at irradiation time less than 40s, no grafting has occurred (table 1). The reaction mixture remains as such even after the radiation process. This can be due to lesser energy / heat production during 40s duration, which may not be able to produce radicals on starch, APS and acrylamide. While from 60-120s, it has been observed that there was a decrease in % grafting with increase in MW radiation time. It can be due to following two major reasons:

- Degradation of polysaccharide at high temperature.
- More homopolymerization as compared to copolymerization

Increased radiation time causes elevation in temperature, which further produces more radical sites on acrylamide. These radicals then react with one another to produce a competing homopolymer rather than participating into grafting process and leading to poor % GE.

Similarly excessive heat generated by microwave may also cause the breakdown of polymeric backbone and decrease the grafting.

### D. Intrinsic viscosities of graft copolymers:

The intrinsic viscosity was evaluated for hydrolyzed copolymers GH1 to GH4. It is obvious that intrinsic viscosity of each set of modified starch is greater than that of plain starch (Table 1). This can be explained by higher molecular weight of graft copolymer than simple maize starch, due to the grafting of PAM branches on polysaccharide backbone. This is a good agreement with the Mark-Houwink-Sakurada relationship (intrinsic viscosity  $\eta_i = KM^\alpha$ , where K and  $\alpha$  are constants, both related to stiffness of the polymeric chains). Further the results show increase in the intrinsic viscosities of polysaccharide with increase in % grafting<sup>3</sup>. This is obvious since higher % grafting translates to higher molecular weight, which in turn leads to higher intrinsic viscosity.

Further, it can also be explained by the repulsion mechanism of introduced groups. After partial alkaline hydrolysis, the  $-\text{CONH}_2$  group of grafted

polyacrylamide chains are converted to  $-\text{COO}^-\text{Na}^+$ . There is a huge steric repulsion between these  $-\text{COO}^-\text{Na}^+$  bulky groups, which expands and straighten the chains, ultimately increasing the hydrodynamic volume. Consequently, the intrinsic viscosity (i.e. the hydrodynamic volume of polymer molecule in solution) of hydrolyzed copolymer in solution increases<sup>9</sup>. Further for highly grafted copolymer the number of the introduced carboxyl groups will be more. Therefore, the intrinsic viscosity decreases from G1 to G4.

### E. Neutralization equivalent (NE):

NE has been performed for hydrolyzed copolymers GH1 to GH4.

Results show decrease in NE with increase in % grafting (Table 1). During reaction, the added 0.1 N HCl reacts with sodium salt of carboxylic acid ( $-\text{COO}^-\text{Na}^+$ ) present in the hydrolyzed copolymer and replaces  $\text{Na}^+$  ion with  $\text{H}^+$  because HCl is much stronger than organic acid. The HCl is utilized according to the extent of carboxylic groups present. The remaining HCl is then back titrated by using 0.1 N NaOH. Since HCl is much stronger than organic acid, it will be neutralized. Organic acids are not dissociated in presence of HCl due to common ion effect. Therefore as the carboxyl content increases, more of HCl get consumed and lesser will be the value of neutralization equivalent.

The decrease in NE with increased % grafting can be explained by following fundamentals of NE:

NE value indicates the number of basicity of a polybasic acid. NE is equal to molecular weight of the acid/basicity. During the alkaline hydrolysis of amide group of graft copolymer, molecular weight of polymer would remain same. Hence greater the number of carboxyl groups (basicity) (higher % grafting), lower will be the NE value<sup>10,11</sup>.

### F. Fourier transform infrared spectroscopy (FTIR):

The FTIR spectra of Starch, PAM-g-Starch and HPAM-g-Starch with their characteristic peaks have been obtained as follow:

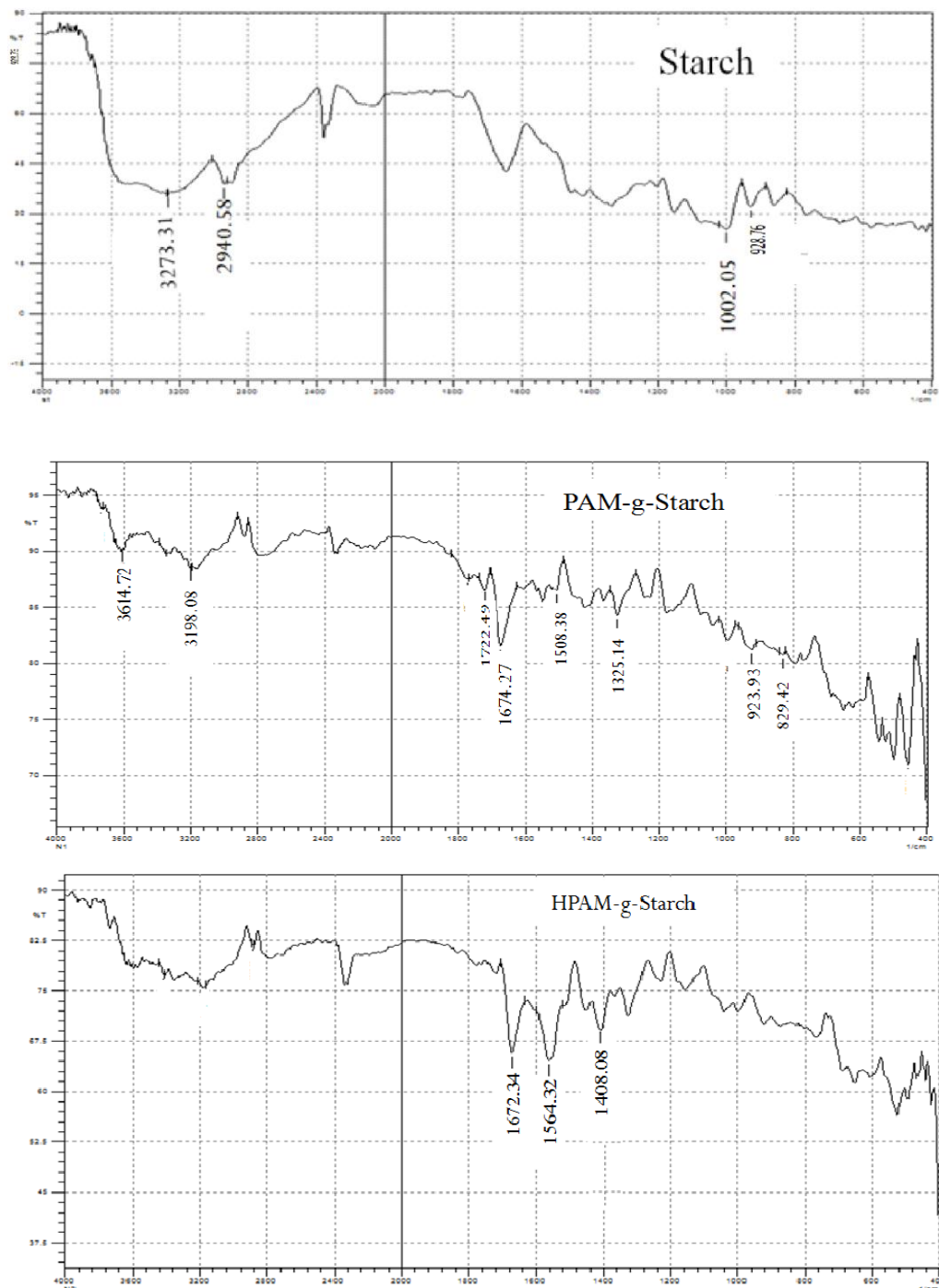


Figure 1: IR spectrum of Maize Starch, graft copolymer (PAM-g-Starch) and hydrolyzed copolymer (HPAM-g-Starch)

IR spectra of Maize Starch:

It shows characteristic peak of O-H stretching (hydroxyl group) at 3273.31 cm<sup>-1</sup> which is an essential group for grafting / target site for acrylamide radical. Further, peaks at 2940.58 cm<sup>-1</sup> for C-H stretching and peaks at 1002.05 cm<sup>-1</sup> and 928.76 cm<sup>-1</sup> for C-O-C stretching give identification of maize starch.

IR spectra of graft copolymer (PAM-g-Starch):

It was found reduction in the hump present in the spectra of Starch at 3273.31 cm<sup>-1</sup>, indicates overlapping of -OH group of Starch and -NH<sub>2</sub> group of Acrylamide with characteristic peak at 3614.72 cm<sup>-1</sup>. The introduction of -CONH<sub>2</sub> group due to grafting was identified by characteristic peaks for C=O stretching at

1722.49  $\text{cm}^{-1}$  and 1674.27  $\text{cm}^{-1}$ , for  $-\text{NH}$  stretching at 3198.08  $\text{cm}^{-1}$  and 1508.38  $\text{cm}^{-1}$  and further for C-N stretching at 1325.14  $\text{cm}^{-1}$ . Peak at 923.93  $\text{cm}^{-1}$  and 829.42  $\text{cm}^{-1}$  suggest the presence of C-O-C stretching for graft copolymer. Presence of these additional peaks indicate the successful completion of grafting process.

#### IR spectra of hydrolyzed copolymer (HPAM-g-Starch):

Hydrolysis partially replaces  $-\text{CONH}_2$  groups of graft copolymer by sodium salt of carboxylic acid ( $-\text{COO}^-\text{Na}^+$ ). This has been identified by peaks for  $-\text{COO}$  stretching at 1564.32  $\text{cm}^{-1}$  and 1408.08  $\text{cm}^{-1}$  along with the peak for C=O stretching at 1672.34  $\text{cm}^{-1}$ . This additional peak on grafted product suggests the completion of saponification.

#### 4. CONCLUSION

Based upon result, the following remarks or conclusion can be drawn:

In the present investigation a novel biopolymer based on starch as natural polysaccharide was developed by microwave assisted grafting method. Four sets of graft copolymer G1 to G4 were prepared by just differing microwave irradiation time, keeping all other factors constant. They were then converted into anionic polymers by alkaline hydrolysis to impart mucoadhesive property.

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