

SYNTHESIS AND CHARACTERIZATION OF pH SENSITIVE AMPIPHILIC NEW COPOLYMER OF METHYL METHACRYLATE GRAFTED ON MODIFIED STARCH: INFLUENCES OF REACTION VARIABLES ON GRAFTING PARAMETERS

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ABSTRACT

Objectives: The present study was performed to investigate the effect of graft copolymerization on modified maize starch for providing a pH sensitive matrix system along with the moisture, chemical and thermal resistance.

Methods: Starch was acetylated with acetic anhydride using 0.5N NaOH as a catalyst and methyl methacrylate (MMA) was grafted onto acetylated starch (Ast) by using ceric ion induced free radical polymerization. Different reaction parameters such as initiator concentration, monomer concentration, reaction time and temperature were optimized to get maximum graft yield (45.93%) and graft efficiency (94.64%). Physicochemical properties were characterized by FTIR, ¹H NMR, [¹³C] NMR, SEM, X-RD and TG-DTA analysis.

Results: Structures of acetylated starch and graft copolymer was confirmed by IR and NMR spectroscopic analysis. Particle shape and morphology were studied by SEM which shows that graft copolymerization leads to enhancement of roughness of carbohydrate surface. Moreover, graft copolymer has been found to be more moisture and chemical resistant. TG-DTA studies revealed that the decomposition temperature of grafted starch was higher than that of native starch and acetylated starch. A suitable mechanism to explain initiation, propagation and termination of graft copolymerization has also been proposed.

Conclusions: Graft copolymer showed higher acid, base and thermal resistance, lower crystallinity and increased moisture resistance as compared to the acetylated and native starch. Swelling behavior at pH 1.2-10 revealed that graft copolymer provided a pH sensitive matrix system and may be useful to overcome the harsh environment of the stomach.

Keywords: Maize starch; Methyl methacrylate (MMA); Acetylated starch; Graft copolymerization.

INTRODUCTION

Hybridization of the natural polymers with synthetic polymers received considerable attention because of its applicability in controlled drug delivery system. Controlled drug delivery technology represents one of the most rapidly advancing areas of pharmaceutical science in which chemists and chemical engineers are contributing to human health care, such delivery systems have numerous advantages compared to conventional dosage forms including improved efficacy, reduced toxicity, and improved patient compliance and convenience [1]. It offers several advantages such as the achievement of an optimum concentration usually for prolonged time, enhancement of the activity of labile drugs by protecting them against hostile environments and diminishing the side effects of drug by reducing its high initial blood concentration [2]. Natural polysaccharides and their derivatives represent a group of polymers widely used in the pharmaceutical and biomedical fields for the controlled release of drugs. Grafting of the natural polymers with synthetic polymers is of great interest because of its application to biomedical and bio-degradable materials. It is one of the methods to increase the compatibility between synthetic polymers and natural polymer like starch. Because of biocompatibility, degradability and low costs, starch and starch derivatives are used as excipients for tableting and matrices for delivery systems in pharmaceutical industry [3, 4]. Starch shows poor flow property and enormous swelling owing to their hydrophilic nature, which results in premature release of drug in the stomach/upper intestine, and therefore, they should be protected while gaining entry into stomach and small intestine. These problems can be rectified by the modification of starch in order to improve their rheological behavior and stability in acidic environment with a variety of useful hydrophobic monomeric and polymeric products by physical and chemical means [5]. Graft copolymerization of synthetic polymers onto a polysaccharide backbone offers one of the best ways to get new co-polysaccharides with enhanced properties for important applications [6, 7]. There is a strong interest in chemically modified

starch derivatives possessing functional groups that allow easy binding of ligands to the surface. Commercial poly acrylic acid (PAA)-based polymers, has been known to possess good adhesion and biocompatibility. However, these PAA polymers alone cannot be used because they tend to be irritant [8]. Therefore, research efforts have been directed to combine the useful properties of PAA polymers, such as bioadhesion and stability in acidic environment with those of polysaccharides (starch), such as swelling characteristics and biodegradability. Among the grafted starch products, polyacrylate group advantageously serve as cross-linker in starch which enhances bioadhesion property of starch. Hence, these starch derivatives can be used in coatings and stabilizers, they can also serve as carriers for delivery systems and controlled drugs release.

MATERIALS AND METHODS

Materials

Maize starch, was obtained from Meru Chem Pvt. Ltd., India. Glacial acetic acid and acetic anhydride was purchased from Sigma Aldrich, U.S.A. Methyl methacrylate (MMA) monomer and solvents of analytical grade were obtained from Merck Ltd., Germany.

Methods

Synthesis of acetylated starch

Acetylated starch was synthesized by the method previously reported by Mark and Mehlretter with some modifications [9]. The starch was dried at 50°C for 24 h before reaction to avoid the interference of moisture. Solution of 100 gms maize starch in 200 ml of acetic acid was placed in 500 ml double mouth flat bottom flask equipped with a mechanical stirrer, a reflux condenser. Reaction mixture was stirred for 1 h at 25°C and maintained at pH 11 with 0.5 N NaOH. After stirring for 1h, 200 ml of cooled acetic anhydride was added drop wise to the mixture and mixture was allowed to reflux for 2 h and then allowed to cool. Ethanol was poured into the

solution with vigorous agitation to precipitate a white to off-white solid. The product was filtered and washed twice with water. The esterified starch was dried at ambient temperature.

Determination of degree of substitution (DS)

Degree of substitution is the average number of hydroxyl groups substituted per anhydroglucose unit. Determination of DS of acetylated starch by titration involved complete basic hydrolysis of the ester linkages and titration of the excess alkali. Degree of substitution depends upon the reaction time and ratio of acetic anhydride to starch [10]. According to previously reported method, the % of acetylation was determined by titrimetric analysis [11]. Esterified starch (1.0 g dry sample) was allowed to swell by placing it in 50 ml of an acetone-water mixture (1:1 by volume) for 24 h at room temperature. Five milliliters of a 1M ethanolic potassium hydroxide solution were added to the suspension to start the complete deacetylation for 24 h at ambient temperature. The same procedure was done for 2 blank values without starch ester. Excess potassium hydroxide was titrated with 0.5M aqueous hydrochloric acid back to a pH of 7.0 by using 1% thymolphthalein as indicator. The consumption of hydrochloric acid was ascertained and acetyl content (w) was calculated according to the following equation:

$$w = \frac{V_1 - V_2 \times M_{\text{sub}} \times C_{\text{HCl}}}{m} \times 100\%$$

Acetyl content (w) was used to calculate the degree of substitution (DS), according to the following equation

$$DS = \frac{162w}{43 - 42w}$$

Where: V_1 the volume of 0.5 M HCl in ml used for titration of blank; V_2 the volume of 0.5 M HCl in ml used for titration of 1.0 g sample; C_{HCl} the molarity of HCl solution (mol/L); m the weight of the sample in mg; M_{sub} , the molecular weight of the acetyl group (43 g/mol); 162 the molecular weight of anhydroglucose unit.

Synthesis of graft copolymer with methyl methacrylate

Copolymer was synthesized via free radical copolymerization of MMA on acetylated starch by following the procedure reported by

Echeverría *et al.* [12]. In 750 ml flask, acetylated starch (40 gm) was dispersed in 500 ml of distilled water. The medium was purged with purified nitrogen and flask was placed in a water bath with desired temperature (30-80°C). Next varying concentration (0.2-0.8 mol/L) of MMA was added, followed by the 50 ml of initiator solution (0.02-0.1M ceric ammonium nitrate in 1N nitric acid) over a period of 15 min. The polymerization reaction proceeded under N_2 atmosphere for varying time intervals (1-3 h) at varying temperature (30-80°C). After completion of the reaction, the product was filtered and filtered product was further extracted with the dimethylformamide to remove the traces of homopolymer. The final product was dried under vacuum until constant weight. Percentage polymer loading (%PL), percentage graft yield (%GY), percentage graft efficiency (%GE) and percentage photopolymer (%HP) formed were calculated by equations mentioned below [13] and results are depicted in Table.1.

$$\text{Percent polymer loading (\%PL)} = \frac{W_2 - W_1}{W_1} \times 100$$

Where W_1 and W_2 are the initial and final weights of the sample, respectively, (before photopolymer extraction).

$$\text{Percent graft yield (\%GY)} = \frac{W_3 - W_1}{W_1} \times 100$$

The quantity of the grafted polymer is evaluated as the weight increase of the sample (W_3) after extraction of the photopolymer.

% Add-on computed the weight in percentages of MMA added in the graft copolymer

$$\% \text{ Add on} = \frac{W_3 - W_1}{W_3} \times 100$$

Percent grafting efficiency (% GE) was used to quantify the amount of photopolymer formed during the grafting reaction:

$$\% \text{GE} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Graft efficiency expresses the selectivity of the main reaction with respect to the most important byproduct photopolymer.

$$\text{Percent homopolymer (\%HP)} = 100 - \% \text{GE}$$

Table 1: Overview of the results-responses of various selected reaction parameters for grafting of MMA onto acetylated starch

S. No.	Initiator conc. (M)	Monomer conc. (mol/L)	Reaction Time (h)	Reaction Temp. (°C)	%PL ^a	%GY ^b	%Add-on	%GE ^c	%HP ^d
1.	0.02	0.2	1	30	35.90	23.15	18.80	64.48	35.52
2.	0.04	0.2	1	30	33.78	25.68	20.43	76.02	23.98
3.	0.06	0.2	1	30	38.08	30.93	23.62	81.22	18.78
4.	0.08	0.2	1	30	31.43	27.28	21.43	76.79	23.21
5.	0.1	0.2	1	30	25.78	17.08	14.58	66.25	33.75
6.	0.06	0.4	1	30	43.48	37.35	27.19	85.91	14.09
7.	0.06	0.6	1	30	26.79	21.06	17.40	78.61	21.39
8.	0.06	0.8	1	30	25.93	15.85	13.68	61.13	38.87
9.	0.06	0.4	2	30	46.45	40.60	28.88	87.41	12.59
10.	0.06	0.4	3	30	26.53	20.68	17.13	77.95	22.05
11.	0.06	0.4	2	40	42.83	41.38	28.25	91.94	8.06
12.	0.06	0.4	2	50	48.53	45.93	31.47	94.64	5.36
13.	0.06	0.4	2	60	40.68	34.78	25.80	85.49	14.51
14.	0.06	0.4	2	70	38.73	30.28	23.24	78.18	21.82
15.	0.06	0.4	2	80	34.53	25.28	20.18	73.21	26.79

^a%PL: Percent polymer Loading

^b%GY: Percent graft yield

^c%GE: Percent grafting efficiency

^d%HP: Percent photopolymer

Characterization

IR spectra of pure starch, modified starch and graft copolymers were recorded with SHIMADZU Infrared-spectrophotometer, FTIR-8400S using KBr pellet technique. The $^1\text{H-NMR}$ and $[^{13}\text{C}]$ NMR spectra's were measured by JEOL, AL300, FT-NMR spectrophotometer with operating frequency, 300 MHz at temperature 25°C using DMSO-d_6 as solvent containing TMS as internal standard and chemical shift was reported in δ values (ppm). Scanning electron microscopic study was carried out using ZEISS Supra 40 scanning electron microscope at operating voltage 15.0 kV. Powder X-ray diffraction (X-RD) were recorded using Rigaku-DMAX-III diffractometer with $\text{Cu-K}\alpha$ radiation from a Cu target at $2\theta = 5\text{--}40^\circ$ range with operating voltage of 40 kV and current of 30 milliamperere. Data were recorded at a scanning rate of $1^\circ/\text{min}$ at 6 kW energy. The TG-DTA analysis was carried out on Labsys TG-DTA16 instrument from ambient temperature to 700°C in atmospheric air with heating rate $10^\circ\text{C}/\text{min}$.

Moisture absorbance study

Moisture absorbance studies at various relative humidities (RH) were carried-out as per the standard method reported by Kaith *et al.* [14]. Moisture absorbance (%) was found out by placing a known weight (W_i) of dry grafted and ungrafted acetylated samples in a humidity chamber for 2 h and the final weight (W_f) of the samples exposed to a specific RH was taken and calculated as:

$$\% \text{ moisture absorbance } (\%M_{\text{abs}}) = \frac{W_f - W_i}{W_i} \times 100$$

Chemical resistance analysis

As per previously reported method, studies of chemical resistance were carried-out as a function of percentage weight loss of native, modified starch and its grafted copolymer when treated with acid and base [14]. A known amount (W_i) of the sample was separately treated with a fixed volume of 5N hydrochloric acid and 5N sodium hydroxide and the weight of the sample was noted down at 12h intervals until a constant weight was reached. The sample were then washed 2-3 times with distilled water and finally dried in an oven at 70°C to a constant weight. The samples were weighed again to obtain the final weight (W_f) and % weight loss was calculated as:

$$\% \text{ weight loss} = \frac{W_i - W_f}{W_i} \times 100$$

Swelling studies

The equilibrium swelling was measured according to the conventional "tea bag" method. The completely dried preweighed samples (native, modified starch and graft copolymer) were placed in fixed volume of various polar and non-polar solvents such as water, buffer solutions (pH 1.2-10), ethanol, dimethylformamide and carbon tetrachloride at 37°C . The tea bag was taken out at regular time intervals, wiped superficially with filter paper to remove surface water, weighed, and then placed in the same bath. The mass measurements were continued until the equilibrium was attained. The percentage mass swelling was determined using the following expression [15]:

$$(\%SM) = \frac{M_t - M_0}{M_0} \times 100$$

Where M_0 and M_t are the initial mass and mass at different time intervals, respectively.

RESULTS AND DISCUSSION

Degree of substitution (DS) in acetylated starch

Acetylated starch was synthesized by the reaction of starch with acetic anhydride in the presence of acetic acid and sodium hydroxide. This is an example of nucleophilic substitution at an unsaturated carbon atom of acetic anhydride. The DS of acetylated starch was determined by back titration and it is defined as the number of hydroxyl groups per anhydroglucose unit, which have

been substituted by other groups. Since anhydroglucose units contain originally three hydroxyl groups, the maximum possible DS is three. According to Xu *et al.*, degree of substitution increases with increasing the reaction time and increasing ratio of acetic anhydride to starch [10]. The results indicated that starch acetate with low degree of substitution (DS 0.97) was synthesized by the method employed in this work having ratio of acetic anhydride to starch 2:1 and reaction time 2 hrs. DS value <1 indicates acetylation of less than one hydroxyl group of anhydroglucose unit. Furthermore, the three free hydroxyl groups of anhydroglucose unit have different reactivities. The primary OH on C_6 is more reactive and is acetylated more readily than the secondary ones on C_2 and C_3 due to steric hindrance. So with DS value <1 , the attack was supposed to take place at hydroxyl group substituted on C_6 of anhydroglucose unit [10].

Mechanism of graft copolymerization

The proposed mechanism for grafting of methyl methacrylate onto acetylated starch backbone is shown in scheme 1. This mechanism is supported by previous finding of some other authors [16, 17]. With ceric ammonium nitrate, grafting reaction proceeds through redox mechanism in which Ce(IV) ions are reduced to Ce(III) ions by the transfer of electron from the acetylated starch molecules, and hence active sites are generated on the polymeric backbone at which monomer radicals can attack to form graft copolymers. Ceric ions form complexes with the carbon chain of polymer backbone and then breaking of $\text{C}_2\text{-C}_3$ bonds of anhydroglucose unit in acetylated starch results in the formation of free radical sites on the polymeric backbone. These free radicals initiate the monomer grafting and the polymerization reaction of MMA.

Reactions between growing MMA chains also resulted in the termination of reaction with the formation of photopolymer [13]. Hence, concentrations of initiator and monomer, variations of time, and reaction temperature affect the graft yield as these factors determine the relative population of various radical species generated in different steps during the course of reaction.

Effect of reaction parameters on grafting efficiency

In order to study the efficiency of the graft copolymerization reaction, the poly methyl methacrylate homopolymer (PMMA) was removed from the total reaction product, with the dimethylformamide, by soxhlet extraction.

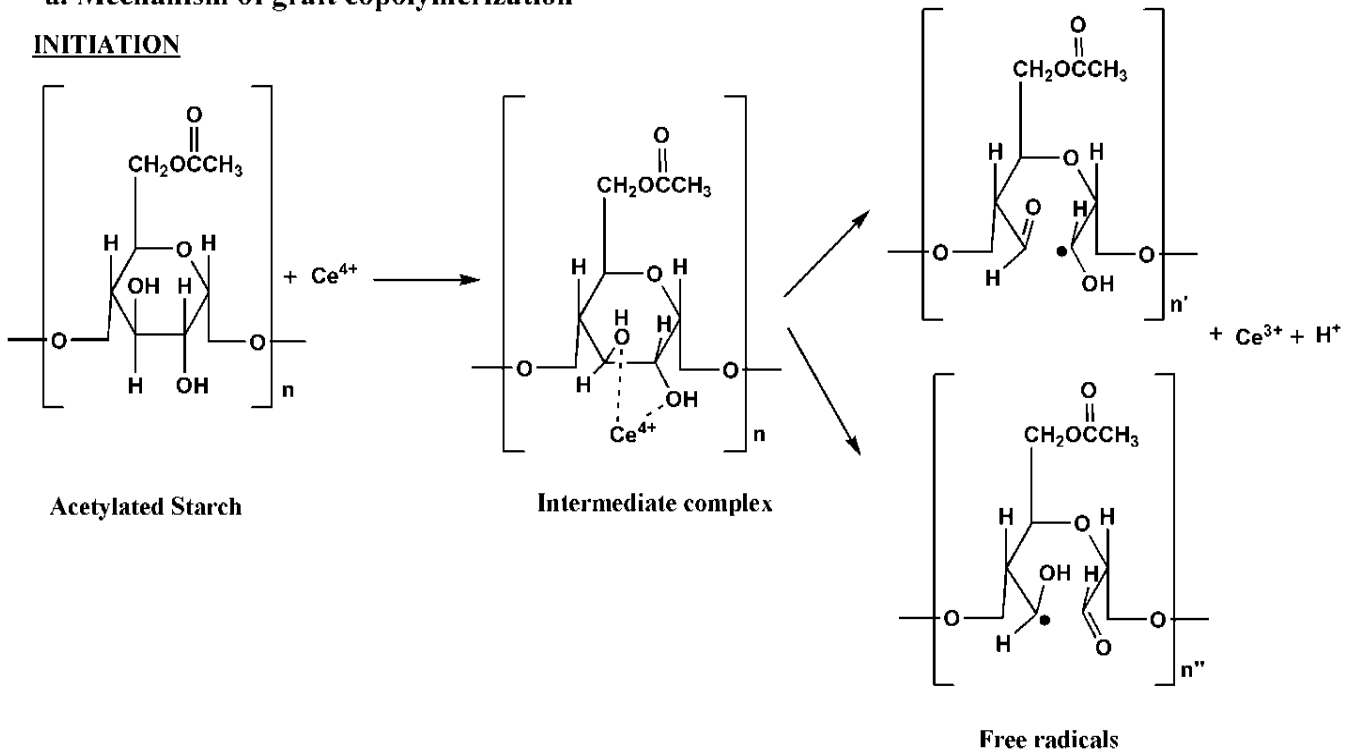
Graft copolymer of methyl methacrylate with acetylated starch was synthesized by free radical polymerization utilizing ceric ammonium nitrate as a redox initiator and relative population of various radical species generated in different steps during the course of reaction. These free radical species affect the grafting efficiency; hence to optimize the conditions for grafting of MMA on acetylated starch, the initiator concentration, monomer concentration, reaction time and temperature were varied. Effect of variable parameters on grafting yield and grafting efficiency are shown in Table.1.

Effect of CAN (Ceric Ammonium Nitrate) Concentration

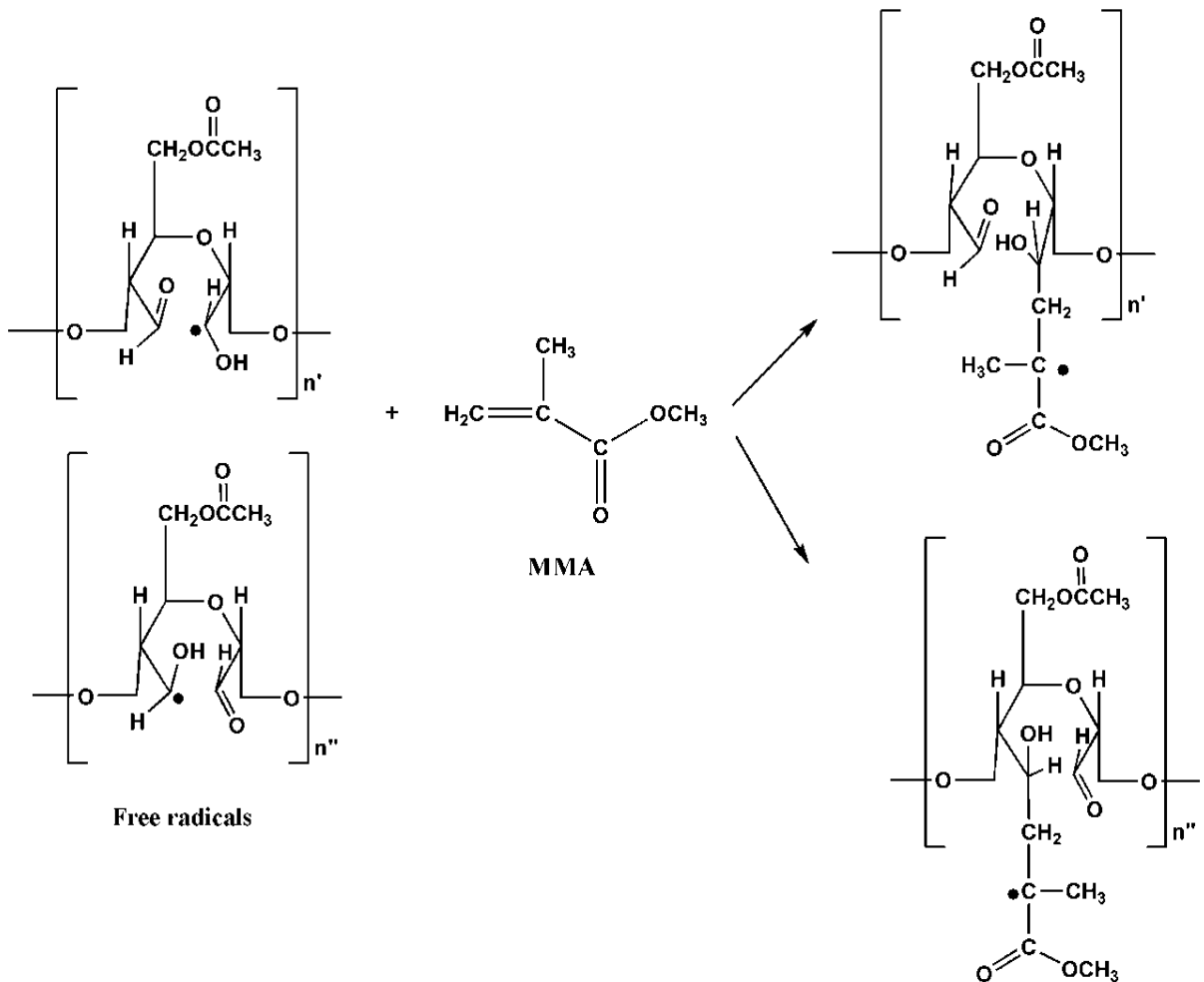
It has been observed that percentage graft yield and graft efficiency were found to increase with increase in CAN concentration, but after reaching critical value (0.06 M) further increase in concentration resulted in decreased graft yield and graft efficiency. The initial increase in percentage graft yield and efficiency may be ascribed to the increase of the active sites on the backbone of the acetylated starch arising from the attack of Ce^{4+} . The decrease of grafting parameters at higher concentration of CAN may be due to (a) oxidative degradation of modified starch chains by excess Ce^{4+} ions, (b) an increase in the termination reaction of the chain radicals via bimolecular collision because of an increased population of macro radicals produced. Another factor which could contribute to a decrease in the grafting percentage at higher concentration of initiator is the increase the photopolymer formation, which competes with the grafting reaction for the available monomer. These observations are in agreement with similar observations reported by others [18, 19].

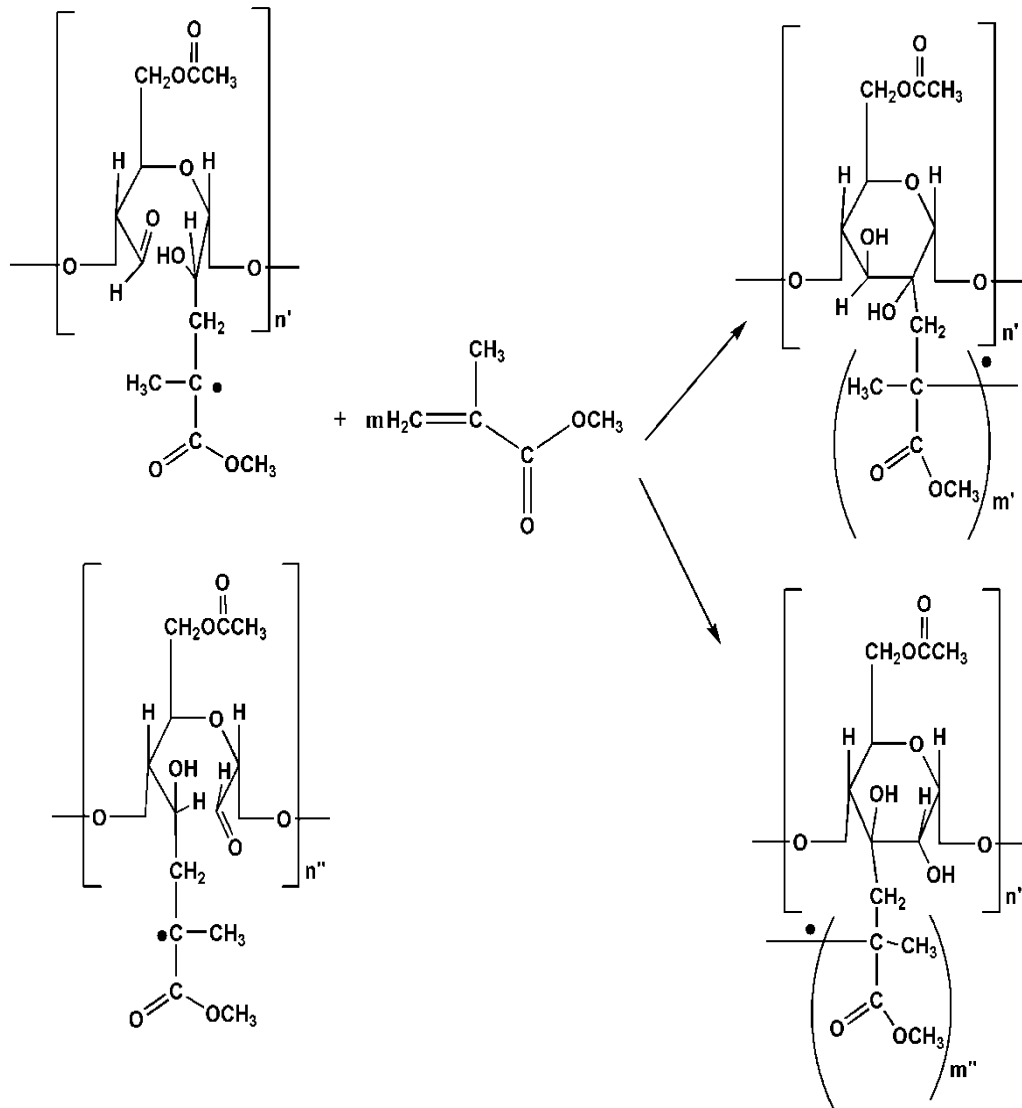
a. Mechanism of graft copolymerization

INITIATION

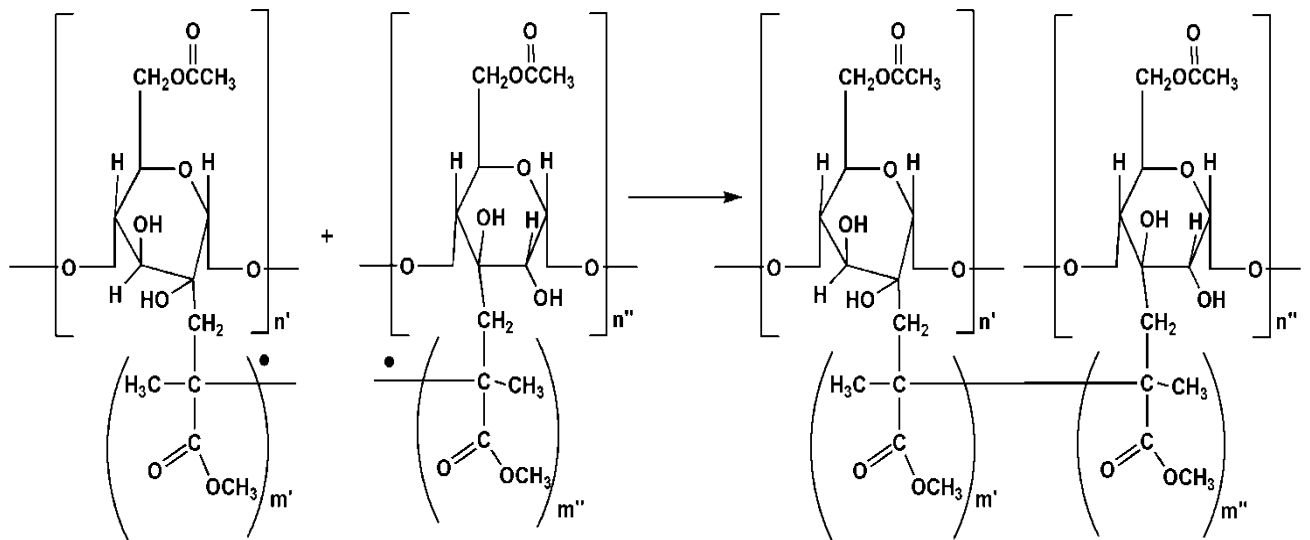


PROPOGATION





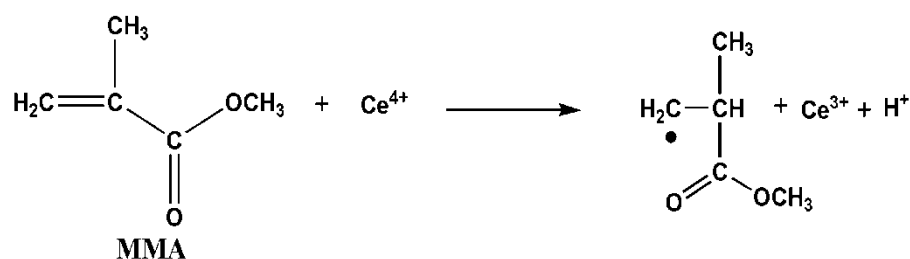
TERMINATION



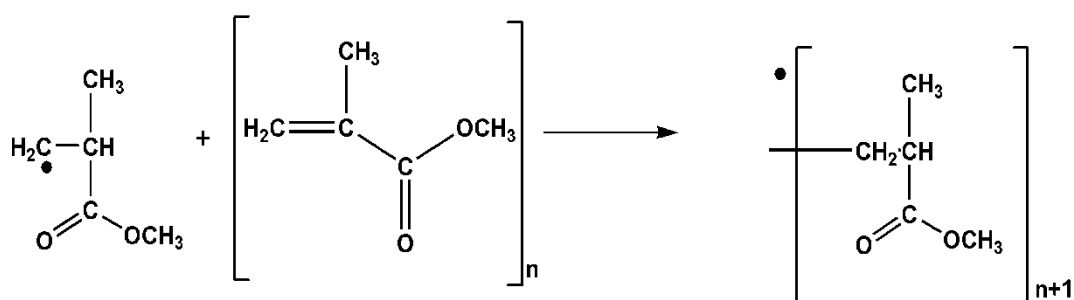
Graft copolymer (Ast-g-PMMA)

b. Mechanism of homopolymer formation

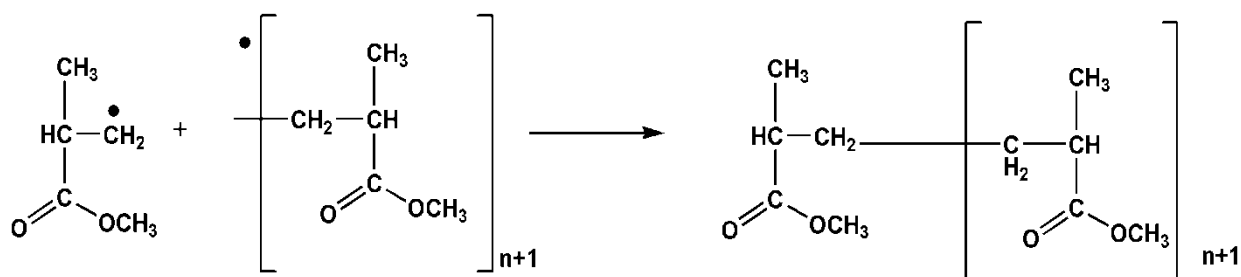
INITIATION



PROPOGATION



TERMINATION



Homopolymer

Scheme 1: Proposed mechanism for (a) graft copolymerization of MMA onto acetylated starch (b) homopolymer formation

Effect of monomer concentration

It was observed that with increase in concentration of MMA, %GE increased continuously and reached maximum value (85.91%) at 0.4 mol/L, thereafter it decreases (Table.1). This could be ascribed to the increase of monomer concentration in the vicinity of the acetylated starch backbone as well as greater availability and enhanced chances for molecular collisions of the reactants, which leads to graft copolymerization. However, after a certain level of MMA, %GE decreases probably due to preferential homopolymerization over graft copolymerization as well as increasing the viscosity of reaction medium (gel effect), which hinders the movement of free radicals. Needless to say, the increase in the viscosity of reaction medium inhibit the diffusion of Ce^{4+} and the monomer into acetylated starch for further grafting. Similar observations have been reported for the grafting of ethyl acrylate onto cellulose [20], methyl acrylate onto starch [21] and

methyl methacrylate onto acetylated *Saccharum spontaneum L.* fiber [13].

Effect of reaction time

It was found that with increase in reaction time % GY and % GE increased gradually and reached maximum value 40.60% & 87.41%, respectively in 2 h. This is attributed to the increase of the extent of initiation and propagation of the graft copolymerization with time (Table.1). However, beyond the optimum reaction time, the graft percentage decreases and levels off, which may be attributed to the decrease in monomer and initiator concentrations and retardation of diffusion when polymer formed on the acetylated starch surface. The grafting loss may also be attributed to the decrease in the number of sites available for grafting, disintegration of poly (MMA) chains grafted on back-bone and predominance of homopolymerization

over graft copolymerization. Similar time dependency of grafting parameters was reported by other authors [22, 23].

Effect of reaction temperature

According to Witono et al., optimal temperature for the grafting reaction is much dependent on the initiation system [24]. Lutfur et al. reported that, for graft copolymerization with ceric ammonium nitrate, the optimum temperature was 50°C [25]. It can be seen from the results that the % GY and % GE were increased with increasing of reaction temperature from 30 to 50°C. Further increase of reaction temperature led to a decrease in grafting yield and efficiency. Increase of the grafting percentage from 30 to 50°C could be attributable to an increase in the number of the free radicals formed on the backbone, increase in the mobility of monomer molecules and their collision with backbone macro radicals, and enhancement of diffusion of monomer and initiator onto the back bone structure [26]. The decrease in % GY and % GE beyond 50°C can be ascribed both to the premature termination of growing grafted chains due to excess of free radical formation and the increased chance of chain transfer reaction, accounting for the increase in the amount of homopolymers.

Characterization

Infrared spectroscopy (IR)

Infrared spectroscopy confirms the changes of chemical structure of the native starch molecule resulting from acetylation and subsequent grafting with MMA. In the spectrum of native starch, the peaks at 3000-3600 cm^{-1} and 2900-2950 cm^{-1} correspond to OH and CH stretchings, while peaks at 1645 cm^{-1} and 1420 cm^{-1} correspond to OH and CH bendings (fig.1a). In comparison to the spectrum of starch, acetylated starch showed a strong absorption band at 1744.43 cm^{-1} that was attributed to stretching of the ester carbonyl C=O group and intensity of the broad peak due to hydrogen bonded -OH was found to decrease which confirmed the acetylation of starch (fig.1b). On grafting with MMA, IR spectrum of Ast-g-PMMA showed an additional stretching vibration band at 1750-1735 cm^{-1} due to aliphatic carbonyl group which confirms the grafting of MMA in anhydroglucose moiety of acetylated starch (fig.1c). The typical stress (=C-H) and flexion (C=C) bands of unsaturated carbonyl compounds at approximately 3040-3010 cm^{-1} and 1650-1630 cm^{-1} are not evident, confirming the absence of unreacted MMA [27]. Moreover, the copolymer showed typical bands in the range of 1150-1270 cm^{-1} characteristics of C-O stretch vibration from ester.

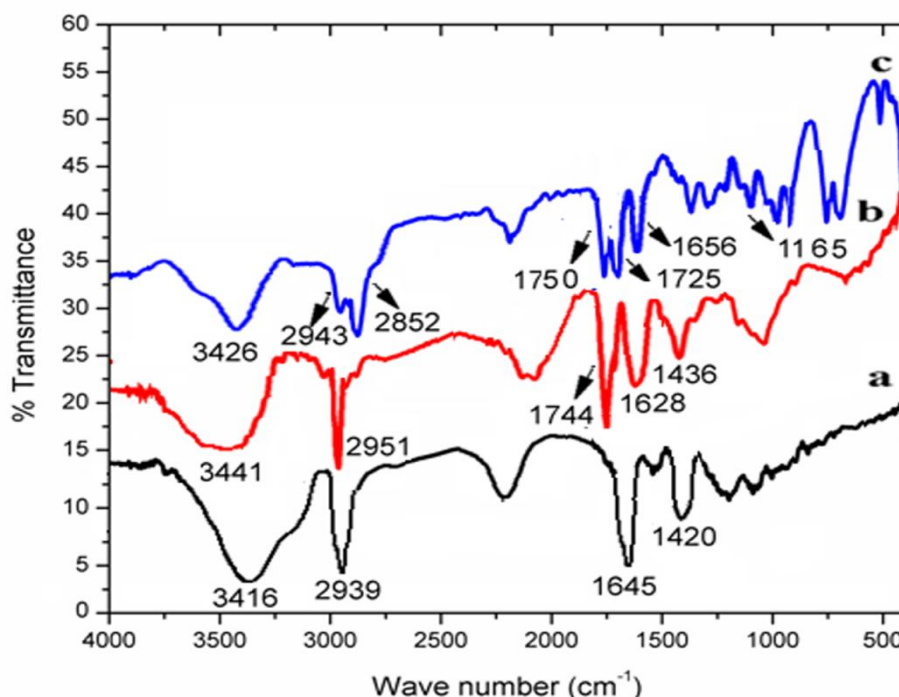


Fig. 1: FTIR spectra of (a) native starch (b) acetylated starch (c) Ast-g-PMMA

^1H and $[^{13}\text{C}]$ NMR spectral analysis

Furthermore, the graft copolymer and its parent carbohydrates were characterized by ^1H and $[^{13}\text{C}]$ NMR spectroscopy. Findings of NMR spectroscopy support the results obtained by IR spectroscopy. In the ^1H -NMR spectrum of native starch, the characteristic peaks located in the range of 4.59-5.50 ppm assigned to the proton signals of hydroxyl group on C-2, C-3 and C-6 while the proton signals assigned to CH and CH_2 of the starch unit are centered at about 3.60-3.8 ppm. Presence of characteristic peaks in ^1H NMR of acetylated starch in the range of 2.02-2.11 ppm confirms the presence of protons of acetyl group as well as proton resonances of hydroxyl group of anhydroglucose unit showed some changes compared with that of native starch which indicate the participation of hydroxyl group in esterification and it has been supported by Chi et al. [28]. On graft copolymerization of acetylated starch with MMA new peaks were observed in the range of 0.8-1.4 ppm indicating the presence of methyl group and presence of some new peaks at 3.6-3.9

ppm representing the protons of methoxy groups present in MMA [29]. The $[^{13}\text{C}]$ NMR spectrum of the unreacted starch showed characteristic peaks at 63.24 (C_6), 66.29-73.73 ($\text{C}_2, \text{C}_3, \text{C}_5$), 81.03 (C_4) and 101.78 (C_1) due anhydroglucose units of starch. In modified starch, acetyl group was introduced into starch with esterification process and the NMR spectra showed new intense signals at 21.29 ppm attributed to methyl carbon and 175.37 ppm assigned to the carbonyl carbon ($>\text{C}=\text{O}$) of acetate. $[^{13}\text{C}]$ -NMR spectra of the grafted copolymer Ast-g-PMAA showed new peaks around 18-19 ppm, representing the methyl carbon, 54-55 ppm representing the methoxy carbon and 178-180 ppm assigned to the carbonyl carbon. Moreover the lack of bands at 110-140 ppm of olefinic carbon atoms ($\text{C}=\text{C}$) proves the absence of MMA without grafting [30].

Scanning electron microscopy (SEM)

The microphotographs of the native starch, acetylated starch and its grafted copolymer used in this study illustrate the morphological

alteration in the granule morphology and surface texture. SEM micrographs of native starch revealed that most of the starch granules are round in shape and having smooth surfaces without scratches (fig.2a) while the surfaces of acetylated starch granules were highly eroded with various cracks (fig.2b). This morphology has also been detected by Xu et al. [10]. The particles, after copolymerization, showed more irregular morphology and appeared as aggregate of various size granules (fig.2c), [12]. Exposure of heat

during drying may cause slight gelatinization of the surface of the grafted copolymer and granules adhere together to form aggregates whereas surface roughness of the grafted copolymer is probably due to the milling process carried out after drying [30]. Rough surfaces of the grafted copolymer are expected to be favorable for improvement of adhesion because with an increase in the roughness of surface leads to enhancement of surface area for bonding and mechanical interlocking [13].

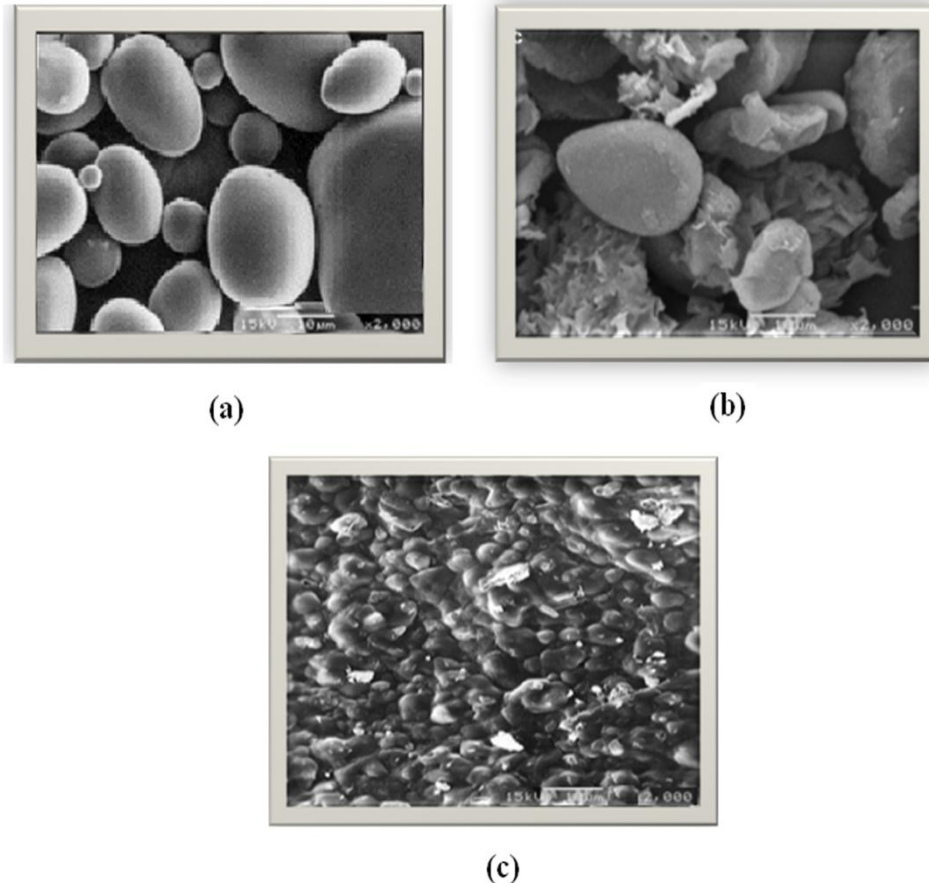


Fig.2: SEM images of (a) maize starch (b) acetylated starch (c) Ast-g-PMMA

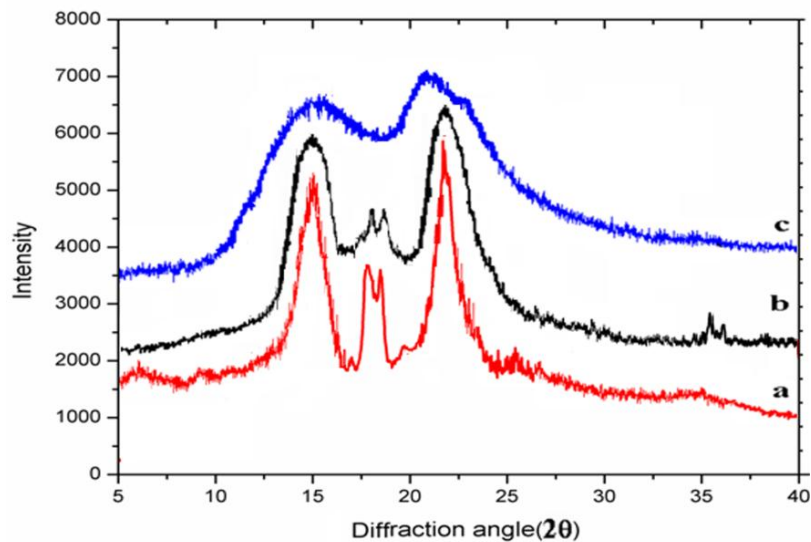


Fig. 3: X-ray diffraction patterns of (a) maize starch (b) acetylated starch (c) Ast-g-PMMA

X-ray diffraction (XRD) studies

X-ray diffraction analyses were performed to study the effect of chemical modification on crystallinity of starch. The X-ray diffraction spectra of native starch, acetylated starch and grafted copolymer are presented in fig.3. In agreement with other authors native starch showed a typical pattern with major reflexes at $2\theta = 15^\circ$ and 23° , and an unresolved doublet at 17° and 18° which demonstrated moderate crystallinity of starch [27, 31]. In agreement with Chi et al., acetylated starch showed similar profile of native one but it had broad diffraction peaks at 9° - 18° which indicates incorporation of acetyl group in the back-bone of starch impairs its crystallinity [28]. On graft copolymerization with MMA further decrement in the intensity of diffraction peaks of copolymer confirms that the graft reaction leads to amorphization of the structure.

Thermogravimetric and Differential thermal analysis

The thermogravimetric analysis was used to examine the thermal stability of starch and grafted copolymer. The TG-DTA curves of native starch, acetylated starch and grafted copolymer were depicted in fig.4. During thermal analysis, samples got degraded by dehydration, glycogen formation and depolymerization. It can be noticed that maize starch shows three-step thermal decomposition in the temperature range from 70-130°C with 12.31% weight loss and two consecutive overlapping steps in the temperature range between 255-455°C with 81.2% weight loss. The former stage was ascribed to loss by dehydration and pyrolytic volatilization processes, whereas the later stage was attributed to loss by depolymerization and oxidation of the organic matter. In case of acetylated starch, the onset of degradation started at higher

temperature than native starch with lower weight loss because of less number of free hydroxyl groups available after acetylation, indicating its higher thermal stability. For acetylated starch, two-step decomposition was found in the temperature range from 110 to 200°C with 41.10% weight loss and 325-500°C with 37.71% weight loss. The first step of decomposition begins with the loss of moisture and volatiles such as CO, CO₂ and some hydrocarbon whereas second step of decomposition resulted in carbonization and ash formation. Ast-g-PMMA also showed two-stage decomposition in the temperature range from 225-330°C with 32.56% weight loss and 410-540°C with 41.87% weight loss. The first stage decomposition occurred due to loss of moisture, decarboxylation and chain scissions while the second stage pertains to the breaking up of covalent bonds. The shift to higher decomposition temperature could be accounted due to increase in covalent bonds in the graft copolymers. DTA curves confirmed the TG results; maize starch exhibited three endothermic peaks at 86 °C, 315°C and 418°C respectively. Endothermic peak at 86°C corresponds to decomposition stage between 70-130°C while the endothermic peak at 305°C and 418°C corresponds to second decomposition stage (255-455°C) in TGA (Fig.4a). However, acetylated starch showed endothermic decomposition at 160 and 445°C (Fig.4b), broad endotherm in DTA curve indicated that acetylated starch was thermally more stable than native one. In case of Ast-g-PMMA, endothermic peak arising at 281 °C corresponds to the thermal decomposition between 225-330 °C and endothermic peak at 492°C corresponds to decomposition stage between 410-540°C in TGA (Fig.4c). TG-DTA studies revealed that the decomposition temperature of grafted copolymer was higher than that of native and modified starch.

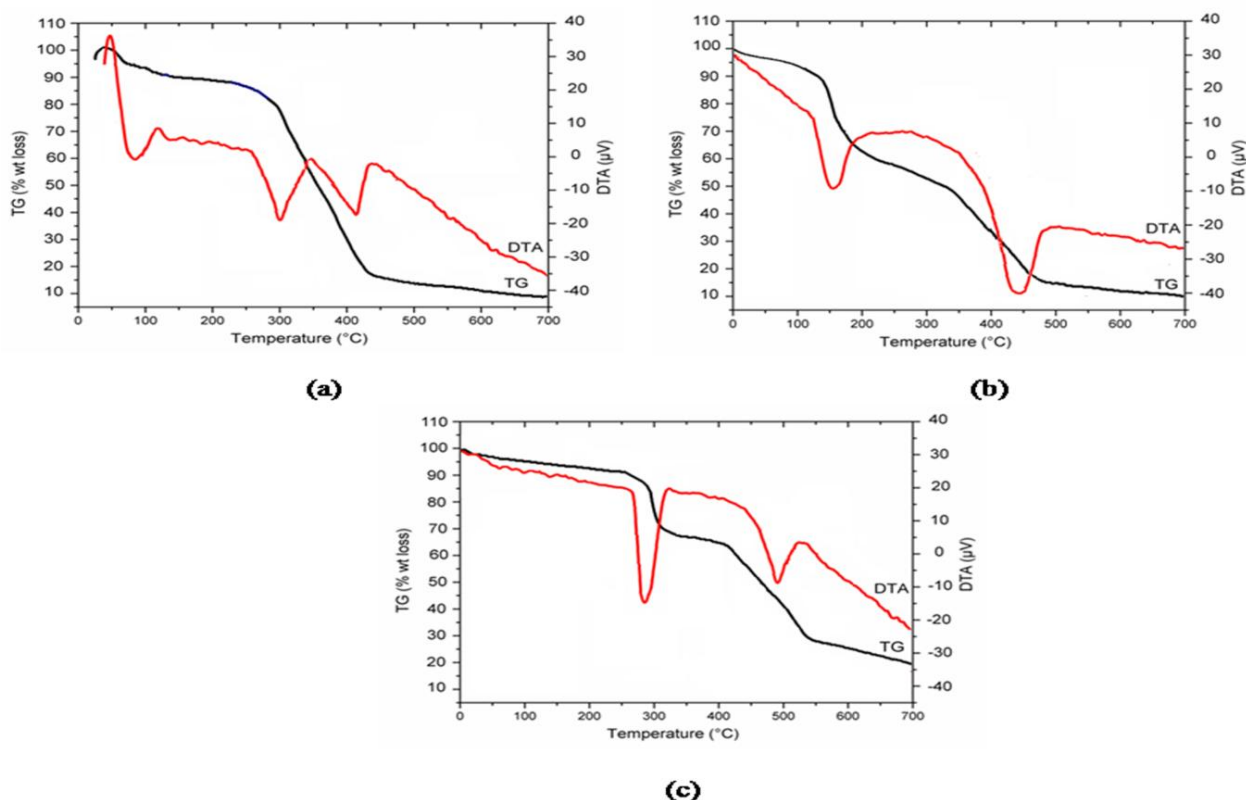


Fig. 4: TGA and DTA curves of (a) native starch (b) acetylated starch (c) Ast-g-PMMA

Moisture absorbance study

In comparison to native and acetylated starch, grafted copolymer showed higher moisture resistance (fig.5a). The extent of moisture absorbance was found to be decrease with

increasing the % graft yield (fig.5b). These results can be supported by the fact that the sites prone to be attacked by moisture were blocked with hydrophobic poly methyl methacrylate chains on grafting, thereby, decreasing the sensitivity of starch towards moisture.

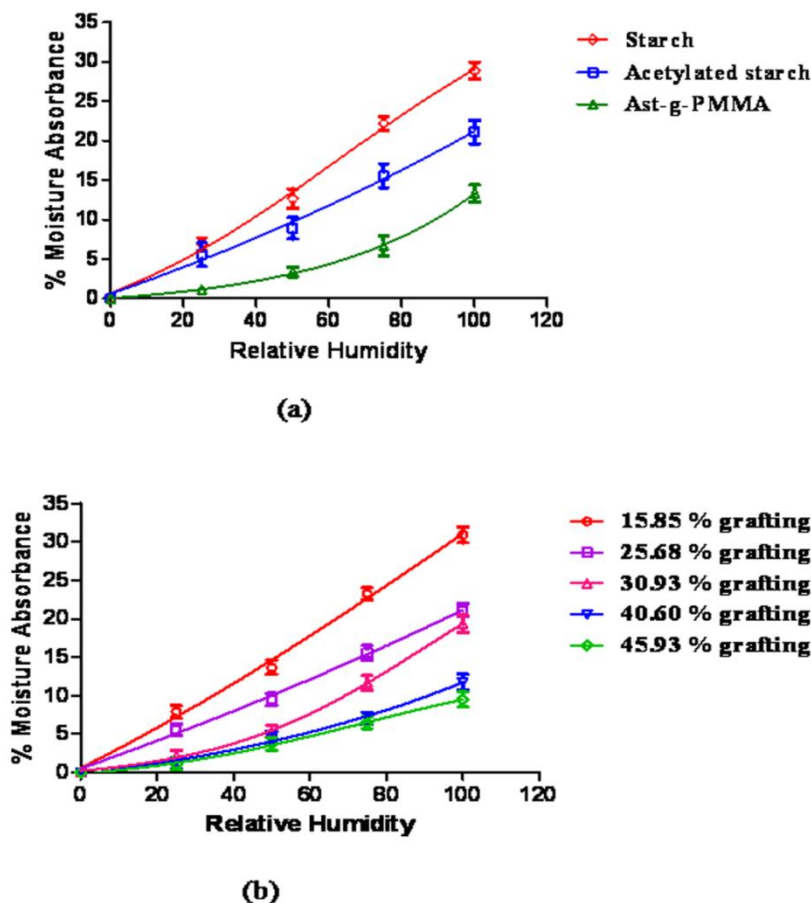


Fig. 5: (a) Moisture absorbance capability of starch, acetylated starch and Ast-g-PMMA (b) Effect of grafting on moisture absorbance at different humidity levels.

Chemical resistance

Acid and base resistance of the native starch was found to be increased on acetylation and then subsequent grafting with MMA. As revealed in fig. 6, for a given strength of acid (5N HCl) and base (5N NaOH), native starch showed maximum weight loss 66.3% and 70.9% respectively. It has been also observed that chemical resistance of the acetylated starch enhanced with increase in degree of grafting. The weight loss decreased with the increase in % graft yield, reaching to minimum values i.e. 44.3% in acid and 48.9% in base for graft copolymer having 45.93% grafting (fig.6 a & b). This is attributed to less affinity of poly(MMA) chains grafted copolymer for acid and base as compared to hydroxyl and other functional groups present in ungrafted starch. Therefore, the greater resistance of grafted copolymer towards acid could be due to the blocking of reactive sites on the starch backbone by poly(MMA) chains that are incorporated as a result of graft copolymerization.

Swelling studies

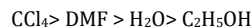
One of the factors governing the drug release is the swelling of the matrix. Therefore, we studied the swelling behavior of the graft copolymer in different solvents such as water, buffer of pH 1.2- 10, ethanol, dimethylformamide (DMF) and carbon tetrachloride.

Fig.7a showed the percent swelling of native, acetylated starch and grafted copolymer in different solvents. In comparison to grafted copolymer, lots of free hydroxyl groups are present in native starch and acetylated starch which has strong affinity for water. Therefore, native and acetylated starch showed maximum swelling in water whereas least swelling was observed in CCl₄, which is a non-polar solvent having little affinity for hydrophilic hydroxyl groups present in native starch and acetylated starch. On the basis of polarity,

swelling order of starch and acetylated starch was observed as follows:

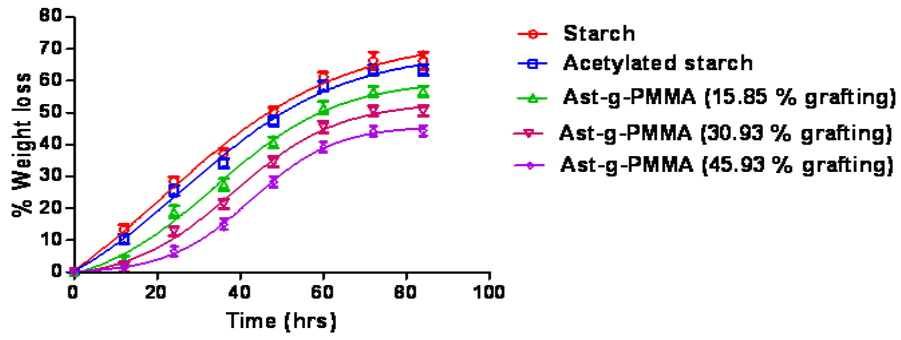


Due to incorporation of hydrophobic poly-MMA chain, grafted copolymer was solvolyzed by non polar solvents such as CCl₄ to a maximum extent and showed maximum swelling in CCl₄. Grafted copolymer exhibit least swelling in C₂H₅OH due to presence of bulky alkyl group, which leads to less solvolyzation of polymeric chains on grafted copolymer. The order of swelling of grafted copolymer in different solvents is given as:

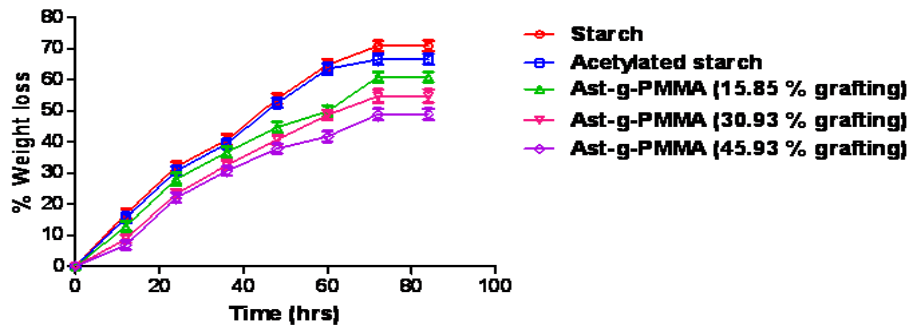


Affinity towards nonpolar solvents increased with increase in degree of grafting (fig.7b) whereas for polar solvents it was found to decrease.

Swelling behavior of graft copolymer in various buffer solution of pH 1.2-10 revealed that swelling is very small when the pH is lower than 5.0 whereas it increases sharply upto pH 8 and then further increases slowly upto pH 10 (fig.7c). The swelling depends on the intermolecular- interaction between the polymer components of grafted copolymer. At lower pH, the carboxyl group of the grafted methyl methacrylate chain remains almost in unionized state and involved in hydrogen bond formation with hydroxyl group of starch which makes the polymer segments rigid, thereby hindering the water uptake and lowering the extent of swelling whereas at higher pH the carboxyl groups get ionized which decreases the starch-acrylic interaction by decreasing H-bonding; the repulsion among similarly charged COO-group increases chain relaxation; and ionic nature of chains facilitates water uptake, with the overall result being increase in the extent of swelling in the medium of higher pH.



(a)



(b)

Fig. 6: Effect of grafting on (a) acid resistance (b) base resistance.

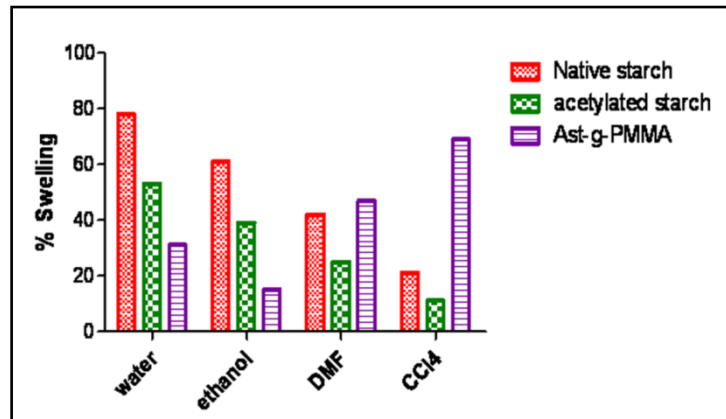


Fig. 7a: Swelling behavior of native starch, acetylated starch and Ast-g-PMMA in different solvents

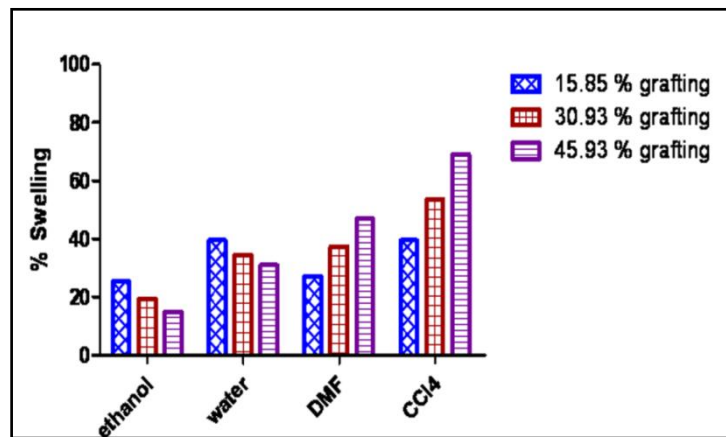


Fig. 7b: Effect of grafting on swelling behavior of Ast-g-PMMA in different solvents

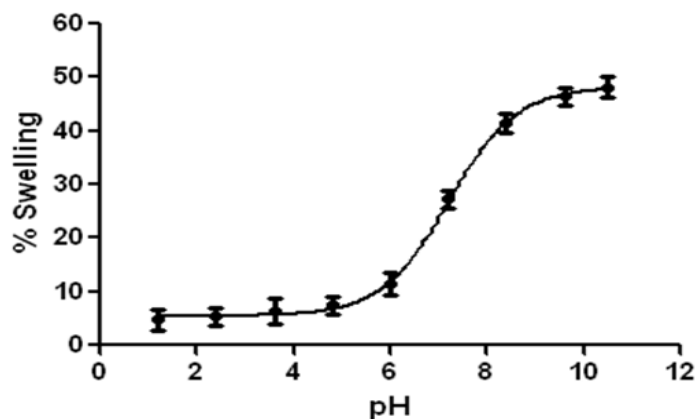


Fig.7c: Effect of pH on swelling behavior of graft copolymer (Ast-g-PMMA), Vertical bar represents mean \pm SD (n=3).

CONCLUSIONS

Acetylated starch with low degree of substitution was synthesized by a method in which maize starch reacted with acetic anhydride using NaOH as catalyst and it has been successfully modified through grafting by CAN induced free radical polymerization. Reaction conditions were optimized for grafting of MMA on acetylated starch by varying the concentration of CAN, concentration of MMA, polymerization time, and reaction temperature. Acetylation and then subsequent incorporation of methyl methacrylate chain is confirmed by IR, ^1H and $[^{13}\text{C}]$ NMR spectroscopies. Large value of % graft efficiency (61–94%) indicated the selectivity of the main reaction with respect to the byproduct photopolymer. Graft copolymer showed modification in terms of higher acid, base and thermal resistance, lower crystallinity, higher hydrophobicity, more irregular morphology and decrease in moisture absorbance as compared to the acetylated and native starch. Swelling behavior revealed that graft copolymer provided a pH sensitive matrix system for site-specific drug delivery. So, it may be concluded that methyl methacrylate grafted copolymer of acetylated starch, can be a good candidate to overcome the harsh environment of the stomach and can possibly be used in future as excipient in the controlled drug delivery system, which is being investigated.

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REFERENCES

- Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM. Polymeric system for controlled drug release. *Chem Rev* 1999; 99: 3181-3198.
- Maiti, S, Ranjit S, Sa B. Polysaccharide based graft copolymers in controlled drug delivery. *Int J Pharm Tech Research* 2010; 2: 1350-1358.
- Dumoulin Y, Cartilier LH, Mateescu MA. Cross-linked amylose tablets containing alpha-amylase: an enzymatically-controlled drug release system. *J Control Release* 1999; 60: 161-167.
- Röper H. Renewable raw materials in Europe-industrial utilization of starch and sugar. *Starch/Stärke* 2002; 54: 89-99.
- Shaikh MM and Lonikar SV. Starch-acrylics graft copolymers and blends: Synthesis, characterization, and applications as matrix for drug delivery. *J Appl Polym Sci* 2009; 114: 2893-2900.
- Goñi I, Gurruchaga M, Valero M, Guzman GM. Graft polymerization of acrylic monomers onto starch fractions. I. Effect of reaction time on grafting methyl methacrylate onto amylose. *J Polym Sci: Polym Chem Ed* 1983; 21: 2573-2580.
- Athawale VD, Rathi SC. Synthesis and characterization of starch-polymethacrylic acid graft copolymers. *J Appl Polym Sci* 1997; 66: 1399-1403.
- Adriaens E, Ameye D, Dhondt MM, Foreman P, Remon JP. Evaluation of the mucosal irritation potency of co-spray dried Amioca/poly(acrylic acid) and Amioca/Carbopol 974P mixtures. *J Control Release* 2003; 88: 393-399.
- Mark AM, Mehlretter CL. Facile preparation of starch triacetates. *Starch/ Stärke* 1972; 24: 73-76.
- Xu Y, Miladinov V, Hanna MA. Synthesis and characterization of starch acetates with high substitution. *Cereal Chem* 2004; 81: 735-740.
- Volkert B, Lehmann A, Greco T, Nejad MH. A comparison of different synthesis routes for starch acetates and the resulting mechanical properties. *Carbohydr Polym* 2010; 79: 571-577.
- Echeverría I, Silva I, Goñi I, Gurruchaga M. Ethyl methacrylate grafted on two starches as polymeric matrices for drug delivery. *J Appl Polym Sci* 2005; 96: 523-536.
- Kaith BS, Jindal R, Jana AK, Maiti M. Characterization and evaluation of methyl methacrylate-acetylated Saccharum spontaneum L. graft copolymers prepared under microwave. *Carbohydr Polym* 2009; 78: 987-996.
- Kaith BS, Singha AS, Gupta SK. Graft copolymerization of flax fibers with binary vinyl monomer mixtures and evaluation of swelling, moisture absorbance and thermal behavior of the grafted fibers. *J Polym Mater* 2003; 20: 195-199.
- Dubey S, Bajpai SK. Poly(methacrylamide-co-acrylic acid) hydrogels for gastrointestinal delivery of theophylline. 1. Swelling characterization. *J Appl Polym Sci* 2006; 101: 2995-3008.
- Singha AS, Rana AK. Kinetics of graft copolymerization of acrylic acid onto *Cannabis indica* fiber. *Iran Polym J*. 2011; 20: 913-929.
- Routray CR, Tosh B. Graft copolymerization of methyl methacrylate (MMA) onto cellulose acetate in homogeneous medium: effect of solvent, initiator and homopolymer inhibitor. *Cell Chem Technol* 2013; 47: 171-190.
- Carrillo F, Defays B, Colom X. Surface modification of lyocell fibres by graft copolymerization of thermo-sensitive poly-N-isopropylacrylamide. *Europ Polym J* 2008; 44: 4020-4028.
- Sadeghi M, Hosseinzadeh H. Studies on graft copolymerization of 2-hydroxy ethylmethacrylate onto kappa-carrageenan initiated by ceric ammonium nitrate. *J Chil Chem Soc* 2010; 55: 497-502.
- Gupta KC, Sahoo S, Khandekar K. Graft copolymerization of ethyl acrylate onto cellulose using ceric ammonium nitrate as initiator in aqueous medium. *Biomacromolecules* 2002; 3: 1087-1094.
- Rahman L, Silong S, Zin WM, Ab Rahman MZ, Ahmad M, Haron J. Graft copolymerization of methyl acrylate onto sago starch using ceric ammonium nitrate as an initiator. *J Appl Polym Sci* 2000; 76: 516-523.
- Ojah R, Dolui SK. Graft copolymerization of methyl methacrylate onto *Bombyx mori* initiated by semiconductor-based photocatalyst. *Bioresour Technol* 2006; 97: 1529-1535.

23. Gupta KC, Sahoo S. Graft copolymerization of acrylonitrile and ethyl methacrylate comonomers on cellulose using ceric ions. *Biomacromolecules* 2001; 2: 239-247.
24. Witono JR, Noordergraaf IW, Heeres HJ, Janssen LP. Graft copolymerization of acrylic acid to cassava starch-evaluation of the influences of process parameters by an experimental design method. *Carbohydr Polym* 2012; 90: 1522-1529.
25. Lutfor MR, Sidik S, Haron J, Rahman MZA, Ahmad M. Modification of sago starch by graft copolymerization: Effect of reaction conditions on grafting parameters. *Int J Polym Mater Biomater* 2003; 52: 189-201.
26. Abu-Ilaiwi FA, Ahmad MB, Ibrahim NA, Ab. Rahman MZ, Md Dahlan KZ, Yunus W Md ZW. Graft copolymerization of methyl methacrylate onto rubber-wood fiber using H₂O₂ and Fe²⁺ as an initiator system. *J Appl Polym Sci* 2003; 88: 2499-2503.
27. Marinich JA, Ferrero C, Jiménez-Castellanos MR. Graft copolymers of ethyl methacrylate on waxy maize starch derivatives as novel excipients for matrix tablets: Physicochemical and technological characterization. *Eur J Pharm Biopharm* 2009; 72: 138-147.
28. Chi H, Xu K, Wu X, Chen Q, Xue D, Song C, Zhang W, Wang P. Effect of acetylation on the properties of corn starch. *Food Chem* 2008; 106: 923-928.
29. Kriz J, Masar B, Pospisil H, Plestil J, Tuzar Z, Kiselev MA. NMR and SANS study of poly(methyl methacrylate)-block-poly(acrylic acid) micelles and their solubilization interactions with organic solubilizates in D₂O. *Macromolecules* 1996; 29: 7853-7858.
30. Casas M, Ferrero C, Violante De Paz M, Jiménez-Castellanos MR. Synthesis and characterization of new copolymers of ethyl methacrylate grafted on tapioca starch as novel excipients for direct compression matrix tablets. *Eur Polym J* 2009; 45: 1765-1776.
31. Xie X, Liu Q, Cui SW. Studies on the granular structure of resistant starches (type 4) from normal, high amylose and waxy corn starch citrates. *Food Res Int* 2006; 39: 332-341.