ENZYMATIC BIODEGRADATION OF POLY (METHYL METHACRYLATE) GRAFTED BIOPOLYMER

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ABSTRACT: The graft copolymerization of poly (methyl methacrylate) (PMMA) onto sago starch biopolymer (sago starch-g-PMMA) was carried out using ceric ammonium nitrate (CAN) as an initiator. PMMA was grafted onto sago starch using CAN as an initiator under nitrogen gas atmosphere. The maximum percentage of grafting (%G) was determined to be 246% at the optimum conditions. The copolymers produced were characterized by Fourier Transform Infrared Spectrophotometry (FTIR). The FTIR spectra of the copolymers clearly indicated the presence of characteristic peaks of PMMA and sago starch, which suggested that PMMA had been successfully grafted on the sago starch. Biodegradability studies of sago starch-g-PMMA and sago starch were carried out by α-amylase enzyme. Maximum biodegradation of the biopolymer was achieved after 3 days of incubation, while for the product (sago starch-g-PMMA) was 7 days. The maximum production of glucose was obtained when the concentration of α-amylase reached 10 ppm.

Keywords: *Sago Starch, Free Radical, Ceric Ion, Methyl Methacrylate, α-Amylase.*

1. INTRODUCTION

Biodegradation or biological degradation of polymers consists of those processes with result from an attack on the material by living organisms. The study of biodegradation has two opposite facets. On the one hand, there are many applications where biological resistant materials are needed. In all of these applications, the polymer is expected to serve for a long time, it must be bioresistant. On the other hand, there is an increasing demand for biodegradable plastics. Large amounts of polymers are used in packaging applications

and will be discarded after use. Environmental protection requires self-destructing polymers which will degrade and disappear completely when subjected to the combined effects of various environmental factors including microorganisms [1]. It is estimated that about 60 million tons of sago starch, extracted from sago palms, are produced per annum in South-East Asia. In a view of its abundance, sago may be utilized for the production of fermentable sugars. Utilization of sago starch as part of a sustainable agrotechnological system will provide a source of starch and lead to conservation of marshy areas of land [2].

Starch, in its native form, exists in relatively inert granular structures which are composed of macro molecules arranged in a polycrystalline state. These granules are insoluble in water and resistant to many chemicals and enzymes. Disruption of the granular structure by heating in water (gelatinization) enhances its chemical reactivity towards hydrolytic enzymes [3-5]. Starches are biopolymers of α-(1-4) linked glucose units with 0-5% α-(1-6) linked branches. Its biodegradability appears more outstanding and important in the current world in which attention has been drawn to the protection of the environment from pollution. The use of enzyme to produce starch hydrolysis is largely to replace acid hydrolysis because the degree of hydrolysis can be more precisely controlled. By using different enzymes, the composition of the product can be manipulated [6-8]. Starch is formed by a α-glucosidic chain. Such a compound yields only glucose on hydrolysis, which is called glucosan or glucan. However, using α-amylase in the starch hydrolysis can produce only glucose (1 unit of glucose + 1 unit of maltose). The amount of glucose liberated from starch can be detected by the phenol-sulfuric method to estimate the total sugar. Further more, the glucose liberated from starch by the action of α-amylase enzyme can be reduced and produce sorbitol and mannitol by the action of another enzyme to form n-hexane under special conditions. α-Amylases are extensively used in industrial processes because they do not only break down the starch into a mixture of glucose, maltose, oligosaccharides and dextrin, but their mode of action also results in rapid reduction in the viscosity of the gelatinized starch. The amylases act on starch, glycogen, and derived polysaccharides to hydrolyze the α-(1,4) glucosidic linkages. The amylases may be divided into three groups: α-amylases which hydrolyze glucosidic bonds in the interior of the substrate, β-amylases which hydrolyze maltose units from the non-reducing terminal end of the substrate, and finally the glucoamylases which hydrolyze glucose units from the non-reducing terminal end of the substrate. The initial interaction between amylases and starch granules can vary. Fungal amylases may first adsorb through the granule surface for efficient hydrolysis and the degree of adsorption is directly correlated to the extent of digestion. However, the native sago starch is a poor substrate to the enzyme and the hydrolysis patterns are surface erosion, pitting and crevassing. The rate of hydrolysis for gelatinized starch is faster compared to the rate for starch without gelatinization [2],[5],[9], [10-12]. The main objective of this paper is to investigate the laboratory scale production of glucose by using gelatinized sago starch as a raw material and sago starch attached to poly (methyl methacrylate) polymer.

2. MATERIALS AND METHODS

Sago starch (S.S) was purchased from Wee Kwong Sdn. Bhd., Malaysia and used without any purification. D(+)–glucose monohydrate were purchased from Merck

(Germany). The commercial α-amylase enzyme, produced from malt (Type V-A, 2.7 units/mg solid at pH 6.9 at 20°C), was supplied by Sigma Chemical Co. (USA). All chemicals and reagents were of analytical grade.

2.1 Graft Copolymerization

The grafting reaction was carried out in a round bottom flask equipped with a reflux condenser and a nitrogen gas inlet. To control the reaction temperature, the flask was placed in a thermostatic water bath equipped with a magnetic stirrer. Gelatinized sago starch was used in all experiments and was obtained by heating its slurry in the flask for 30 min at 80 °C. The grafting reaction procedure was well established and carried out in the pervious studies [13-16].

2.2 FTIR Spectra of Polymers

FTIR spectra of S.S, grafted copolymer and side chain polymer were recorded on the Perkin–Elmer 1725X FT-IR spectrophotometer, using the potassium bromide pellet technique.

2.3 Enzymatic Degradation of PMMA Grafted Sago Starch by α-Amylase

Enzyme stock solution was prepared by dissolving 0.1 g of α-amylase and 0.1950 g of sodium azide in 1 L of 8.3 mM phosphate buffer at pH 7.2. The sodium azide was added into the stock solution to inhibit the growth of fungi. The starch and the S.S-g-PMMA samples used in this study were sterilized by washing with 2% solution of sodium azide. This was followed by washing with 70% (v/v) ethanol/water, and dried in the oven at 60 °C. To study the degradation, 0.8 g of the polymer sample was immersed in 45 ml of the enzyme solution in a sterilized 250 ml volumetric flask. The sample was incubated at 30 °C and shaken at the speed of 150 rpm. At a chosen time interval, 1.0 ml sample's supernatant was taken for the glucose content analysis by using phenol sulfuric acid method [12], [17]. Five different enzyme concentrations, i.e. 10, 20, 30, 40 and 50 ppm, were used for this study.

2.4 Estimation of Total Sugar

Phenol sulfuric acid method was used to estimate the sugar content. A stock glucose solution (1.0×10^{-3} M) was used to prepare 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, and 110 ppm of the glucose standard solution. To convert the glucose (standard or sample) solutions into a coloured compound, 1.00 ml of this solution was first mixed with 1.00 ml of 5% phenol reagent and then 5.00 ml of concentrated (98%) H_2SO_4. The addition of the H_2SO_4 was carried out as fast as possible and the solution was vigorously shaken. The solution was then cooled at room temperature for 30 minutes before its absorption at 490 nm was measured by a UV-vis spectrophotometer. The standard curve was obtained by

plotting absorption versus glucose concentration and the glucose concentrations in the sample solutions were estimated from this curve [12].

3. RESULTS AND DISCUSSION

3.1 Preparation of The Copolymer and Optimization Study

The effects of the operating parameters such as reaction temperature, reaction periods, initiator concentrations, nitric acid concentration, and monomer concentration on the grafting of PMMA onto S.S prepared by using CAN as an initiator were deeply investigated by varying the parameters mentioned above [13-16]. The maximum percentage of grafting (%G) was determined to be 246% when the optimum conditions (reaction temperature: 70 °C, reaction period: 2 hrs, the amount of CAN: 2.0 mmoles, the amount of nitric acid: 0.4 mmoles, and the amount of monomer (MMA): 141 mmoles).

3.2 FTIR Spectrum Analysis

FTIR spectra of S.S., PMMA and S.S-g-PMMA are shown in Fig. 1 (A1 to A3). As it can be seen in the spectrum of PMMA (Fig. 1, (A1)) the presence of C=O group (1732 cm^{-1}). The FTIR spectrum of S.S (Fig. 1, (A2)) shows a band at 3447.78 cm^{-1}, indicating the presence of OH group and a band at 1646.20 cm^{-1} that suggests the existence of the C-C group. FTIR spectrum of S.S-g-PMMA (Fig. 1, (A3)), indicates the presence of the C=O group (1736.70 cm^{-1}) and 3447.78 cm^{-1} which was assigned to the band of the OH group. The appearance of the absorption band at 1736.70 cm^{-1} indicated the presence of the C=O group whereas this group did not appear in the spectrum of S.S [18, 19].

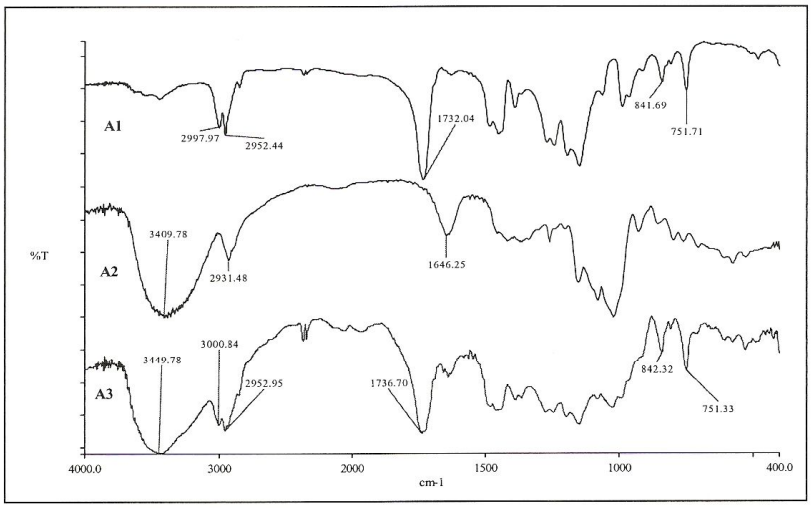

Fig 1: FTIR spectra of PMMA (A1); S.S (A2) and PMMA grafted S.S (A3)

3.3 Degradation of the Copolymer by α–Amylase Enzyme

Figure 2 shows the standard curve for the quantitative analysis of sugar by phenol sulfuric acid method. The degradation study was carried out by using sago starch and 90% of sago starch-g-PMMA prepared by using CAN as an initiator. The amount of glucose liberated from the copolymer of sago starch-g-PMMA using different concentrations of enzyme (10, 20, 30, 40 and 50 ppm) is presented in Fig. 3. The maximum amount of glucose produced was on the 7th day. After the 7th day the amount of glucose produced decreased (Fig. 4).

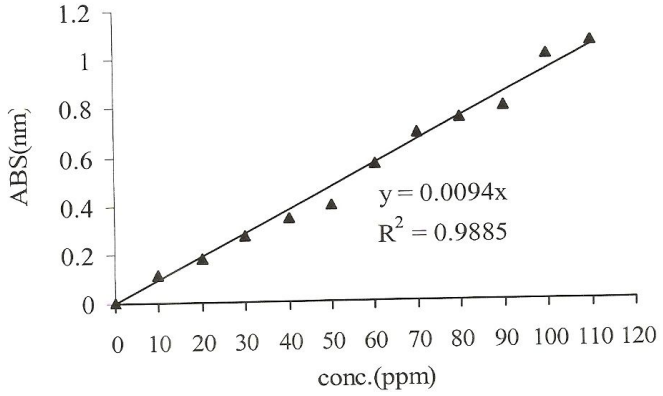

Fig 2: The standard curve of glucose (reaction conditions: volume of glucose solution, 1.00 ml; phenol reagent, 1.00 ml of 5%; H_2SO_4 (98%), 5.0 ml; wave length, 490 nm).

On the other hand, the amount of glucose, which was liberated from sago starch at different enzyme concentrations (10, 20, 30, 40, 50 ppm) (Fig 5) was maximum on the 3rd day and gradually decreased as the incubation period, increased further (Fig 6). The increase in the amount of glucose before reaching its maximum concentration was due to the hydrolysis process of sago starch. The highest glucose production was obtained when the α-amylase enzyme concentration reached 10 ppm and further increase in enzyme concentration up to 50 ppm led to slightly increase to level off in glucose amount. The decrease of the glucose amount could be due to the fermentation of glucose to ethanol and water [10], [12], [20].

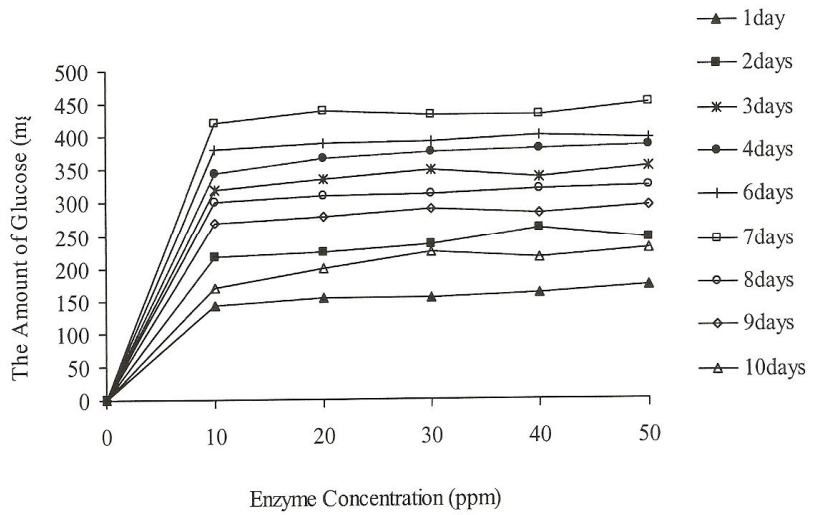

Fig 3: The amount of glucose liberated from S.S–g–PMMA at different concentrations of enzyme.

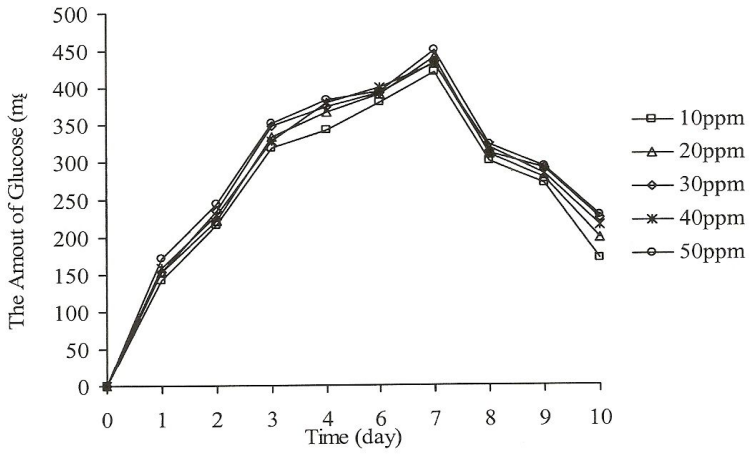

Fig 4: The maximum amount of glucose liberated from S.S-g-PMMA.

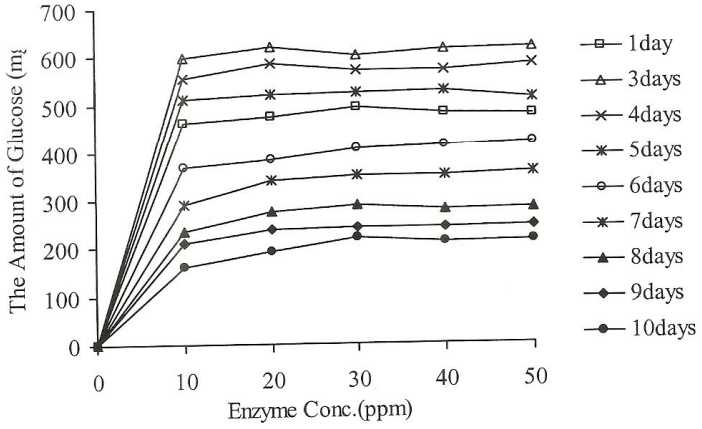

Fig 5: The amount of glucose liberated from S.S at different concentrations of enzyme.

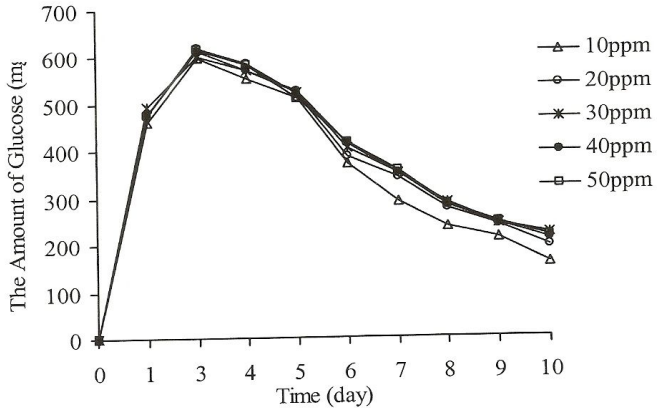

Fig 6: The maximum amount of glucose liberated from S.S.

4. CONCLUSION

This study concluded that the sago starch biopolymer could be grafted with MMA using CAN initiator. The biodegradation study using the commercial α-amylase showed that the maximum production of glucose produced from gelatinized S.S biopolymer was achieved after 3 days of incubation, while for the product (sago starch-g-PMMA) was 7 days. The highest glucose production was obtained when the α-amylase enzyme concentration reached 10 ppm, by increasing enzyme concentration up to 50 ppm, glucose amount was close to level off.

REFERENCES

[1] K. Tibor, (1983)"Polymer Degradation" Published by Van Nostrand Reinhold Company Inc. New York.

[2] W. J. Wang, A. D. Powell & C. G. Oates "Pattern of enzyme hydrolysis in raw sago starch: effects of processing history", *Carbohydr Polym.*, 26, 1995, 91-97.

[3] S., Ueda, (1981)"Fungal Glucoamylases and raw starch digestion". *Trends Biochem.*, 6, 89-90.

[4] F. W., Bergmann, J. Abe, & S. Hizikuri, (1988) "Selection of microorganisms which produce raw-starch degrading amylases." *Appl. Microbiol. Biotechnol.*, 27, 443-6

[5] W. M. Fogarty and C. T. Kelly, "Recent Advances in Microbial amylases. In Microbial Enzymes and Biotechnology", Fogarty W.M. & Kelly C. T. Eds., Elsevier, London, 1990, pp. 71-132.

[6] D. French, "In Starch: Chemistry and Technology" 2nd Ed. Whistler R.L., Ed., Academic Press, Orlando, FL, 1984, pp. 184.

[7] J. C. Shannon, and D. L. Garwood, "In Starch: Chemistry and Technology" 2nd Ed. Whistler R.L., Ed., Academic Press, Orlando, FL, 1984, pp. 25.

[8] J. G. Yu, J. P. Gao and T. Lin, "Biodegradable Thermoplastic Starch", *J. Appl. Polym. Sci.*, 26, 1996, 1491-1494.

[9] B. A. Arbakariya, B. A. Asbi, M. N. Azudin and J. F. Kennedy, "Effect of Mixing on enzymatic liquefaction of sago starch", *Carbohydr. Polym.*, 33, 1987, 101-108.

[10] R. K. Murray, D. K. Granner, P. A. Mayes, and V. W. Rodwell, "Harper's Biochemistry", California, USA, Appleton & Lange, 1988, pp. 125.

[11] T. ITO, Y. Arai and T. Kaneko, "Rheological Properties of Sago Starch and The Formation of Hexane from Mannitol for Utilization of Sago Starch", In: "Sago-'85:"Proceeding of the Third International Sago Symposium, Tokyo, 1986, pp 153-160.

[12] T. M. Wood, and K. M. Bhat, "Methods for Measuring Cellulase Activity", *Methods in Enzymology*, 160: 1988, 87-111.

[13] I. Y. Qudsieh, W. M. W. Wan Yunus, A. Fakhru'l-Razi, B. A. Mansor and M. Z. Rahman, "Graft Copolymerization onto Sago Starch: Ceric Ion Initiated Graft

Copolymerization of Methyl Methacrylate". *4th International Conference-Chemistry in Industry,* supported by *ACS,* Manama, Bahrain, (2000).

[14] I. Y. Qudsieh, W. M. W. Wan Yunus, A. Fakhru'l-Razi, B. A. Mansor and M. Z. Rahman, "Graft copolymerization of methyl methacrylate onto sago starch using ceric ammonium nitrate and potassium persulfate as redox initiator systems" *J. Appl. Polym. Sci.*, 83: 2275, 1375-1381 (2002)

[15] I. Y. Qudsieh, A. Fakhru'l-Razi, S. A. Muyibi, W. Y. Wan Md Zin, "Investigation on Flocculation Characteristics of Sago Starch Grafted Polyacrylamide in Kaolin Suspension". Proceeding *2nd World Engineering Congress, Biochemical and Environmental Engineering Symposium*, Kuching, Sarawak, Malaysia, 22-25 July, (2002b), pp. 283-287.

[16] I. Y. Qudsieh, A. Fakhru'l-Razi, S. A. Muyibi, B. A. Mansor, M. Z. Rahman and W. M. W. Wan Yunus "Preparation and Characterization of Poly(methyl methacrylate) Grafted Sago Starch Using Potassium Persulfate as Redox Initiator" J. Appl. Polym. Sci., vol. 94, 5, (2004), pp. 1891-1897.

[17] B. G. Kang, S. H. Yoon, S. H. Lee, J. E. Yie, B. S. Yoon, and M. H. Suh, "Studies on the physical properties of Modified Starch Filled HDPE Film", *J. Appl. Polym. Sci.,* 60, 1996, 1977-1984.

[18] A. Lagos, and J. Reyes, "Grafting onto Chitosan. I. Graft copolymerization of methyl methacrylate onto chitosan with Fenton's reagent (Fe^{2+}-H_2O_2) as a redox initiator", *J. Polym. Sci., Polym. Chem.,* 26, 1988, 985.

[19] J. Retuert and M. Yazdani-Pedram, "Cocatalyst effect in potassium persulfate initiated grafting onto Chitosan", *Polymer Bulletin*, 31, 1993, 559.

[20] Y. Li, J. Nothnagel and T. Kissel, "Biodegradable brush-like graft polymers from poly(D,L-Lactide) or poly(D,L-Lactide-co-glycolide) and modified, hydrophilic dextrans as backbone-synthesis, characterization and invitro degradation properties", *Polymer* 38, 25, 1997, 6197-6206.