BIODEGRADABLE POLYMERS BASED ON NATURAL SUBSTANCES

A THESIS

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By

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MAY 2004

CERTIFICATE

Certified that the work incorporated in this thesis entitled **"Biodegradable Polymers Based on Natural Substances"** submitted by Mrs. Padmaja Prasad Galgali, was carried out under my supervision. Such material as has been obtained from sources has been duly acknowledged in the thesis.

Dr. A.J. Varma

(Research Guide)

Dedicated to the memory of my father

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Padmaja Galgali

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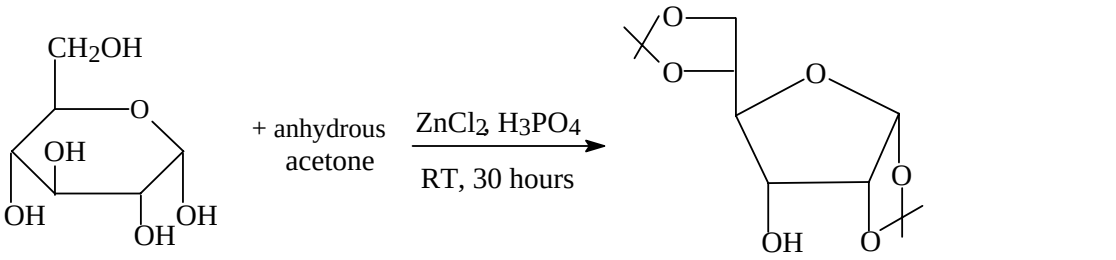
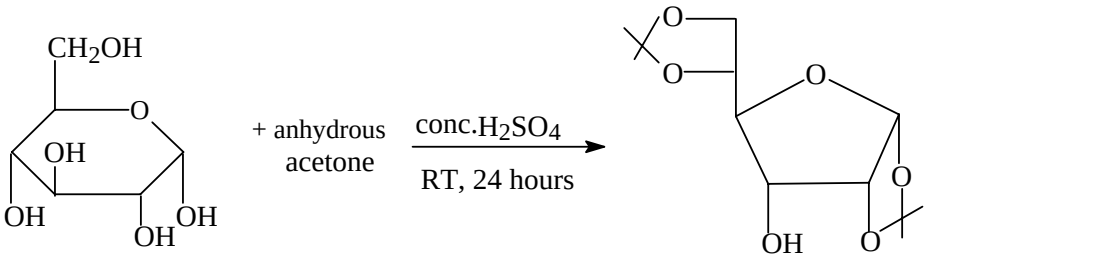
Synthesis of Sugar Derivatives:



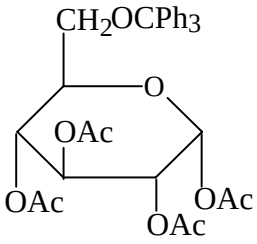
D-Glucose

Diacetone D-glucose
(1,2: 5,6- diisopropylidene D-glucose)



D-Glucose

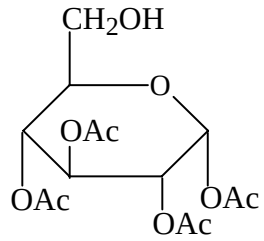
Diacetone D-glucose
(1,2: 5,6- diisopropylidene D-glucose)



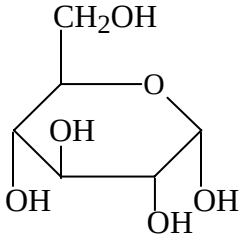
6-O- trityl, 1,2,3,4-tetraacetyl D- glucose

HBr/ gl.CH_3COOH

10^0C, 1 min.



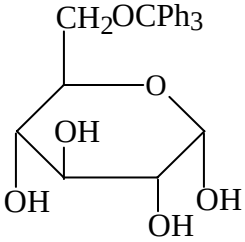
1,2,3,4- Tetraacetyl D-glucose



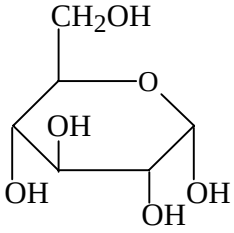
D-Glucose

trityl chloride/ pyridine

40^0C, 16 h



6-O- trityl D- glucose



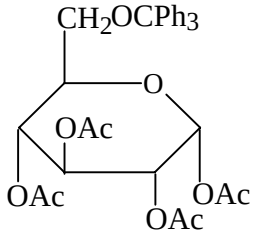
D-Glucose

1. trityl chloride/ pyridine

40^0C, 16 h

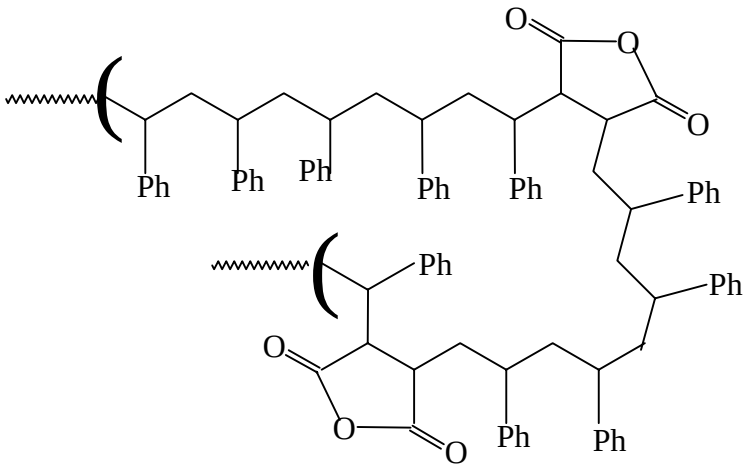
2. acetic anhydride

25^0C, 2 h

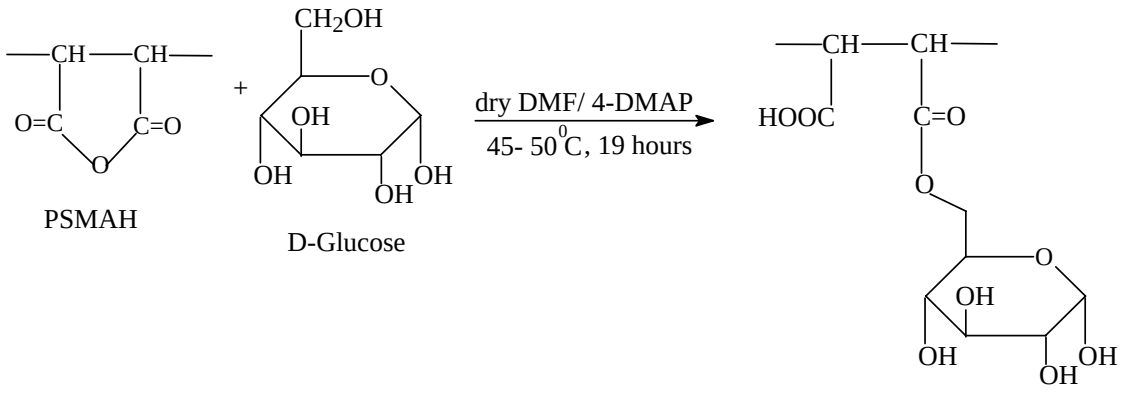


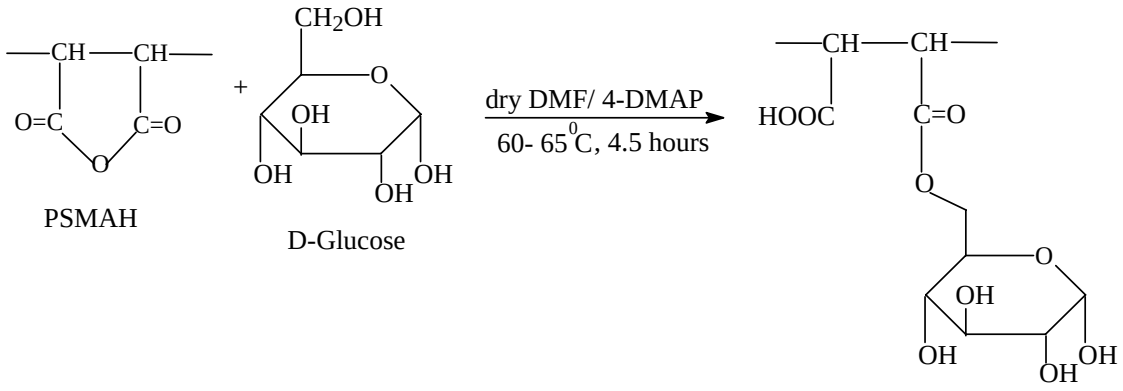
6-O- trityl, 1,2,3,4-tetraacetyl D- glucose

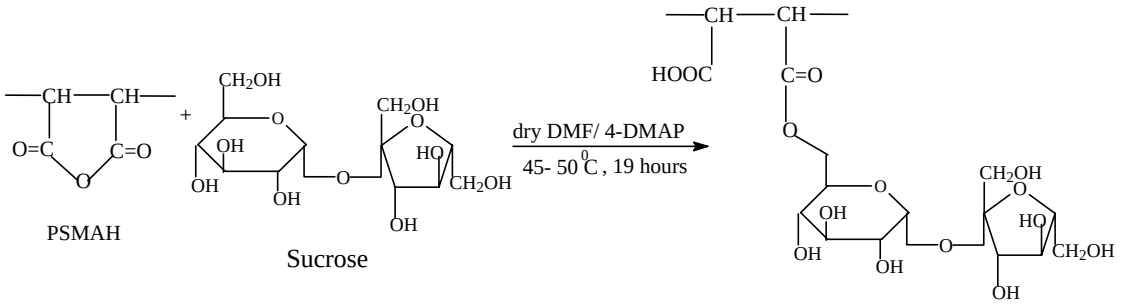
Structure of the polymer viz. poly(styrene maleic anhydride)

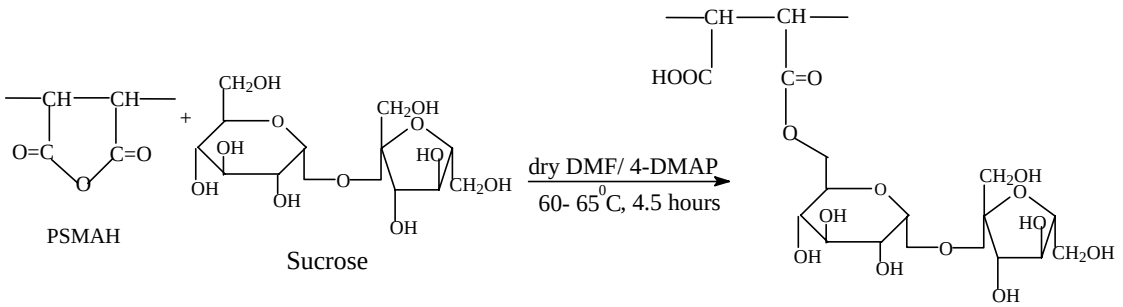


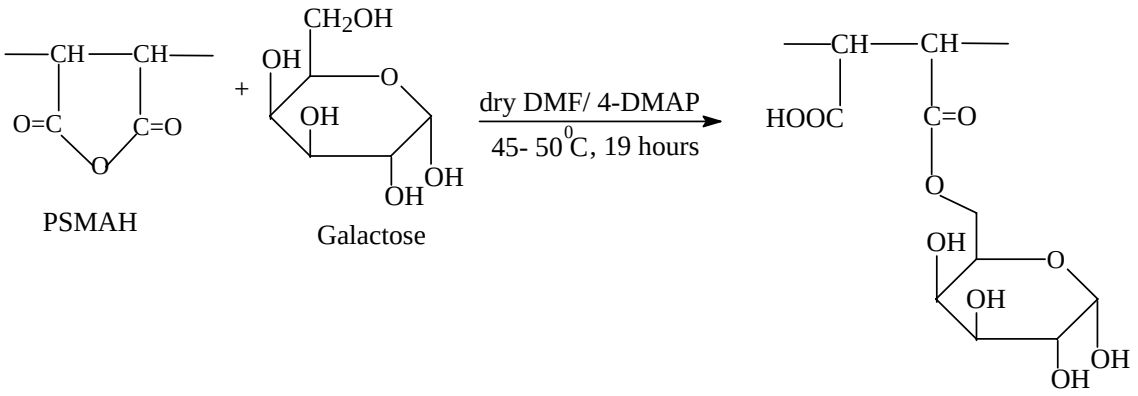
Reactions of poly(styrene maleic anhydride):



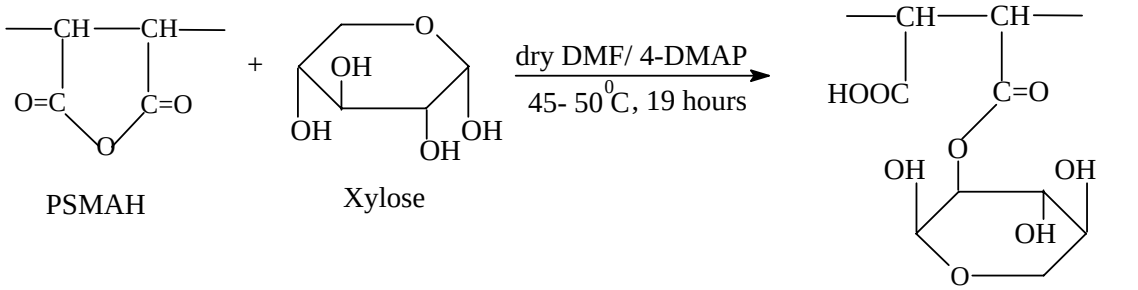

CH
CH
O=C
C=O
O
PSMAH
+
CH2OH
O
OH
OH
OH
OH
D-Glucose
dry DMF/ 4-DMAP
60- 65 0C, 4.5 hours
CH
CH
HOOC
C=O
O
O
OH
OH
OH
OH
Glucose- linked PSMAH


CH
CH
O=C
C=O
O
PSMAH
+
CH2OH
O
OH
OH
O
CH2OH
O
HO
CH2OH
OH
Sucrose
dry DMF/ 4-DMAP
45- 50 0C , 19 hours
CH
CH
HOOC
C=O
O
O
OH
OH
O
CH2OH
O
HO
CH2OH
OH
Sucrose- linked PSMAH

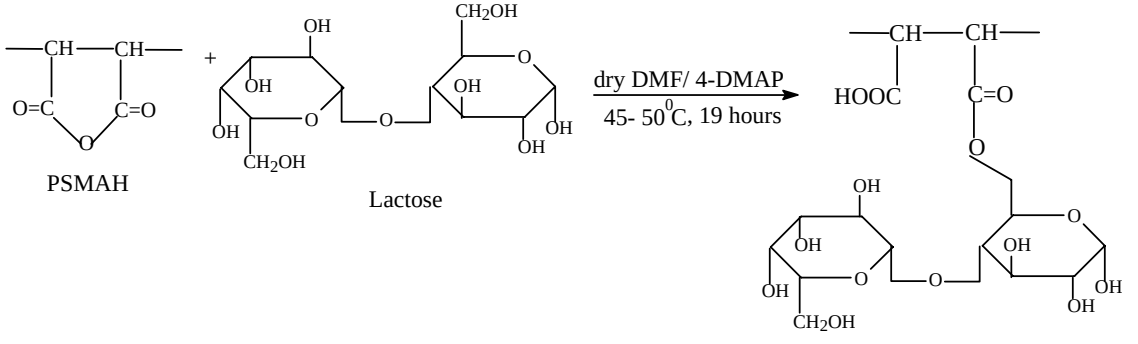

CH
CH
O=C
C=O
O
PSMAH
+
CH2OH
O
OH
OH
O
CH2OH
O
HO
CH2OH
OH
Sucrose
dry DMF/ 4-DMAP
60- 65 0C, 4.5 hours
CH
CH
HOOC
C=O
O
O
OH
OH
O
CH2OH
O
HO
CH2OH
OH
Sucrose- linked PSMAH



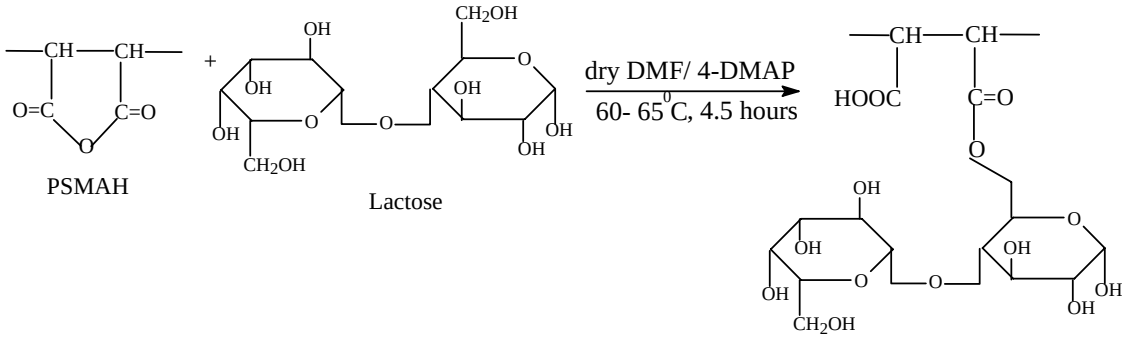
Galactose- linked PSMAH



Xylose- linked PSMAH

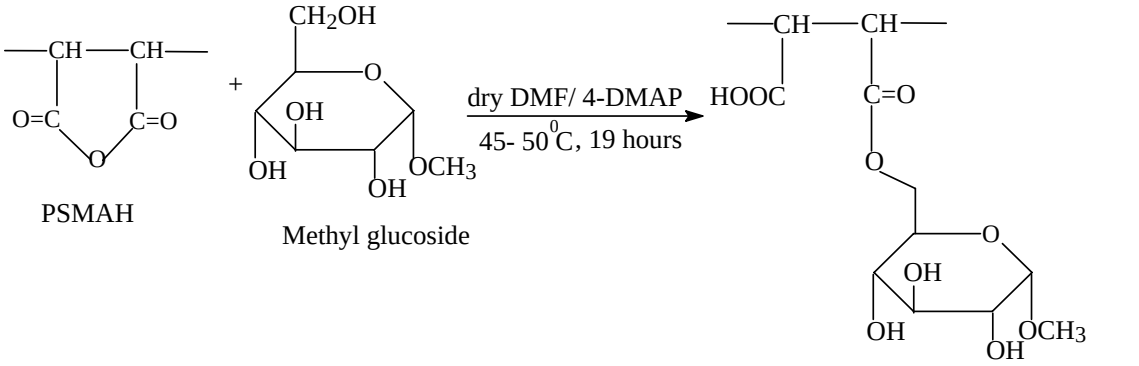


Lactose- linked PSMAH



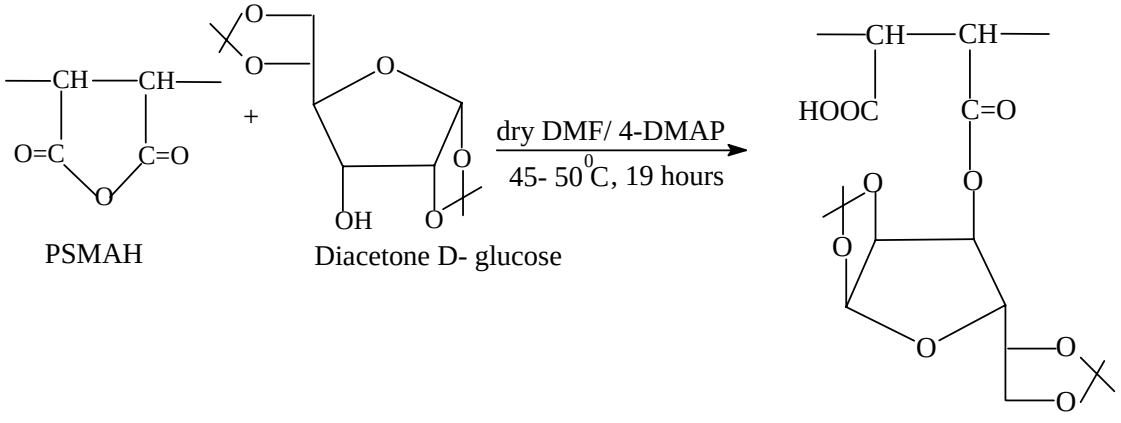
PSMAH Lactose

Lactose- linked PSMAH



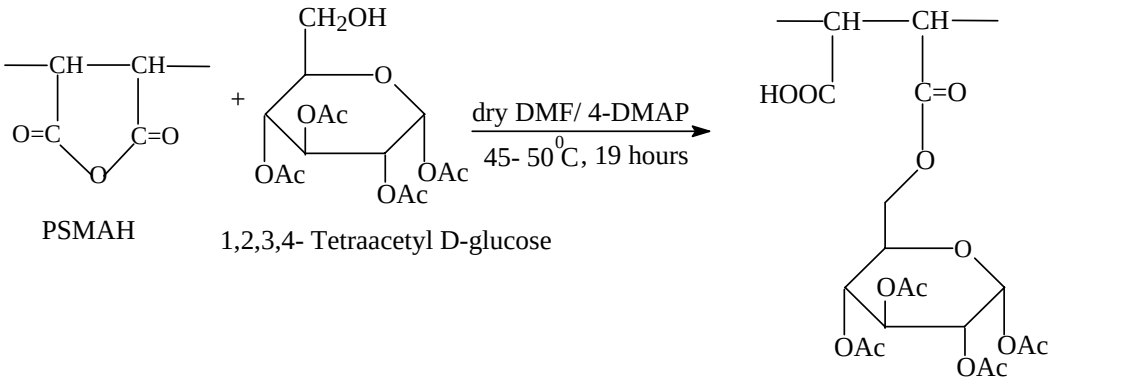
PSMAH Methyl glucoside

Methyl glucoside- linked PSMAI



PSMAH Diacetone D- glucose

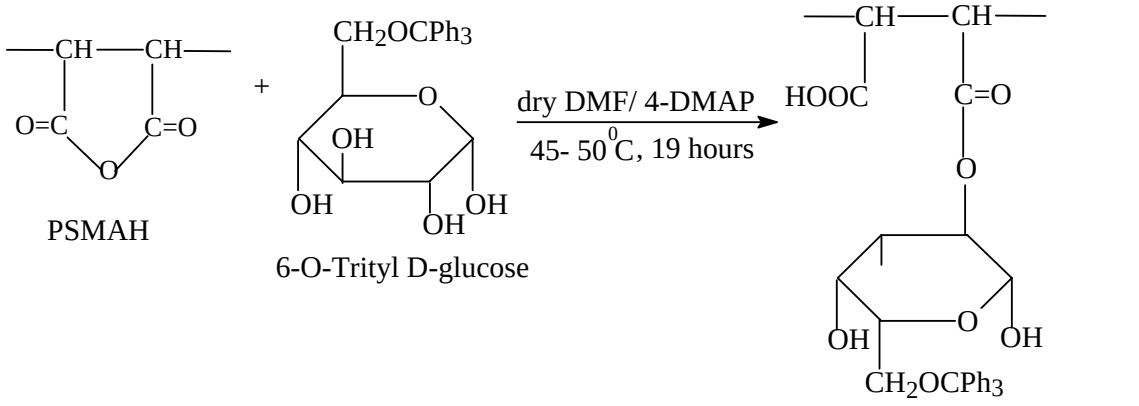
Diacetone D- glucose- linked PSMAH



PSMAH

1,2,3,4- Tetraacetyl D-glucose

1,2,3,4- Tetraacetyl D-glucose-linked PSMAH



PSMAH

6-O-Trityl D-glucose

6-O-Trityl D-glucose- linked PSMAl

Abbrevations

TGA: Thermogravimetric Analysis

FTIR: Fourier Transform Infra Red Spectroscopy

PSMAH: Poly(styrene maleic anhydride)

MM: Mineral Medium

DTG: Differential Thermogravimetry

TG: Thermogravimetry

NMR: Nuclear Magnetic Resonance Spectroscopy

BSA: Bovine Serum Albumin

SEM: Scanning Electron microscopy

OECD: Organisation for Economic Co-operation and Development

ISO: International Standars Organisation

ASTM: American Society for Testing and Materials

MITI: Ministry of International Trade and Industry

DIN: German Institute for Standardisation

CEN: European Standardisation Committee

ORCA: Organic Reclamation and Composting Association

ISR: Institute for Standards Research

OD: Optical Density

Abstract of the thesis:

Introduction:

Plastics and polymers have become a part and parcel of our day-to-day life. They are readily processable they can be molded into various articles. The toothbrushes we use, the disposable teacups, the carry bags we use for carrying vegetables and various electronic components are all made up of plastic. The world production of plastic is around 100 million tones annually. In a large developed country like USA, about 10 million tonnes of plastics are consumed (article in World & I, Dec 1990), while in a small developed country like Australia itself, around 1.5 million tones of plastic materials are consumed each year. A developing country like India consumes over 4 million tonnes of plastics, but this consumption is increasing rapidly and is expected to go upto 8 million tones per annum by the year 2007 according to a TIFAC report on Biodegradable Plastics, 2003. Packaging, the largest market for plastics, accounts for over a third of the consumption of raw plastics. The high consumption of plastics has led to an acute solid waste disposal problem. By the year 2005, 15% of the total municipal solid wastes in developed countries would be contributed by commodity plastics. Thus researches on biodegradable polymers worldwide have become a topic of intense activity and importance. A variety of new ideas, methodologies, and interdisciplinary work are being undertaken to tackle the material property requirements of the new generation of consumer polymers**:** adequate mechanical properties, processability, economic viability, and biodegradability. Our research incorporates methodologies which takes into account known structure-property relationships needed for obtaining biodegradable polymers and harmonizing them with the chemistry of natural monomers (which are known food sources for microorganisms)**.** The expected result is to have new polymers having a synergy of properties: mechanical strength, along with biodegradability.

The use of abundantly available and replenishable sources such as monosaccharides and disaccharide in the design of semi synthetic polymers is a strategy that needs to

be explored in greater depth, and we have made a beginning with new scientific approaches in this regard. These results will be discussed at length in this thesis. Indeed, synthetic polymers bearing carbohydrate moieties have been topics of intense research, as they can have a wide range of applications. They are potentially biodegradable in the time scale required for commercial applications. Most of the syntheses of these polymers are based on polymerization of vinyl saccharide monomers or ionic ring-opening polymerizations. However these procedures have several drawbacks for commercial synthesis. These drawbacks include multi-step synthesis involving tedious protection- deprotection chemistry of the hydroxyl group, unfavorable reactivity ratios of the monomers and comonomers, and high costs. Hence their applications have been restricted to specialized fields. In our study we chose to develop alternative strategies to design and develop biodegradable synthetic polymers based on carbohydrates, by linking carbohydrate moieties onto synthetic polymers by polymer analogous reactions. The latter methodology is simple, one-step procedure, and hence commercially viable. The amounts of carbohydrates linked to the polymer are in minute quantities (typically less than 3% by weight), thereby keeping the processable characteristics of the polymers intact.

Biodegradation

A degradable polymer is a polymer, which undergoes physical, chemical and mechanical changes. The physical changes include yellowing of the polymer and embrittlement with loss of its integrity as a whole. The mechanical changes include loss of impact resistance and tensile properties of the polymer, whereas the chemical changes include formation of new functional groups caused by oxidation, hydrolysis, etc., along with possible loss of some already existing functional groups. As already mentioned in the earlier section, biodegradation is a process in which the degradation of the polymer is effected by micro- or macro- organisms or by enzymes secreted by them. It involves two processes, namely bioassimilation and biomineralization. In the process of bioassimilation, the large fragments of the polymer chain are broken down either randomly or successively into smaller fragments, which are then transported

into the microbial cells. In the process of biomineralization, the polymeric carbon enters the biochemical pathways that take place in the microbial cells and gets finally converted to either carbon dioxide and/ or methane. The carbonaceous end product of such aerobic metabolism is carbon dioxide and that of anaerobic metabolism are carbon dioxide and methane. The equations can be summarized as follows:

Aerobic biodegradation: carbon dioxide + water + biomass + minerals

Anaerobic biodegradation: carbon dioxide + methane + water + biomass + minerals

The final fate of the polymer is that its gets converted to carbon dioxide and/ or methane, which finally reenter the geochemical cycle. One of the important criteria to be fulfilled by the degrading polymer is that it should not produce any toxic products during its degradation. Different polymers have different compositions and find themselves in different environments after their disposal. Hence, after disposal, polymers are subjected to degradation under different conditions. Therefore, there is a great need to investigate the mechanism of biodegradation of different polymers in different environments. This has led to a variety of different test methods for evaluating biodegradation. Several test standards have been designed to address the biodegradability of the polymer. These include:

- American Society for Testing and Materials (ASTM)
- European Standardisation Committee (CEN)
- International Standars Organisation (ISO)
- Institute for Standards Research (ISR)
- German Institute for Standardisation (DIN), and
- Organic Reclamation and Composting Association (ORCA)

These test methods define the biodegradation of a polymer in different environments and under different composting conditions.

There have been several attempts to modify these methods from time to time. However, no test method has been found suitable to evaluate the biodegradability of all the polymers. It is for this reason that scientists all over the world have been greatly debating on choosing a particular test method as a standard one. Furthermore, there have been several changes in the acceptance limits of biodegradability, e.g. according to the OECD standard, a degradable polymer should degrade by 60% in a time span of 28 days under the set of conditions mentioned in the standard. On the other hand, according to the standards set by the CEN, a polymer can be considered to be a degradable polymer if it mineralizes to 90% in case of polymeric blends or copolymers and to 60% in case of a homopolymer, in a time span of 6 months (ICS-UNIDO Information Package on Environmentally Degradable Plastics, 2001).

In our study we chose functionalized polystyrene, viz. poly(styrene maleic anhydride) and sugar- linked poly(styrene maleic anhydride) for our study. Polystyrene base polymers are known to be non- biodegradable (Enc. Chem. Technol., Kirk & Othmer (Edn. Mary Howe- Grant, Wiley Interscience, New York, vol. 17, 4^{th} Edn., 1997). So, our set of polystyrenes were first screened for biodegradation using different microorganisms. The screening tests were carried out in 10 mL of minimal medium with the polymer (50 mg) as the sole source of carbon. The composition of the mineral medium is such that it contains all the essential salts required by the microorganisms except the carbon course. The carbon source is derived from the organic substrate, whose biodegradability is under study. The microorganisms tested included bacteria, fungi and yeasts. The microorganisms and their visual growth patterns are listed below:

Table 1: Bacterial growth pattern recorded after 15 days:

	A	B	C	D	E
Bacillus sp. (NCIM 2812)	++	+	+	+	+++
S. aureus (NCIM 2079)	-	-	-	-	+++
E. coli (NCIM 2068)	+	-	-	-	+++
P. aeruginosa (NCIM 2863)	+	-	+	-	+++
Pseudomonas sp. (NCIM 2220)	++	+	++	++	+++
Serratia marscecens (NCIM 5061)	++	-	+	_	+++
P. resinivorans (NCIM 2599)	-	-	-	+	+++

Table 2: Fungal and yeast growth recorded after 28 days:

	A	B	C	D	E
A. niger (NCIM 596)	++	+	++	+	+++
A. niger (NCIM 1025)	+	++	++	+	+++
A. niger (NCIM 584)	-	-	+	-	++
A. niger (NCIM 598)	+	-	+	-	+++
A. niger (NCIM 1222)	-	-	-	+	++
A. terreus (NCIM 1220)	-	+	-	+	++
P. ochro-chloron (NCIM 1219)	+	++	++	-	+++
Saccharomyces sp. (NCIM 3304)	-	+	-	-	++
Candida sp. (NCIM 3433)	-	+	-	-	++
Aeureobasidium (NCIM 1049)	-	+	-	-	++
T. viride (NCIM 1221)	+	-	-	+	+++
Gliocladium virens (ATCC 9645) / Trichoderma sp. (NCIM 1297)	+	-	++	++	+++

Pullularia pullulans (ATCC 9348)	+	++	+	++	+++

A = lactose- linked poly(styrene maleic anhydride)

B = glucose- linked poly(styrene maleic anhydride)

C = sucrose- linked poly(styrene maleic anhydride)

D = poly(styrene maleic anhydride)

E = glucose

- \- no growth
- \+ little growth
- ++ good growth
- +++ excellent growth

The microorganisms which showed positive growth were chosen for further studies. These include

Bacteria: Serratia marscecens (NCIM 5061), Pseudomonas sp. (NCIM 2220) and Bacillus sp. (NCIM 2812)

Fungi: A. niger (NCIM 1025), Trichoderma sp. (NCIM 1297), P. ochro-chloron (NCIM 1219) and Pullularia pullulans (NCIM 1049).

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The further tests were carried out in conical flasks using 100 mL minimal medium in each of the conical flasks and the polymer as the sole source of carbon. The growth of the bacteria was monitored over a period of 25 days by turbidimetry (optical density measurements). Turbidimetry could not be employed for monitoring the fungal degradation. The composition of the minimal medium was different for both bacterial and fungal cultures, the details of which along with the experimental details and results have been discussed in the subsequent chapters.

It was established from the results of the tests conducted in the shaker flasks, that the sugar- linked polymers viz. sugar- linked poly(styrene maleic anhydride) polymers were more biodegradable as compared to unmodified poly(styrene maleic anhydride)

by both bacterial and fungal cultures. Hence, we decided to evaluate the biodegradability of these polymers by a standard OECD method. However, instead of the activated sludge used in the OECD standard, we used pure strains of bacteria in our experiment. The advantage of using a pure strain is that it helps to identify a particular organism responsible for effecting the biodegradation. The experimental details and the results have been discussed in chapter 7.

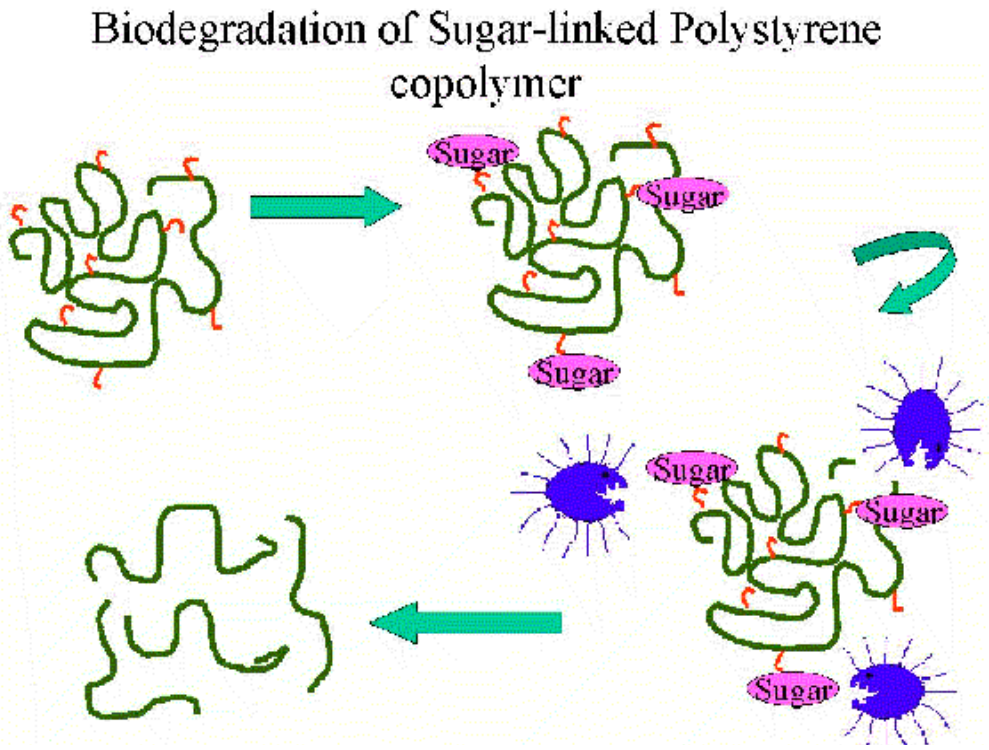


Figure 1: Schematic representation of biodegradation process.

The work is presented in nine chapters. The outline of each chapter is as follows:

Chapter 1: Synthetic Polymers Functionalized by Carbohydrates : A Review

This chapter includes detailed literature search on synthetic polysaccharides, regarding the methods of preparation, applications, potential applications, characterization, and the advantages and disadvantages associated with each method.

The conclusions from this literature review are that polymer analogous reactions need to be explored further and the products of their reactions need to find application in industry. The details have been reported in a review (Accepted for publication in "Carbohydrate Polymers", 2004).

Chapter 2: Towards biodegradable polyolefins : strategy of anchoring minute quantities of monosaccharides and disaccharides onto functionalized polystyrene, and their effect on facilitating polymer biodegradation.

This chapter discusses the general method for the synthesis of linking minute quantities of monosaccharides and disaccharides onto poly(styrene maleic anhydride) by polymer analogous reaction and their degradation by bacteria such as Serratia marscecens, Pseudomonas sp. And Bacillus sp. The synthesized polymers were characterized by FTIR, colorimetry and NMR spectroscopy of the silylated product. The biodegradation was monitored by the optical density measurements. The degraded products were characterized by FTIR and GPC and the weight losses of the degraded polymers were determined. It was observed that incorporation of even minute quantities of sugars into the polymer, increased the rates of biodegradation greatly as compared to the unmodified polymer. The details are also discussed in a publication (Padmaja Galgali, Anjani J. Varma, Ulka S. Puntambekar, Digambar V. Gokhale, *Chem. Commun.,* 2884-2885, 2002).

Chapter 3: Biodegradable synthetic polymers and process for preparation thereof

This chapter gives a description of the various procedures employed for the linking minute quantities of various monosaccharides and disaccharides onto poly(styrene maleic anhydride). This chapter also overviews the various methods that have been employed previously for the syntheses of biodegradable polymers. The possibility of using other functionalized polymers, other solvent systems, catalysts and work-up procedures have also been discussed. The synthesis of biodegradable polymers by linking of sugars onto functionalized polystyrene by polymer analogous reaction has

been covered in a patent (Biodegradable synthetic polymers and process for preparation thereof, Patent Application: Nov. 2002)

Chapter 4: Fungal degradation of carbohydrate-linked polystyrenes

This chapter describes the degradation of sugar (glucose, sucrose and lactose)-linked polystyrene maleic anhydride by fungi such as Aspergillus niger, Pullularia pullulans, Trichoderma sp. and Penicillium ochro-cloron. Weight loss measurements after fungal treatment confirmed the biodegradability of the carbohydrate-linked polymers, and it was observed that the degree of susceptibility to degradation varied with the type of test organism as well as with the type of sugar. The conclusion drawn was that the otherwise non-biodegradable polymers could be made biodegradable by incorporation of certain structural features into the polymers. The synthesized polymers were characterized by FTIR and colorimetry. Weight losses of the degraded polymers were recorded and the degraded polymers were characterized by FTIR. The results are presented in a publication (P. Galgali, U. S. Puntambekar, D. V. Gokhale, and A. J. Varma, Carbohydrate Polymers, 55, 393-399, 2004).

Chapter 5: Elucidation of the ester peak positions and reactivities of the different glucose hydroxyls: An FTIR study of the reaction of glucose and glucose derivatives with poly(styrene maleic anhydride).

This chapter includes characterization of the characterization of the sugar-linked poly(styrene maleic anhydride) by FTIR spectroscopy with special emphasis on glucose-linked poly(styrene maleic anhydride) and derivatives of glucose linked to poly(styrene maleic anhydride). The amounts of sugars incorporated into the polymer being minute, it was difficult to establish which of the sugar hydroxyls was involved in the esterification reactions. Hence different derivatives of glucose were synthesized by blocking one or few of the different hydroxyls present in the glucose molecule and these derivatives were reacted with the functionalized polymer and their reactivity ratios were calculated from the ratios of the anhydride peak to that of the >C=C<

stretching frequency of the aromatic ring of polystyrene fraction. It was found that the primary hydroxyl was the most reactive one followed by the secondary.

Chapter 6: Thermal analysis of sugar- linked poly(styrene maleic anhydride)

The thermal analysis of the sugar-linked poly(styrene maleic anhydride) was studied using thermogravimetry. The sugar-linked poly(styrene maleic anhydride) polymers were also treated at various temperatures (150°C, 250°C and 350°C) and the thermally treated samples were characterized by FTIR to evaluate the different changes that occur at those temperatures. The main aim of this study is to check the conditions for processability of the sugar-linked polymers.

Chapter 7: Biomineralization of the sugar-linked poly(styrene maleic anhydride)

The evolution of carbon monoxide by the breakdown of the sugar-linked poly(styrene maleic anhydride) by micro-organisms was studied by a standard OECD method. In this method carbon dioxide free air was bubbled through a mineral medium containing the polymer as the sole source of carbon and the medium inoculated with the concerned microorganism. The evolved carbon dioxide was trapped in barium hydroxide solution and then titrated with HCl solution and the percent biodegradation was calculated from the standard calculations.

Chapter 8: Biodegradation of acylated sugar-linked poly(styrene maleic anhydride)

This chapter discusses the general method for the acylation of the sugar-linked poly(styrene maleic anhydride) polymers, the characterization of these polymers by FTIR and thermogravimetry. The bacterial and fungal degradation of these acylated polymers was also monitored by the optical density measurements, weight loss measurements and SEM.

Chapter 9: Suggestions for the future work

The present work has opened up new avenues for further research and they will be discussed in this chapter.

CHAPTER 1

Literature Review

"Synthetic Polymers Functionalized by Carbohydrates"

(reproduced from our paper published in Carbohydrate Polymers, 2002, Article in press)

Abstract:

Polymers and plastics based on carbohydrates have re-emerged as exciting topics of polymer research, due to a worldwide focus on sustainable materials. However, multi-step synthesis of such polymers have made their use as commodity plastics uneconomical, and currently their applications are restricted to biomedical and other highly specialized low-volume high value fields. Functionalization of polymers has emerged as another important area of polymer science and technology. Chemically linking sugar moeities onto synthetic polymers is a unique method of functionalization of synthetic polymers, whereby not only is the synthetic polymer functionalized for further chemical modifications, but it can also get other desirable properties such as biodegradability – a property much debated and researched in modern times. This paper reviews several methods of anchoring carbohydrates onto polymers and the advantages and the disadvantages associated with each method, their current and potential applications, and their characterization methods.

1.1. Introduction:

There has been a worldwide realization that nature-derived monosaccharides, disaccharides, oligosaccharides and polysaccharides can provide us the raw materials needed for the production of numerous industrial consumer goods (Kunz, 1993; Varma, 2003; Pacitti, 2003). While functionalization of polymers (especially polyolefins) is a particularly attractive area of polymer research today (Chung, 2002), using sugar functionalized petrochemical polymers such as polystyrene for use as biodegradable polymers is a newly discovered application of a sugar-linked synthetic polymer (Galgali et al., 2002, 2003). The class of sugar based polymers, generally known as poly(vinylsaccharide)s, have also been investigated in some detail for a variety of applications, particularly in the biomedical field (Fraser & Grubbs, 1995; Kallin, Lonn & Norberg, 1989; Caneiro, et al, 2001; Kobayashi K., Sumitomo & Ina, 1985; Nishimura, et al, 1991; Nishimura, Matsuoka & Kurita, 1990).

Structurally, the poly(vinylsaccharide)s have a synthetic C—C backbone with pendant carbohydrate molecules, whereas modified polysaccharides have a carbohydrate backbone with synthetic molecules attached as pendants. Since sugars are a good source of food for micro-organisms, many poly(vinylsaccharide)s have the potential to be utilized as biodegradable polymers. There are four general methods of preparing synthetic polysaccharides: polymerization of vinyl sugars (polyvinylsaccharide)s, polymerization of anhydro-sugars (polyanhydrosugar)s, enzyme-mediated synthesis of carbohydrate polymers, and grafting of sugars onto functionalized synthetic polymers by polymer analogous reactions. In few cases, olefin metathesis reactions have also been employed for synthesis of poly(vinylsaccharide)s.

Poly(vinylsaccharide)s are most commonly synthesized by either homopolymerization of the vinyl sugars or by copolymerization of the vinyl sugar with other polymerizable vinyl monomers. Anhydro sugars have been polymerized cationically by ring-opening polymerization to obtain stereoregular synthetic polysaccharides. This strategy has been extended to the synthesis of synthetic polymers with pendant sugars by graft copolymerization of anhydro sugars onto halogenated polymers. Enzymatic reactions have been used to synthesize linear polymers with sugar as part of the main chain (Patil, Rethwisch & Dordick, 1991). Enzymatic reactions are very specific and do not involve any protection and deprotection of the sugar hydroxyls, but are extremely slow (Martin, et al., 1992). Hence chemo-enzymatic methods, or in other words, enzyme mediated polymerizations, have been used to synthesize poly(vinylsaccharide)s and were found to be better alternatives, wherein the polymerizable vinyl saccharides were synthesized enzymatically and the polymerizations had been carried out by conventional chemical methods. Another method of obtaining polymers of carbohydrates is by grafting saccharides onto synthetic polymers. A recent review elaborated the various synthetic methods for the preparation of sugar containing polymers and their applications (Wang, Dordick and Linhardt, 2002). The current

review also discusses the advantages and disadvantages associated with each method as well as their characterization methods.

1.2. History of Carbohydrate Based Polymers:

The synthesis of the poly(vinylsaccharide)s dates back to as early as the 1930's. Reppe (1930) was the first person to synthesize vinyl saccharide monomers. He synthesized vinyl ethers from glucose and fructose by alkali catalyzed addition of protected sugars to acetylene. He synthesized two vinyl saccharide monomers viz. 1-O-vinyl1,2:5,6-disopropylidene fructopyranose and 3-O-vinyl1,2:5,6-diisopropylidene glucofuranose which he later polymerized (Reppe & Hecht, 1936) to obtain insoluble polymers.

In the 1940s, Yanovsky worked on the synthesis of poly(vinylsaccharide)s (Treadway, 1945; Nichols & Yanovsky, 1944 & 1945). However, he was successful in getting only crosslinked polymers since the polymerization involved glucose pentamethacrylate and glucose allyl ether monomers. Haworth, Gregory & Wiggins, (1946) polymerized substituted carbohydrates, containng two acrylate or methacrylate groups to obtain hard products, which were also crosslinked. The reactions were carried out in absence of a catalyst. Helferich & Hofmann (1952) & Helferich & Jung (1958) were successful in synthesizing water soluble poly(vinylsaccharide)s viz poly(p- hydroxystyrene-α-D-galactoside) and poly(p-hydroxy β-D-glucoside). They studied the adsorption of three enzymes viz. β-D-glucosidase, α-D-galactosidase and β-D-galactosidase, all of which are present in sweet almonds, on these polymers. (Wolfrom, Swan, Ennor & Chaney, 1959) reported the polymerization of 3-methacryloyl-D-mannitol pentanitrate into a hard solid. Until the 1950s more stress was laid on synthesizing monomer derivatives and checking their polymerizability rather than obtaining polymers with properties for specific applications.

The synthesis of a linear vinyl saccharide polymer was seldom reported until 1960. The fist high molecular weight poly(vinylsaccharide) which was also soluble in water

was poly(methacryloyl glucose), which was first reported by Bird, Black, Dewar & Rutherford (1960). Poly(methacryloyl glucose) has been synthesized as both a homopolymer (Kimura & Hirai, 1962; Imoto & Kimura, 1962; Black, Dewar & Rutherford 1963) and as a copolymer (Kimura & Imoto, 1961). In the1960s two groups Kimura & Imoto, (1961) and Bird, Black, Dewar & Rutherford (1960) were simultaneously working along similar lines to synthesize poly(methacryloyl glucose)(Figure1).

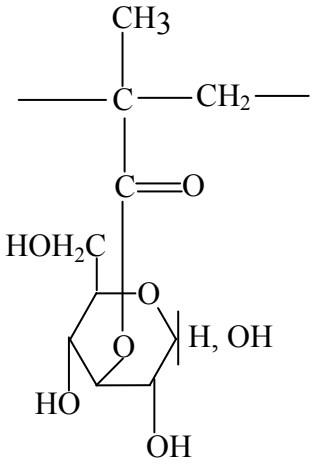


Figure 1. Poly(methacryloyl D-glucose)

They polymerized 1,2:5,6 di-0-isopropylidene-D-glucofuranose methyl methacrylate by free radical polymerization to obtain poly(3-O-methacryloyl 1,2:5,6 di-O-isopropylidene-D-glucofuranose) (Figure2).

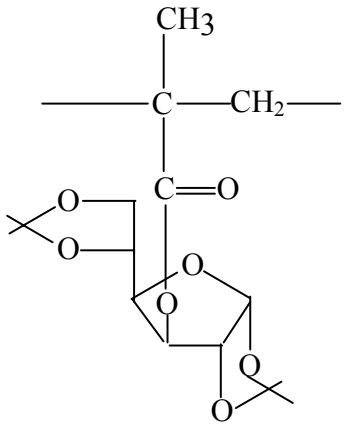


Figure 2. Poly(3-O-methacryloyl 1,2:5,6-diisopropylidene D-glucofuranose)

Removal of the isopropylidene groups afforded poly(methacryoyl glucose).These polymers could be dyed by a water-soluble dyestuff. 1-acrylamido and 1methacryl amido-1-deoxy-D-glucitol were synthesized and polymerized to obtain a new type of vinyl polymer with pendant sugar residues (Panzer & Whistler, 1959). Poly(N-acryloyl-D-glucamine) which was synthesized in 1961 (Whistler, Panzer & Roberts, 1961) displayed a high tolerance for electrolyte. In the early 60s 6-O-vinyl ether of 1,2:3,4 di-O- isopropropylidene-D-galactopyranose (Figure3) (Whistler, Panzer & Goatley, 1962; Black, Dewar & Rutherford, 1963) and the corresponding 6-O-vinyl ether of 1,2:5,6 di-O-isopropylidene glucofuranose (Black, Dewar & Rutherford, 1962) have been reported which were polymerized by cationic polymerization.

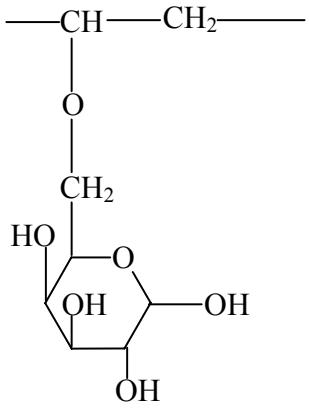


Figure 3. Poly(6-O-vinyl 1,2: 3,4-di-O-isopropylidene D-galactopyranose

However, no special attention was paid to the characterization of the resulting polymers and solution properties of the deprotected water-soluble products until 1986. Klein (1986) prepared poly(6-O-vinyl-1,2:3,4-di-O-isopropylidene-D-galactopylanose) by cationic polymerization and characterized it by ^{13}CNMR. He also studied the solution viscosities of the polymers formed after the deprotection of the isopropylidene groups viz. poly(6-O-vinyl-D-galactopyranose).

Polymers having molecular weights upto 100,000 were successfully synthesized in 1963 (Black, Dewar & Rutherford, 1963). Klein (1982) obtained molecular weights upto 715,000 by solution polymerization. The same group of workers (Klein, Herzog & Hajibegli, 1985) extended their work on the synthesis of poly(vinylsaccharide)s to obtain further higher molecular weights by emulsion polymerization wherein they obtained molecular weights upto 2.9 x 20^7. The methodolgy of grafting onto synthetic backbones, however, has since not been pursued vigorously, due to synthetic challenges. Polymeric derivatives of methacrylic acid containing aromatically substituted glycopyronoside side chains were reported for the first time by (Carpino, Ringsdorf &Ritter, 1976).

Another strategy was adopted for obtaining poly(vinylsaccharide)s by the ring openinig polymerization of the anhydro sugars using macromolecular halides as

initiating systems (Uryu et al., 1981). Refer to note on cationic polymerizations. In the 1990s the importance of poly(vinylsaccharide)s in biological systems was elucidated. It was only in the 1990s that the role of poly(vinylsaccharide)s in biological systems gained impetus. Many articles dealt with the use of these polymers in cell recognition processes, for binding of hepatocytes, synthetic antigens etc. They were mainly useful for elucidating the role of carbohydrates in the biochemical processes. Poly(vinyl alcohol) containing glucose linked by adipic acid as a spacer was shown to be biodegradable (Tokiwa, et al., 2000). Furuike, Nishi, Tokura & Nishimura, (1995); Nishimura, et al., (1991 & 1994), Nishimura, Matsuoka & Kurita, (1990); Matsuoka & Nishimura, (1995), were instrumental in synthesizing a number of glycoconjugates. They studied their binding specificities with lecithins and they found that glycopolymers having related disaccharide derivatives in their side chains (Nishimura et al., 1991) showed enhanced binding capacity with lecithins based on a polymer sugar cluster effect whereas synthetic trisaccharide or smaller sugar derivatives showed only weak affinity to the hemaglutinin molecules (Sauter et al., 1989). Kobayashi A., Akaike, Kobayashi K. & Sumitomo (1986) have reported the synthesis of polystyrene having pendant lactose residues by polymerizing the oligosaccharide lactones with p-vinylbenzylamine by using radical polymerization methods and its application as substratues for liver cell cultures. They also synthesized poly (N-(p-vinyl benzyl)-4-O-β-D-galactopyranosyl D-gluconamide) as a substratum for culture of hepatocytes (Kobayashi K., Kobayashi A. & Akaike, 1994; Kobayashi K., Kobayashi A., Tobe & Akaike, 1994; Kobayashi A., Goto, Kobayashi K., & Akaike, 1994). Several pseudopolysaccharides, or in other words, synthetic polymers with pendant carbohydrates have been synthesized and their potentials as polymeric drugs and in solid phase synthesis have been exploited (Kraska & Mester, 1978; Nishio, Nakaya & Imoto, 1978; Nolte, Zomeren & Zwikker, 1978; Horejsi, Smolek & Kocourek, 1978; Andresz, Richter & Pfannemuller, 1978). An amphiphilic glycopolymer was synthesized (Kobayashi K., Kakishita, Okada, Akaike & Usui, 1994) consisting of a poly(acryl(aminophenyl)) backbone and chemoenzymatically synthesized oligosaccharides (Kobayashi K., et al., 1994). A recent review article by Kobayashi K., (2001) describes various applications of glyconjugate polymers in

biological and biomedical fields. He also studied the micellar behaviour of glycoconjugates in water by various techniques such as fluorescent spectroscopy, dynamic light scattering and fluorescence energy transfer experiments and also measured the average particle size of these micelles (Goto et al., 2001). Glycoproteins have been synthesized in the Uryu laboratory to study the binding of oligosaccharide chains to enzymes and immune active proteins to study the activation and stabilization of natural proteins. They also studied low molecular weight AIDS drug carrying carbohydrate moieties. A very recent article, reported the synthesis and its hydrolysis under physiological environment, of poly(ester amide)s based on arabinose (Pinilla et al., 2002).

The history of the success of anhydro sugar polymerizations dates back to the mid 60s. Ruckel & Schuerch (1966) were the first to successfully synthesize a regular polysaccharide by anhydro sugar polymerizations. Ruckel & Schuerch (1966, 1967); Zachoval & Schuerch, (1969); Uryu & Schuerch, (1971); Lin & Schuerch, (1972) studied the synthesis of D-glucan (Figure 4) in different solvent systems, at different temperatures and effect of various electrophilic reagents in detail. They polymerized 1,6-anhydro-β-D-glucopyranose tribenzyl ether (Figure 5) followed by its debenzylation to obtain high yields and stereoregular polymers.

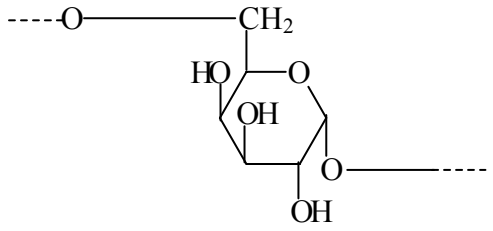


Figure 4. 1,6 D- Glucan

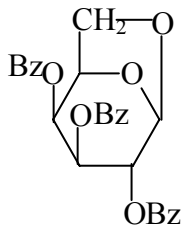


Fig 5. 1,6-Anhydro-β-D-glucopyranose tri benzyl ether

Schuerch was also instrumental in the synthesis of D-galactan (Uryu & Schuerch, 1971; Lin & Schuerch, 1972; Uryu, Libert, Zachoval & Schuerch, 1970) and D-mannan (Lin & Schuerch, 1972; Frechet & Schuerch, 1969; Tkacz, Lampen & Schuerch, 1972). Glucomannans were also synthesized which were linear and stereoregular (Kobayashi K., Eby & Schuerch, 1977). The strategy of ring-opening polymerization of anhydro sugars was extended to the synthesis of glycoconjugates, wherein, disaccharides were linked to various proteins (Eby & Schuerch, 1982).

The anhydro sugars which can be synthesized and polymerized are 1,2-, 1,3-, 1,4-, & 1,6- anhydropyranoses and 1,2-, 1,3-, 1,5- & 1,6- anhydrofuranoses.

The first attempt at polymerizing a 1,6-anhydro sugar dates back to 1918 (Pictet, 1918). Ever since then several attempts, particularly in the late 50s, were made to synthesize linear polysaccharides but were not fruitful (Goldstein & Hullar, 1959; Wolfrom, Thompson & Ward, 1959). Bredereck and Hutten (1963), were first successful in polymerizing perbenzyl ether and peracetate of levoglucosan using organic halides and silver perchlorates. Comb-like glucans were synthesized from 1,6 anhydro-maltose and 1,6-anhydro cellobiose (Veruovic & Schuerch, 1970; Masura & Schuerch, 1970). The polymerization reactivities (polymerizabilities) of isomeric forms of 1,6-anhydroaldoxoses were studied and the rate was found to

be maximum for the mannose sugar (Schuerch, 1981; Uryu, Sakamoto, Hatnaka & Matsuzaki, 1984). The 1,6-anhydro sugars (1,6-anhydro-2,3,4-tri-O-benzyl-β-D-glucopyranose and the corresponding tri-O-methyl derivative) were not only homopolymerized but also have been successfully copolymerized with other monomers like epichlorohydrin, 3,3-bis (chloromethyl) oxetane, and 1,3-dioxolane (Uryu, Hatanaka & Matsuzaki, 1980). 1,6 anhydro-β-D-galactopyranose and 1,6 anhydro-β-D-mannopyranose were also copolymerized to obtain branched heteropolysaccharides viz. α-[1®3] branched dextrans (Ito & Schuerch, 1979). Precipitin activity of synthetic mannan synthesized by polymerization of 1,6-anhydro mannose derivative was compared with mannan isolated from S. cerevisiae and their activities were corelated to their phosphate contents (Okubo et al., 1980). Ring opening polymerization of 1,6 anhydro sugars were carried out using macromolecular halides, Lewis acids or silver hexafluorophosphate to generate carbenium or oxonium ions which initiated the polymerization of anhydro sugars (Uryu et al., 1981).

Uryu, Koyama & Matsuzaki, 1979; Uryu, Kitano, Ito, Yamanouchi & Matsuzaki, 1981) reported the synthesis of 2,3-O-benzylidene[1®5]-α-D-ribofuranan and 2,3-O-benzylidene[1®4]-α-D-ribopyranan by ring opening polymerization of the corresponding 1,5-anhydro2,3-O-benzylidene-β-D-ribofuranose and 1,4-anhydro-2,3-O-benzylidene-α-D-ribopyranose.

The earliest reports of polymerization of 1,4-anhydrosugar was by Kops & Schuerch, 1965. They polymerized 1,4-anhydro-2,3,6-tri-O-methyl-β-D-galactopyranose and 1,4-anhydro-2,3-di-O-methyl-α-L-arabinopyranose. 1,4-Anhydro-2,3,6-tri-O-benzyl-α-D-glucopyranose was also synthesized in 1974 using different catalysts like phosphorous pentafluoride, antimony pentafluoride (Micheel, Brodde & Reinking) and triethyl oxonium tetrafluoroborate (Micheel & Brodde). Difficulties are encountered while polymerizing 1,4-anhydro sugars since these sugars contain fused 1,3-dioxolane, tetrahydrofuran and

tetrahydropyran ring systems (Mark & Bikales, 1988). (1→4)-β-D-ribopyranan was synthesized using the benzylidene derivative of 1,4- anhydroribose (Uryu, et al., 1981), whereas, the furanan form was synthesized from dibenzyl ether of 1,4-anhydro-α- D-ribopyranose (Uryu et al., 1983). More recently arabinofuranan and xylofuranan were prepared by corresponding 1,4- anhydro sugar polymerizations and sulfonated to various extents (Yoshida et al., 2000). The highly sulfonated ones (having degrees of sulfonation of 1.4-1.9) showed potent anti HIV activities and also exhibited higher blood anticoagulant activities.

1,3 Anhydro 2,4,6-tri-O-benzyl and 1,3 anhydro-2,4,6-tri-O-(p-bromobenzyl)β-D-mannopyranose were synthesized by Varma & Schuerch (1980) which were later polymerized to obtain stereoregular mannans by Kong & Schuerch, (1984). Stereoregular (1→3) α-D-glucopyranan and mannopyranan were also synthesized using using triflic anhydride or silver triflate as the catalyst (Kong & Schuerch, (1984); Good & Schuerch, (1985))

Schuerch reported synthesis of glucopyranans and mannopyranans by polymerization of corresponding 1,2- anhydro sugars (Sharkey, Eby & Schuerch, 1981; Yamaguchi& Schuerch, 1980). 1,2 anhydro mannose derivative was also polymerized by Trumbo & Schuerch, (1985) and their results were compared with other 1,2 anhydro sugar polymerizations and mechanisms were proposed.

Other than these few other anhydro sugar polymerizations have been carried out. 5,6-Anhydro-1,2-O-isopropylidene-α-D-glucofurnose (Uryu et al., 1978) was polymerized by ring opening of anhydrous sugars. Uryu, Ito & Matsuzaki, (1979) also reported the polymerization of 3,5- anhydro sugars viz. 3,5-anhydro-1,2-O-isopropyliden eα-D-xylofuranose.

1.3. Methods of preparation:

There are four methods of preparation of synthetic polysaccharides. They are as follows:

1. Polymerization of the vinyl sugar monomers to obtain poly(vinylsaccharide)s.
2. Cationic polymerization of anhydro sugars to obtain synthetic polysaccharides.
3. Enzymatic or enzyme- mediated (chemo- enzymatic) polymerization methods to obtain polymers containing sugars.
4. Grafting of sugars onto functionalized polymeric backbone by polymer analogous reactions.

Each of the methods is described in brief along with the advantages and disadvantages associated with each method.

1.3.1. Polymerizations of the vinyl sugar monomers to obtain poly(vinylsaccharide)s.

Cationic polymerization methods have been employed to polymerize the vinyl ether derivatives of saccharides. An example of this is the polymerization of 6-O-vinyl ether of 1,2:3,4 -di-O-isopropylidene-D-galactopyranose (Whistler, Panzer & Goatley, 1962; Black, Dewar & Rutherford, 1963) and the corresponding 6-O-vinyl ether of 1,2:5,6 di-O-isopropylidene glucofuranose (Black, Dewar & Rutherford, 1962). The above monomers were prepared by bubbling acetylene gas into a mixture containing the corresponding diisopropylidene derivatives of the sugars viz. glucose and galcactose, and potassium hydroxide. The polymerizations of the vinyl ether derivatives of these sugars were carried out using boron trifluoride- etherate catalyst in hydrocarbon solvent. The removal of the isopropylidene protecting groups was effected with 80% formic acid.

The most widely used method of synthesis of poly(vinylsaccharide)s was based on free radical polymerizations of the vinyl sugars. A sugar is attached to a polymeric

backbone through various optional linkages such as ether (Furuike, Nishi, Tokura & Nishimura, 1995; Nishimura et al., 1991; Nishimura, Matsuoka & Kurita, 1990) amide (Nishimura et al., 1994; Fraser & Grubbs, 1995; Kobayashi A., Akaike, Kobayashi K., & Sumitomo, 1986), urea (Klein, 1989; Klein, 1990) or ester (Chen, Dordick & Rethwisch, 1995) linkages. The sugar and the polymer can also be separated by a spacer (Figure 6) (Mandeville & Garigapati, 1997; Tokiwa et al., 2000; Nishimura, Matsuoka & Kurita, 1990) e.g. an alkyl spacer.

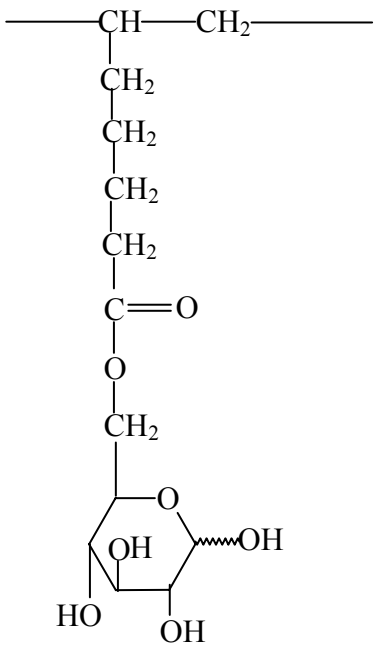


Figure 6. Poly(vinyl alcohol) with pendant glucose separated by adipic acid spacer.

Free radical polymerizations have been carried out in aqueous as well as non-aqueous media. Earlier reports involved polymerizations of the vinyl sugars using the radical initiator viz. AIBN in non-aqueous media (Emmerling & Pfannemuller, 1983; Carpino, Ringsdorf & Ritter, 1976; Rios & Bertorello, 1997; Kimura & Hirai, 1962; Ouchi, Sakamoto, Jokei & Chikashita, 1984) (Figure 7), or benzoyl peroxide also in non-aqueous media (Bird, Black, Dewar & Rutherford, 1960; Rios & Bertorello, 1997).

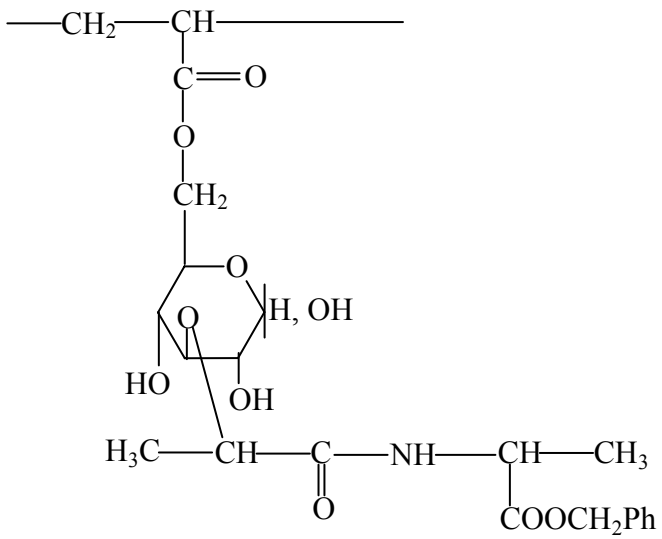


Figure 7. Poly{1-{3-O-[1-(benzyloxycarbonylethylaminocarbonyl)ethyl]-6-O-D- glucopyranosylcarbonyl}ethylene}

Use of benzoyl peroxide gave polymers with higher percentages of sugars as compared to AIBN (Rios & Bertorello, 1997). In one case di-t-butyl nitroxide based alkoxyamine was used as an initiator in nonaqueous medium with dicumyl peroxide as the accelerator (Ohno et al., 1999). Tertiary butyl peroxide was also used for polymerization of poly(vinylsaccharide)s (Moriguchi, 1994). More recently most of the polymerizations of vinyl saccharides have been carried out in aqueous systems using ammonium persulfate or potassium persulfate and N, N, N', N' tetra ethylene diamine (TEMED) (Nishimura et al., 1991; Zhou, Kurth, Hsieh & Krochta, 1999; Lee et al., 1999; Grande, Baskaran & Chaikof, 2000). Ammonium peroxidisulfate was used as a radical catalyst for emulsion polymerization of 3-O-methacryloyl 1,2:5,6 di-O-isopropylidene-D-glucofuranose (Klein, Herzog & Hajibegli, 1985). Redox initiators viz $(NH_4)_2$ S_2O_8 / Na_2 S_2O_8 were used in aqueous medium for polymerizations of poly(vinylsaccharide)s (Klein, 1987, 1989). Vinyl sugar monomers bearing various functional groups were synthesized and these monomers were either

homopolymerized or copolymerized with other polymerizable vinyl monomers to obtain poly(vinylsaccharide)s. Atom transfer radical polymerization of sugar containing radically polymerizable monomers (e.g. acetonide-protected D-glucofuranose methacrylate) in presence of a bromine-containing carbohydate initiator, a ligand and CuBr (Bon & Haddleton, 1999; Ohno, et al., 1998). High-energy radiation was used for polymerization of 1-acrylamido and 1-methacrylamido-1-deoxy-glucitol (Whistler, Panzer & Roberts, 1961). Decomposition of peroxide or azo type catalysts and redox catalyst systems were also tried out (Figure 8).

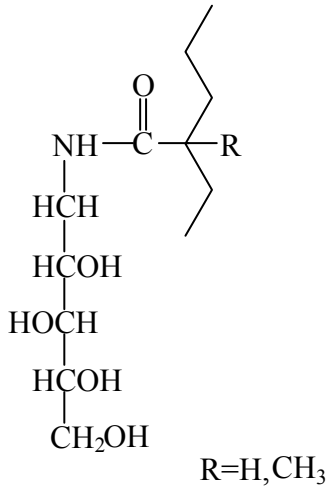


Figure 8. Poly(1-acrylamido/ 1-methacrylamido-1-deoxy-glucitol)

There are various methods to obtain these vinyl sugar monomers so that they can be linked to the polymer through various linkages. These are listed below:

1. Incorporation of acrylic ester onto a sugar moiety and homopolymerizing or copolymerizing it with an acrylate using a radical catalyst (Patil, Dordick & Rethwisch, 1991). This acryloylation can be done either chemically or enzymatically (Figure 9).

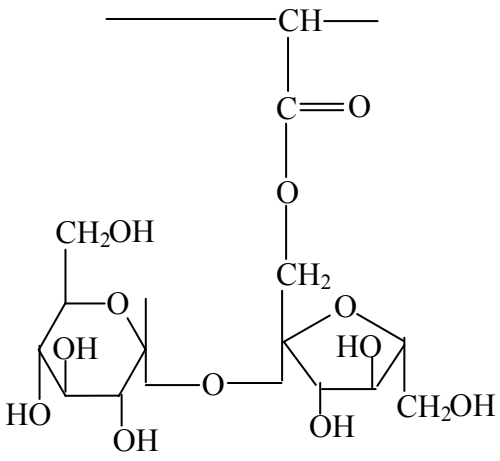


Figure 9. Poly(sucrose acrylate)

2. Converting the sugar into a sugar oxime and homopolymerizing it without the protection of hydroxyl groups (Zhou et al., 1997) (Figure10).

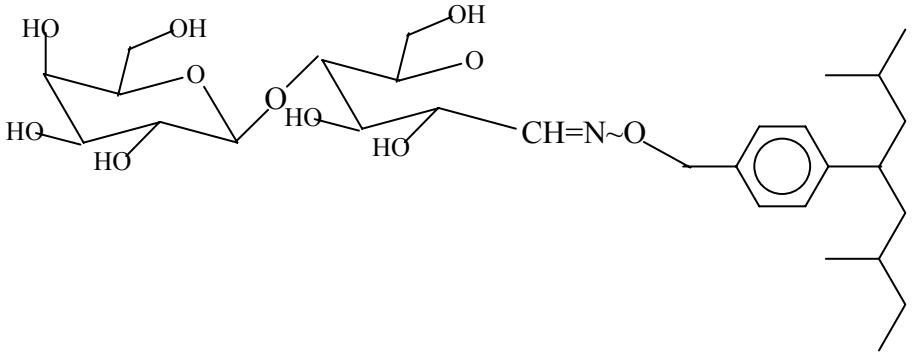


Figure 10. Homopolymer of D-lactose O-(p-vinylbenzyl) oxime.

3. Condensation of an alkyl isocyanate with a sugar amine followed by its free radical polymerization to obtain a poly(vinylsaccharide) with a urea linkage

(Zhou, Kurth, Hsieh & Krochta, 1999) (Figure 11).

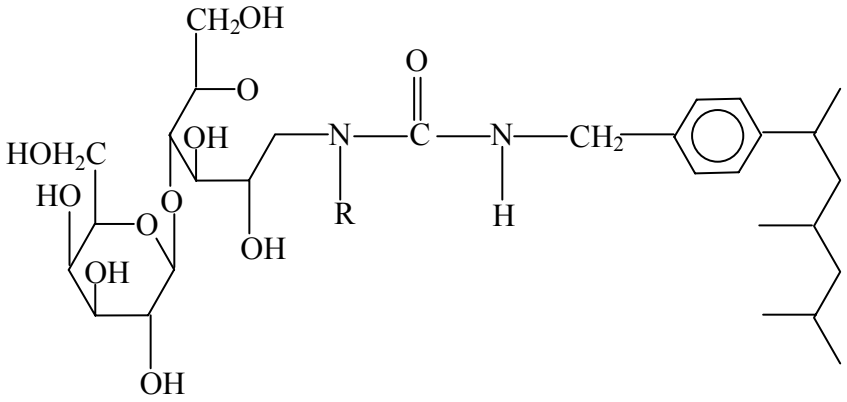


Figure 11. Polystyrene linked to lactose via urea linkage

4. Oxidation of sugars to their corresponding lactones, which in turn are reacted with p-vinyl benzyl amine and polymerization of the adducts by free radical means (Kobayashi K., Sumitomo & Ina, 1985) (Figure 12).

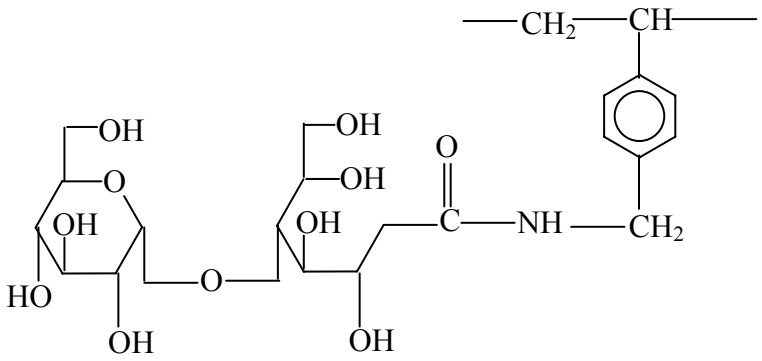


Figure 12. Maltose linked to polystyrene via amide linkage

5. Conversion of sugars to their corresponding glycosyl amines, followed by their conversion to N-acryloyl derivatives, followed by their polymerization by radical polymerization methods. (Kallin, Lonn & Norberg, 1989) (Figure 13).

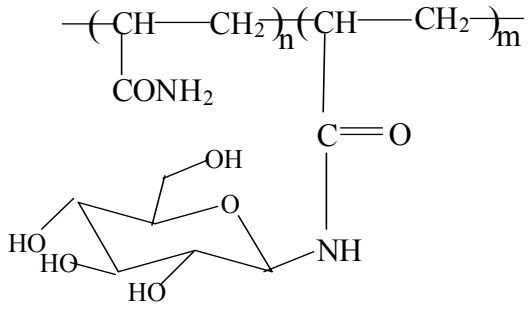


Figure 13. Copolymer of N-acryloyl-4-O-(β-D-galactopyranosy)-β- D-glucopyranosylamine with acrylamide

6. Bulk polymerization of isopropylidene protected vinyl sugars (Wulff, Schmidt & Zhu, 1999) (Figure 2).

There are certain limitations in the syntheses of poly(vinylsaccharide)s involving vinyl sugar polymerizations. Vinyl ethers have to be polymerized cationically only and not by radical polymerization methods.

To synthesize olefins possessing no activating groups Zeigler Natta catalysts have to be employed. However, Ziegler Natta catalysts are not used for polymerizations involving highly functionalized carbohydrate monomers. Another limitation is when a specific percentage of sugar on the polymer is required for a particular application or property, the reactivity ratios of the monomers control the copolymerizaton reactions (Wulff G., et al, 1996). As an illustration, the batch emulsion polymerization of 3-O-methacryloyl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (3-MDG) with butyl acrylate is goverened by their initial monomer ratios in the reaction mixture. 3-MDG has a reactivity ratio as compared to butyl acrylate. Hence the rate of the reaction is directly proportional to the content of the sugar component

(Koch, Yaacoub, 2003)There are again several difficulties associated with protection and deprotection steps, such as multi-step processes, yields, separation of isomers and by-products, crosslinking, and so on. However, this method is invaluable if low amounts of saccharide molecules are to be linked to a functionalized synthetic polymer chain. The deprotection reaction on the monomer is likely to isomerize the double bond. Further, quantitative deprotection of the sugars anchored onto the polymer chain is difficult. Sugars attached to the polymer by ester or glycosidic bond can be hydrolysed under the conditions used for deprotection of the sugars. Ether linkages are susceptible to hydrolysis to certain extent. Only amide bonds are relatively stable (Wulff G., et al, 1996). These, then, are the major difficulties in synthesizing poly(vinylsaccharide)s, which makes their large-scale applications economically unviable. Hence, their applications are restricted only to specialty polymers and high value products, which justify their high costs.

Olefin metathesis reaction, although not a general method of synthesis of poly(vinylsaccharide)s, has also been employed in some cases to obtain poly(vinylsaccharide)s (Mortell, Weatherman & Kiessling, (1996); Fraser & Grubbs, 1995) (Figure 14).

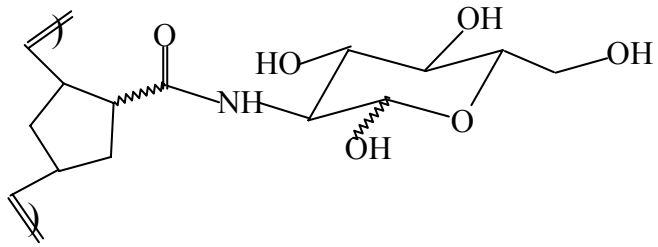


Figure 14. Poly 2-((±)-exo-5-norbornene-2-carboxamido)-2-deoxy-D-glucopyranose

1.3.2. Polymerization of anhydro sugars:

Cationic polymerization methods in carbohydrate chemistry have been more commonly employed for the ring-opening polymerization of an anhydro sugars. The polymerizations are initiated by carbonium ions. The advantage of this method lies in the acquisition of a highly stereoregular polymer with high molecular weight. Such polymerization reactions require extreme purity of the monomers and solvents, and reactions are sensitive to even traces of nucleophilic impurities in the reaction mixture. This has limited the use of these polymers for bulk applications. However this method is definitely useful for obtaining polysaccharides with high stereoregularity (Uryu, Hagino, Terui & Matsuzaki, 1981) which is a prime requirement in cellular studies. The ring strain and the hydroxyl protecting groups also dictate the polymerizations.

1.3.2.1. 1,6-Anhydro sugar polymerizations:

Bredereck & Hutten, 1963 were the first to successfully polymerize the perbenzyl ethers and peracetates of levoglucosan cationically, using organic halides and silver perchlorates as catalysts, to obtain non-stereoregular polymers. The effects of different Lewis acid catalysts on the polymerization of levoglucosan in 1,4-dioxane were studied (Korshak et al., 1961). They obtained branched products with mixed anomeric configuration. They obtained less viscous and crystalline products by carrying out the reactions in toluene at 50°C in presence of boron trifluoride-etherate catalyst. Lowering of reaction temperatures resulted in polymers with higher viscosities. Ruckel and Schuerch (1966, 1967) carried out a series of experiments to determine the optimum conditions, solvents and catalysts required for obtaining stereoregular polymers and they reported that the best results were obtained using phosphorous pentafluoride as the catalyst at a temperature of –78°C in dichloromethane solvent. Number of triether derivatives of levoglucosan do support polymerizations to obtain stereoregular polymers of high molecular weights

(Schuerch, 1981), whereas triester derivatives failed to effect polymerization, particularly below 0°C. Only the trinitrate derivative polymerized at 0°C (Zachoval & Schuerch, 1969). The polymerizibility of 1,6-anhydro-2,3,4-tri-O-benzyl-glucopyranose was tested in dichloromethane solvent at –60 to -78°C using different Lewis acids such as boron trifluoride and its etherate, phosphorous pentafluoride, titanium tetrachloride, antimony pentachloride and antimony pentafluoride, and different initiators such as (triphenylmethyl) antimony hexachloride, 2,3,4,6-tetra-O-acetyl-D-glucopyranosyl hexafluorophosphate, pentamethylbenzyl hexafluorophosphate, acetyl hexafluorophosphate and triethyl oxonium salts with varios anions, and it was found that phosphorous pentafluoride catalysed polymerizations with low catalyst: momomer ratios, i.e. less than 1mol %, gave polymers with highest molecular weights with highest specific rotations (Schuerch, 1981).

1.3.2.2. 1,5-Anhydro sugar polymerizations:

The polymerization of 1,5-anhydro-2,3-O-benzylidene-β-D-ribofuranose was tried out at 0-40°C was carried out in presence of various Lewis acid catalysts such as phosphorous pentafluoride, boron trifluoride etherate and stannic chloride to obtain polymers with molecular weights of 2000-20,000 with mainly α-furanose and α-pyranose units. The (1→4) β-ribopyranans with molecular weights between 26, 000-32,500 were obtained using antimony pentachloride as the catalyst.

1.3.2.3. 1,4-Anhydro sugar polymerizations:

The polymerization of the 1,4-anhydro sugars has been less extensively studied as compared to 1,6-anhydro sugar polymerizations. The polymerization of 1,4-anhydro-2,3,6-tri-O-methyl-β-D-galactopyranose and 1,4-anhydro-2,3-di-O-methyl-α-L-arabinopyranose were the first of the 1,4-anhydro sugar series and were synthesized by Kops & Schuerch,1965). Generally the polymerizations were carried out at a concentration of 10% (g/ mL) in dichloroethane solvent or aromatic hydrocarbons.

The common catalysts used were phosphorous pentafluoride or boron trifluoride etherate at a concentration of 1-5 mol%. The polymerizations were carried out at temperatures of –78 to -97°C for long periods of time to obtain conversions of 50-90%. Polymerizations of 1,4-anhydro-2,3,6-tri-O-benzyl-α-D-glucopyranose reported by Micheel, Brodde & Reinking (1974) with 15-20 mol% of phosphorous pentafluoride gave molecular weights of 21,000 to 41,000. The polymerization of this monomer using antimony pentafluoride was not fruitful and boron trifluoride etherate totally failed.

1.3.2.4. 1,3-Anhydro sugar polymerizations:

1,3-anhydro-2,4,6-tri-O-benzyl-β-D-glucopyranose has been polymerized using various catalysts like phosphorous pentafluoride, boron trifluoride etherate, triethyloxonium hexafluorophosphate, antimony pentachloride and silicon tetrachloride. Of these, phosphorous pentafluoride gave the best results with yields of 60-70%. Polymerizations with basic catalysts failed (Schuerch, 1981).

1.3.2.5. 1,2-Anhydro sugar polymerizations:

Attempts were made in 1956 (Haq & Whelan), to polymerize a 1,2-anhydro sugar, viz. 1,2-anhydro-3,4,6-tri-O-acetyl-α-D-glucopyranose by thermal methods but it resulted into formation of only oligomers. Polymerization of 1,2-anhydro sugars with ether protecting groups, viz., 1,2-anhydro-3,4,6-tri-O-benzyl-β-D-mannopyranose, has been carried out in presence of iodine, methyl borate, phosphorous pentafluoride and triethyloxonium hexafluorophosphate, and produce polymers of mixture of α- & β- anomeric linkages (Yamaguchi & Schuerch, 1980).

1.3.3. Enzymatic and Enzyme mediated Polymerizations (Chemo-enzymatic methods):

Enzymes are highly stereoselective catalysts, which have been effectively used in the synthesis of sugar based polymers, or in specific poly(vinylsaccharide)s (Dordick, Rethwisch & Patil, 1991; Kobayashi K. & Kamiya, 1996), wherein no protection of the hydroxyl groups of the sugar was required. Sucrose contains eight hydroxyl groups, which are all capable of undergoing esterification reactions. However polycondensation of sucrose was affected with diacids using enzyme catalysts to obtain linear polymers, where only two hydroxyls of sucrose were functional (Patil, Rethwisch & Dordick, 1991). The advantages associated with enzymatic reactions are that they can be carried out both in aqueous and non-aqueous media, and as they are selective, protection and deprotection of the sugars can be avoided. There are certain limitations involving enzyme-catalyzed reactions. Most known enzymes catalyze only selective reactions to produce specific sugar derivatives. Therefore, currently only a limited variety of vinyl sugar derivatives can be synthesized by this method. Examples of vinyl sugars that can be synthesized enzymatically are sucrose1-acrylate (Patil, Dordick & Rethwisch, 1991; Patil, Rethwisch & Dordick, 1991), methyl 6-acryloyl-β-galactoside (Martin, Ampofo, Linhardt & Dordick, 1992). The other limitation encountered with enzyme catalyzed reactions is its very slow rate, thereby making use of such reactions less feasible. This problem can be taken care of by using chemo-enzymatic methods of synthesis wherein the vinyl sugar is synthesized in a single step without protection of the sugar hydroxyls using enzymes and then polymerized by chemical means (Patil, Dordick & Rethwisch, 1991; Nishimura, Matsuoaka & Kurita, 1990; Chen, Dordick & Rethwisch, 1995). The chemo-enzymatic method capitalizes both, on the enhanced regio-selectivity over chemical methods and on the speed of conventional chemical methods of polymerizations (Patil, Dordick & Rethwisch, 1991). Sucrose acrylate was synthesized by enzymatic catalysis using an enzyme proleather (a protease from Bacillus. Sp) (Patil, Dordick & Rethwisch, 1991). The sucrose acrylate was polymerized using potassium persulfate/

(H_2O_2) to obtain poly(sucrose acrylate). A review discussed the enzymatic methods of synthesis of sugar containing polymers including both linear polyesters and linear polymers with pendant sugar groups (Ying, Qi, Xingtao, Chenguo, Deshui and Xianfu, 2002). The chemo- enzymatic method was also useful for the synthesis of a glycopolymer viz. Poly(acryl(aminophenyl)) backbone chain with enzymatically synthesized oligosaccharide (Kobayashi K., et al., 1994). Tokiwa et al., (2000) reported esterification of glucose with adipic acid enzymatically and later on effected its polymerization by conventional methods to obtain biodegradable polymers. α-D-galactose was acryloylated with vinyl acrylate enzymatically and later polymerized chemically. Martin, Ampofo, Linhardt & Dordick, (1992) reported variety of monosaccharides with vinyl acrylate in pyridine to obtain 6-acryloyl esters and later on polymerized them in DMF solvent with AIBN as the initiator to give poly(acrylate) products.

1.3.4. Polymer analogous reactions:

Although much has been reported on poly(vinylsaccharide)s, there have been relatively few reports on synthesis of poly(vinylsaccharide)s by polymer analogous reaction, to obtain linear polymers. In this connection, the contributions of Beate Pfannemuller need special mention. His earlier work was based on grafting monosaccharide segments onto natural polymers like amylose to obtain comb like polymers. He later extended this strategy to grafting of sugars onto synthetic polymers. The method of grafting sugars onto synthetic backbones has not been investigated intensively due to perceived difficulties in grafting large monomeric molecules quantitatively onto polymers. However, this method has obvious advantages and the present investigators are of the opinion that this methodology has much to offer in terms of tailored polymer properties. Sugars with protected as well as unprotected groups can also be grafted onto the polymer (Galgali & Varma, 2001, Galgali et al., 2002, Varma et al., 2002). Mild conditions should be chosen in order to avoid formation of cross-linked products. Another advantage is the ease in controlling

the number of sugars being grafted as well as their randomness for low degrees of grafting on the polymer chain. Dramatic improvement in rates of biodegradation of biodegradation of these polymers were reported by these workers, and ha caught the attention of researchers worldwide. Inspite of these advantages, some difficulties have been encountered in compositional analysis (Klein, 1987).

Pfannemueller (1978) reported the grafting of glucose and maltoligomers onto linear polymers like poly (ethylene glycol) having carboxymethyl end groups, poly(acrylic acid) etc via hydrazone linkages (Andresz, Richter & Pfannemueller, 1978). Mono-, di- and oligosaccharides were also linked via amide bonds to synthetic and natural polymers carrying -COOH or - NH_2 groups e.g. poly(acrylic acid), poly(vinyl amine) and polysaccharide derivatives like chitosan. The number and the length of the saccharide branches were varied to obtain polymers exhibiting polyelectrolyte behaviour (Emmerling & Pfannemueller, 1983). A 1981 Japanese patent describes emulsion grafting of glucose onto styrene butadiene rubber but they obtained a crosslinked product (Showa, 1981). Galactose was covalently linked to 2-hydroxyethylmethacrylate-ethylene methacrylate copolymer (Karel, Jiri & Jan, 1980; Jiri, Karel & Jan, 1978). This polymer was used as a stationary phase material for column chromatography of proteins.

A 1985 Japanese patent describes grafting of α-bromo-3,4,6,-tri-O-acetyl-D-glucosamine hydrobromide. The deacetylated product (Koyama, Yoshida & Kurita, 1986) was bactericidal and useful in the treatment of steel (Keisuke, Yoshiyuki & Masaaki, 1985). Usmani & Salyer, (1983) reported grafting of sucrose onto poly(vinyl alcohol) by polymer analogous reactions, which was not supported by any spectral data. However a repetition of the same work by Beereboom (1983) in the same year disproved the above findings giving spectral evidences. Bahulekar et al., (1998) reported synthesis of poly(vinylsaccharide)s by polymer analogous reactions. Glucosamine hydrochloride and galactosamine hydrochloride were reacted with poly(acryloyl chloride) to obtain linear polyacrylamides with pendant sugar residues (Figure 15).

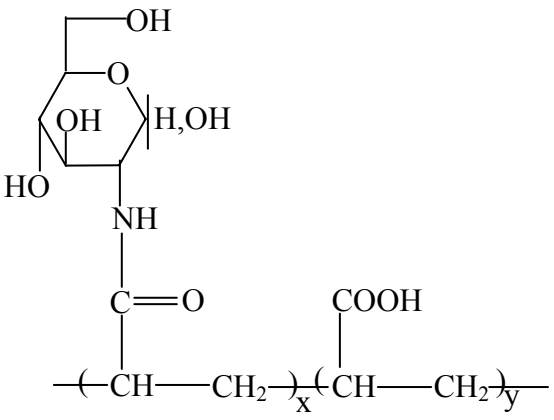
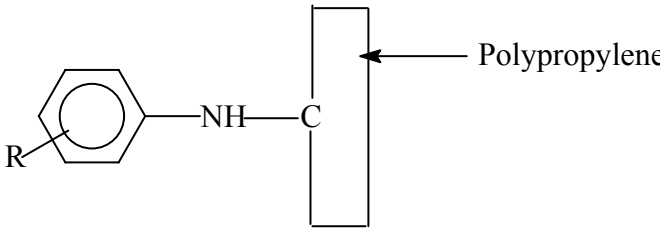


Figure 15. Poly(acryl amide) with pendant glucosamine/ galactosamine

Sucrose was grafted onto butadiene acrylic acid copolymers and poly butadiene carboxylate (Alvarez, Strumia & Bertorello, 1988). The acid chloride derivatives of the polymer were reacted with sucrose in dry DMF using triethyl amine as the catalyst. The esterified polymers were then either reacted with toluene diisocyanate

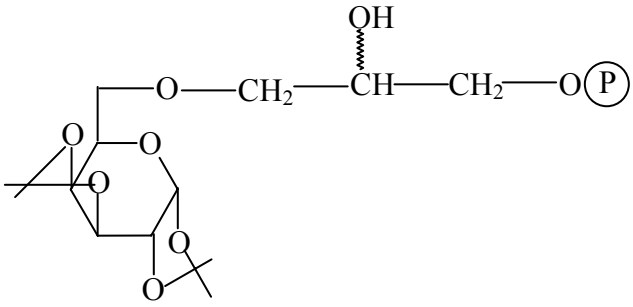


R= sugar (sucrose)

Figure 16. Polypropylene surfaces modified by carbohydrate residues

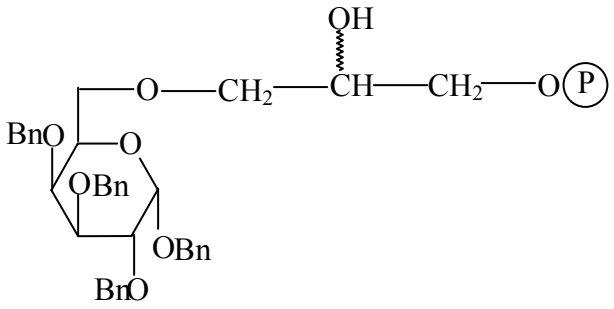
to obtain polyurethanes. The polymers were characterized by IR, PMR. In one of the recent articles (Gruber & Knaus, 2000) polymer surfaces were modified with carbohydrate derivatives by polymer analogous methods (Figure 16).

Partially substituted sucrose esters with 4-azido benzoyl chloride along with swelled polypropylene films were taken in acetone and irradiated by UV radiation. The surface of poly(vinyl chloride) was modified by polymer analogous reactions (Rios & Betorello, 1997). The polymer film was suspended in acetone containing the initiators viz benzophenone and 2,2'- azoisobutyronitrile and sucrose acrylate. The grafting was initiated using ultraviolet radiation. The modification was done in order to improve the interfacial phenomenon between the microorganism and the PVC surface. Kraska & Mester, (1978), reported the base catalyzed adsorption of poly(vinyl alcohol) in DMSO (with traces of KOH) with reducing carbohydrates to obtain pseudopolysaccharides through a chemically and enzymatically inert ether linkage(Figure17, 18 & 19).



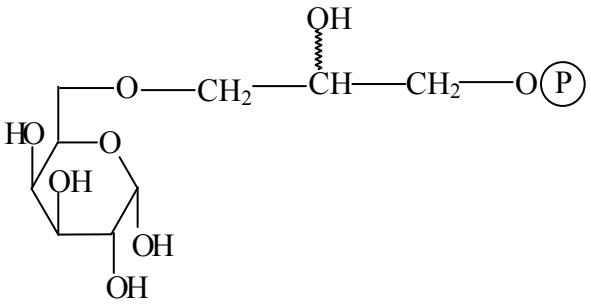
P= poly(vinyl alcohol)

Figure 17. Polyvinylether of 1,2: 3,4-diisopropylidene galactose



P= poly(vinyl alcohol)

Figure 18. Polyvinylether of benzyl galactoside



P= poly(vinyl alcohol)

Figure 19. Polyvinylether of galactose sugar

They are useful in the solid phase syntheses of glycosides, as potential carriers of drugs and they also serve as a useful probe in the study of protein-carbohydrate interactions. 6-O-epoxy propyl derivative of D-galactose-6-O-allyl ether was used for this study. Hemocompatibility of polymer surfaces was improved by grafting of monomers (α-amino acids, peptides and amino sugars like glucamine and D-glucosamine) onto polymers (poly(ether urethanes), poly(ethylene glycol) , poly(tetrahydrofuran), poly (vinyl alcohol and dextran)(Bamford et al., 1990). Pellethane-poly D-glucamine graft was found to be the most inert material towards platelets. Maltamine, which is a mixture of α-D-glucopyranosyl-(1,6)-2-amino-2-deoxy-D-sorbitol and α-D-glycopyroanosyl(1,6)-2- amino-2-deoxy-D-mannitol was

bound to chloroethylated poly(g-Me L-glutamate) and this membrane resolved optically active substances (Nakagawa et al., 1994). A prepolymer prepared from 2-ethyl-2-butyl-1,3-propane diol and hexamethylene diisocyanate was copolymerized with ethyl galactomannan oligomers (Mueller, Peter & Kurt, 1991). Isoamylene and maleic anhydride were polymerized with $Me_3COOCMe_3$ and mixed with D-sorbitol, which showed good dispersibility and Fe-chelating ability (Moriguchi, 1994). Sugars were bound to polymer supports as thiosemicarbazones and they served as means for immobilization of enzymes (Tweeddale, Batley & Redmond, 1994). Glucose and N-acetyl glucosamine hydrazones were reacted with isothiocyanate substituted polystyrene. 3-azido styrene and N-p-vinyl benzyl-(O-B-D-galactopyransoyl (l→4) - D- gluconamide were polymerized and the resulting polymer was applied on a PVC dish and irradiated using ultraviolet radiation to obtain PVC fixed with sugar, which prevented adhesion of blood platelets on the dish (Yura, Goto, Tanaka & Saskurai, 1997). Solvent evaporation technique was also used to prepare nano particles with carbohydrate chains on their surface (Maruyama et al., 1991).

1.4. Methods of characterization:

1.4.1. Elemental analysis has been extensively used in the of analysis of poly(vinylsaccharide)s. In case of copolymers containing N, the nitrogen percentage becomes useful in determining the composition of the copolymer (Ouchi, Sakamoto, Jokei & Chikashita, 1984).

1.4.2. Optical rotation measurements have been used to determine the stereo-regularity of the anhydro sugar homopolymers. They are accurate with a variation of ± 1-2% on different preparations (Schuerch, 1981).

Circular dichroism (c.d.) spectra have been used for determining the configurations at the carbon atoms of the synthetic polysaccharides. As an illustration, both D-glucan different configuration at the C-2 position, the negative sign present in D-glucan and D-galactan is changed to positive sign in D- mannan (Schuerch, 1981).

1.4.3.Intrinsic viscosities of the polymers have been used to calculate the molecular weights of the poly(vinyl sacchahide)s. However intrinsic viscosity does not necessarily give the number average molecular weight. Membrane osmometry in both aqueous and DMSO solvents have been used to determine number average molecular weights for linear and branched D-glucans (Uryu & Schuerch, 1971; Ito & Schuerch, 1979). Light scattering measurements have been used to determine the intrinsic viscosities of the poly(vinylsaccharide)s in 0.1 M sodium sulfate aqueous solution and related to their absolute molecular weights (Klein & Blummenberg, 1988). Sedimentation velocity measurement has been employed to determine the molecular weight distribution in case of tribenzyl ethers of (1→6)-α-D-gluco-, manno-, and galacto- pyranans (Bluhm & Sarko, 1973). Light scattering measurements were also used for determination of molecular weight and hence degree of polymerization (Klein, Herzog & Hajibegli, 1985). Since most of the work on poly(vinylsaccharides) was based on polymerizaton reactions involving vinylsaccharides, the viscosities of the polymer were recorded. The intrinsic viscosity was found to be comparatively small for poly(methacryloyl glucose)(Klein, Herzog & Hajibegli, 1985). Gel permeation chromatography was used for determination of molecular weights and polydispersities of the resultant poly(vinylsaccharide)s (Zhou et al., 1999). Glucose is water soluble, whereas its polymer with acrylic monomers yields an amphiphilic polymer which is water insoluble. Analogous polymers synthesized using disaccharides instead of glucose are water soluble due to increase in the hydrophilicity of the polymer. To study these characteristics, the solution properties of these polymers viz. polymers synthesized by polymerization of N-substituted amides of sugars with 2-isocyanatoethyl methacrylate, were studied by light scattering and viscosity measurements. The value of the Mark- Houwink exponent decreased as the length of the alkyl chain increased, which indicated that the hydrophilic sugar moiety was large enough to keep the sugar moiety in solution, but not strong enough to allow a good solvation of the whole polymer chain by water (Klein, 1990).

1.4.4. Infrared spectroscopy is the most widely used method for analysis of poly (vinyl saccharide. Since most of the work in this area involved polymerization of the vinyl sugars, the disappearance of C=C bond was used as a means for determining the completion of polymerization. In almost all cases IR were done using KBr. The IR was used only for qualitative analysis. Reports on synthesis of poly(vinylsaccharide)s before 1960 did not include analysis by IR spectroscopy. IR spectroscopy was used to monitor the grafting of sucrose acrylate on PVC surface (Rios & Bertorello, 1997). The relative decrease in the band at 616 and 669 cm^{-1} due to ─C─Cl and appearance of new bands at 1720 and 3450 cm^{-1}due to ester bond formation and hydroxyl groups of the sugar. Poly (1,2: 5,6-di –O - isopropylidene- α- D- glucofuranose- 3- oxy- methylstyrene) showed distinct bands at 2980, 1385 and 1375 cm^{-1}, which are the characteristic of the isopropylidene group (Kobayashi K. and Sumitomo, 1980). Poly (D-glucopyranose- 3-oxymethylstyrene) exhibited a broad and strong band at 3400 cm^{-1} due to the hydroxyl group, whereas its sodium salt showed an additional band at 1600 cm^{-1} due to the carboxylate group and its acetylated derivative had bands at 1750, 1375 and 1220 cm^{-1} due to the acetyl group (Kobayashi K. and Sumitomo, 1980). Sucrose linked to carboxylated polybutadiene was characterized by IR spectroscopy. Band at 1700-1720 cm^{-1} was assigned to the carbonyl stretching frequency of the acid and the ester, the band at 3300- 3600 cm^{-1} was assigned to the hydroxyl group of the sucrose and a small band between 1050 and 1100 cm^{-1} was attributed to the –C-O-C bond (Alvarez, Strumia and Bertorello, 1988). The IR spectroscopy of sugar-linked poly(styrene maleic anhydride) polymers was studied. The amount of sugar linked to the polymer was in minute quantities, hence it was not possible to determine which of the sugar hydroxyls was involved in the esterification reaction. Hence to elucidate the role of different hydroxyls in case of glucose in the reaction, different derivatives of glucose were synthesized with varying number of free hydroxyls and their reactivity ratios were determined from the height ratios of the anhydride peak at

1780 cm^{-1} to the >C=C< stretching frequency of the aromatic ring of the polystyrene part at 1492 cm^{-1}.

1.4.5. Nuclear Magnetic Resonance Spectroscopy, both proton and carbon 13 spectroscopy, have been used widely in the analysis of poly(vinylsaccharide)s synthesized after 1980.. Linear structure of the poly (vinyl saccharide) can be confirmed by ^{13} CNMR (absence of peak at δ> 80 PPM indicates absence of branching (Klein, 1989). Sugar region in the ^{13}C NMR is between 40 -100 ppm and in the ^{1}H NMR is between 3.5 - 6.0 δ. Peak at 45 ppm (^{13}C NMR) of poly(1-acrylamido-1-deoxy-D-glucitol) (Klein, 1987) was attributed to branching at the β carbon. Proton NMR spectroscopy was used for determining the grafting of sucrose onto carboxylated butadiene polymer. Where the grafting percentages were high the sugar protons appeared between 3.1 and 4.8 δ and –OH group between 2.4-3.0 δ. However where grafting percentages were low the sucrose peaks did not appear. They were then silylated and then characterized by proton NMR spectroscopy to observe methylic protons at 0.0- 0.4δ. Both CMR and PMR had been used to determine the conformation of poly(N-p-vinylbenzyl-D-gluconamide) in water and attributed the broadening of the signals to intense stacking of the phenyl groups and little mobility of the main chains in water (Kobayashi K., Sumitomo & Ina, 1983). Proton NMR spectroscopy has been instrumental in determining the copolymer compositions in anhydro sugar polymers (Schuerch, 1981). For example, compositional analysis of the copolymer consisting of tri-p-xylyl derivative of glucan and tri-benzyl derivative of mannan was made by comparing the difference in the chemical shifts of the methyl protons of the tri-p-xylyl derivative of glucan homopolymer (Schuerch, 1981). Relative intensities of the peaks corresponding to the α- and the β-anomeric carbon atoms in the CMR spectra are often useful in determining the stereoselectivity in case of anhydro sugar polymers. The assignment of the signals of the anhydro sugar polymers of glucose is made based on a standard linear D-glucan (Colson, Jennings & Smith, 1974).

1.4.6. Absorption spectroscopy: ultra violet spectroscopy has been used for the determination of the copolymer composition. The value of $\varepsilon_{257} = 2751.mol^{-1}cm^{-1}$ in water at room temperature was used for the same (Ouchi, Sakamoto, Jokei & Chikashita, 1984). Absorption spectroscopy has also been used to study the binding of methyl orange and magnesium-1-anilo-8-naphthalene sulfonate to poly(N-p-vinylbenzyl-D-gluconamide) (Kobayashi K., Sumitomo & Ina, 1983).Vacuum-ultraviolet, circular dichroism was used for the study of the conformations of dextran and its oligomers. The linear dextran exhibited a band at 165 nm in contrast to a band at 177 nm for non-linear dextran (Stipanovic & Stevens, 1980).

1.4.7. Scanning electron microscopy was used to study the surface characteristics of the polymers (Rios & Bertorello, 1997; Bahulekar et al., 1998). The weathering behavior of PVC grafted with sucrose acrylate was studied with microorganisms in a growing media and SEM was used to monitor the degradation of the polymer along with IR and weight loss measurements.

1.4.8. Multiple internal reflection fluorescence method: this method was used for studying the interfacial recognition of sugar residues of a sugar carrying block polymers by lecithin (Akira et al., 1999).

1.4.9. Thermal analysis: Form the thermogravimetric curves of poly(glucosamine acrylate) and poly (acrylic acid) it was found that poly(glucosamine acrylate) is thermally more stable than poly (acrylic acid) (Tirkistani, 1997) which was explained on the basis of free amino groups. Differential thermogravimetry was used to determine the initial decomposition temperature and the maximum rate of weight change for PVC and PVC grafted with sucrose acrylate. Both the temperatures were found to lower for PVC grafted with sucrose acrylate as compared to PVC alone (Rios & Bertorello, 1997). Homopolymers of styrene main chain with pendant lactose units were characterized by thermal analysis. The homopolymers showed a two stage

degradation related to the lactose moiety and polystyrene main chain and their glass transition temperatures were 133-134^{o} C (Zhou et al., 1999). The urea linkage increased the glass transition temperatures due to hydrogen bonding. Thermogravimetry showed 25% weight loss between 160-310^{o} C in case of 6-methacryloyl 1,2:3,4 di-O-isopropylidene-D-galactopyranose (Caneiro et al., 2001). Its differential thermal analysis showed that the weight loss occurred in two stages suggesting thermooxidative decomposition of the sample. Copoly(ester amides)s containing L-arabinose units showed a single or two-stage degradation pattern depending on the percentage of the carbohydrate in the polymer, the onset of degradation being 200°C (Pinilla I.M., Martinez M.B., Mata F.Z., Galbis.J.A., 2002). With higher percentages of carbohydrates in the polymer i.e. above 20%, the TGA showed mainly a decomposition pattern with a minor third decomposition stage. The thermal degradation of a copolymer formed by reacting methacryloyl 1,2: 3,4-di-O-isopropylidene-α-D-galactopyranose with ethyl acrylate showed a two-stage degradation pattern with the polymer being stable upto 160°C (Carneiro M.J., et al., 2001). The isopropylidine groups may have been the cause of somewhat lower thermal stability. However, the thermal stability of the unprotected sugar units on the copolymer were not reported. The thermal properties of polyurethane (PU) foams prepared from sucrose polyols and polypropylene glycol mixed with molasses polyols (solution of molasses in polyethylene glycol) were studied by differential scanning calorimeter. It was found that increasing the content of the molasses polyols in case of sucrose based polyols resulted in decrease in the decomposition temperature and in case of polypropylene glycol, there was an increase in the glass transition temperature with an increase in the content of molasses. This suggests that sucrose acts as a hardener in PU molecules and also renders the polymer unstable (Kobashigawa, Tokashiki, Naka, Hirose, Hatakeyama, 1999). In all the above mentioned examples, the thermal studies were carried out on polymers containing significant amount of sugars. Poly(styrene maleic anhydride) linked with minute quantities of saccharide moieties (in the range of 0.1-4.5 weight percent of the polymer) and their biodegraded products were studied by thermogravimetry (unpublished

results). The saccharide-linked polymers and their biodegraded products showed a two-stage degradation pattern as compared to a single-stage pattern in case of the unmodified polymer. The glass transition temperature in case of the copolymer formed by the polymerization of 3-O-methacryloyl 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose with butyl acrylate is reported to be linearly related to the sugar content in the copolymer (Koch, Yaacoub, 2003)

1.4.10. X ray photoelectron spectroscopy has been instrumental in determining the percentage of sugar viz. glucosamine or galactosamine grafted onto poly(acryloyl chloride) using nitrogen percentage values (Bahulekar et al., 1998). The polyacrylamide containing glucose and galactose showed a shoulder for the C1s peak due to presence of acid or ester carbonyl.

1.4.11. Size exclusion chromatography has been used to determine the molecular weight distribution in case of glycopeptide macromers. Unimodal peak indicated narrow molecular weight distribution (Aoi, Tsutsumiuchi, Aoki & Okada, 1996).

1.4.12. Colorimetric titrations were used to determine the sugar contents in the polymers by modified Park-Johnson colorimetric titration (Nishimura et al., 1991).

1.4.13. Contact angle measurements were used for determination of the hydrophilicity of the polymers. Water contact angle decreased substantially for polymers grafted with sugars (Bahulekar et al., 1998).

1.4.14. Gravimetric and dye absorption measurements were used for determining the percentage of grafting of sugars (Rios & Bertorello, 1997). Crystal violet has a tendency to adsorb on hydrophilic surfaces and this property was used to determine the percentage of sugar grafting.

1.5. Applications of polymers containing carbohydrates:

1. They act as cell surface mimics (Fraser & Grubbs, 1995).

   They are used in lecithin or antibody-binding assays, wherein recognition of pendant sugars on the liposomal surfaces by lecithin or enzyme was studied (Kitano & Ohno, 1994, Kitano, Sohda & Kosaka, 1995). Copolymers of N-acryloyl-4-O-(β-D-galactopyranosyl)-β-D-glucopyranosylamine and acrylamide exhibited strong specific binding to antibodies against antigens in ELISA assays. Hence they act as substitutes for glycolipid and glycoprotein antigens in immunological assays (Kallin, Lonn & Norberg, 1989). Kosma & Gass (1987) synthesized artificial antigens based upon polyacrylamide copolymer containing 3-deoxy-D-manno-2-octulopyranosylonic acid residues. Allyl β-cellobiosiduronic acid and allyl β-pseudolaminari biosiduronic acid (Chernyak, Antonov & Kochetkov, 1985) were copolymerized with acrylamide to obtain synthetic antigens. A pseudo polysaccharide that can be useful for binding of monoclonal antibodies by radio-immunoassays and ELISA was developed which contained rhamnose units (Roy & Tropper, 1988). Anhydro sugar polymers have also been used for such studies and concanavalin or lectin were found to react with the terminal α-D-glucopyranosyl and α-D-mannopyranosyl groups (Schuerch, 1981). Since lectin has a valency of four, if two or more of the α-D-glucopyranosyl groups are present in the polysaccharide, it results in he formation of a network. This characteristic has been exploited to determine the linearity of the synthetic polysaccharides (Goldstein, Poretz, So & Yang, 1968; Robinson & Goldstein, 1970; So & Goldstein, 1968).
2. Chromatographic supports for affinity chromatography and for the isolation of proteins with specificity for different sugar residues (Roy & Tropper, 1988; Kobayashi K., Sumitomo & Ina, 1985).
3. Matrices for cell culture (Kobayashi K., Sumitomo, Kobayashi A. & Akaike, 1988; Kobayashi A., et al., 1986).

4. A polymer with pendant sugar residues was conjugated with various enzymes with enhanced stability (Wang et al., 1992).
5. Appended sialic acid residues serving as invitro inhibitors of agglutination of the chicken erythrocytes by the influenza virus (Spaltenstein & Whitesides, 1991; Callstrom, Bednarski & Gruber, 1992). The enzyme horseradish peroxidase was stabilized by the addition of poly(vinyl saccharide), its ability depending upon various factors such as degree of polymerization, various sugars, ionic groups and their degree of substitution in the side chain and also on the concentration of the polymer (Kuhlmeyer and Klein, 2003)
6. Surface grafted lactose, galactose, and N-acetyl glucosamine have been used to obtain high levels of liver cell adhesion to polymers (Hubbell, 1994).
7. Polystyrene plates were coated with polyacrylamide with pendant glucose and galactose, which showed adhesion of rat hepatocytes in primary culture (Bahulekar et al., 1998).
8. They act as artificial glycoconjugates (Nishimura, Matsuoka & Kurita, 1990; Nishimura et al., 1991).
9. They are used in drug delivery systems, dental medicine, bioimplants, contact lenses and tissue engineering (Caneiro et al., 2001).
10. They have pharmacological and biomedical applications (Kobayashi K., Sumitomo & Ina, 1985).
11. Polymer bound oligosaccharides are used as intermediates in synthesis of oligosaccharides (Kobayashi K., Sumitomo & Ina, 1985; Kobayashi K. & Sumitomo 1980).
12. Monosaccharide containing poly(vinylsaccharide)s have strong affinity for organic solutes in water (Kobayashi K., Sumitomo & Ina, 1985).
13. They are used in preparation and use of specialty polymers, which are endowed with specific functions of sugars (Kobayashi K. & Sumitomo 1980).
14. Chiral templates derived from sugars are useful for asymmetric synthesis and optical resolution of organic molecules (Kobayashi K. & Sumitomo 1980). Molecularly imprinted polymers prepared from sugar acrylates have been

used as chiral stationary phases for the resolution of the D- and L-isomers of CBz- Asp in polar organic eluents(Liu & Dordick, 1999)

15. Hydrophilicity of sugar was applied to the design of a reverse osmosis membrane and a selectively permeable membrane (Kobayashi K. & Sumitomo 1980).
16. Potassium N-(p-vinyl benzyl)-6-D-glucasamid-1-ate copolymerized with acrylamide inhibited the activity of β-glucuronidase through a model reaction with p-nitrophenyl β-glucuronide (Hashimoto, Oshawa & Saito, 1999).
17. Silcone rubbers (polysiloxanes) possess highly hydrophobic surfaces, which is a drawback for biomedical applications such as surgical implants of contact lenses. Polysiloxanes containing glucose, sucrose and other carbohydrate derivatives have been reported which give better wettability and biocompability (Mossl, Gruber & Greber, 1993; Gerd & Stadler, 1991; Volker & Stadler, 1998).
18. Polypropylene surfaces were grafted with azido substituted sucrose to increase its wettability (Gruber & Knaus, 2000).
19. Polystyrene plates were coated with polyacrylamide with pendant glucose and galactose to increase the surface wettability (Bahulekar et al., 1998).
20. Used in synthetic fibres of the nylon type from 1,6-diamino sugars and dibasic acids (Bird, Black, Dewar & Rutherford, 1960).
21. Used in optically active poly saccharides (Bird, Black, Dewar & Rutherford, 1960).
22. Poly(p-vinyl phenol) with grafted α-bromo-3,4,6-tri-O-acetyl -D-glucosamine was bactericidal was useful in treatment of steel (Keisuke, Yoshiyuki & Masaaki, 1985).
23. They act as polyelectroytes in absence of salt (Emmerling & Pfannemueller, 1983).
24. N-(2-hydroxypropyl)methacrylamide copolymers linked by fucosylamine/ galactosyl amine with adriamycin drug was oligopeptide spacers are used in cell specific targeting to promote conjugate targeting to L1210 cells and heptatocytes (Duncan et al., 1989).

25. Polyurethane foams especially rigid foams which are prepared by using sucrose as the crosslinking agent are useful as resin binders for foundry use, solid urethane plastics and foam coatings (Kunz, 1993; Faulkner, 1977).
26. Behavior of an amphiphilic chloromethyl styrene polymer containing pendant glucose units behaved as a polysoap in water. Methyl orange was strongly bound to the hydrophobic regions of the polymer in water (Kobayashi K. & Sumitomo, 1980).
27. Polymers formed by homopolymerization of unsaturated sugars and their copolymerization with comonomers like unsaturated carboxylic acids, esters, acrylic compounds, vinyl heterocycles, styrenes or maleic acid compounds are useful as thickeners which are also biocompatible (Buchholz et al., 1995).
28. An inter polymer formed by reactive blending of a synthetic polymer (maleic anhydride-styrene copolymer) having functional groups with starch was suitable for molding various articles (Vaidya & Bhattacharaya, 1993).
29. Copolymers of glucose or sucrose with comonomers like acrylic acid, sodium methallyl sulfonate, sodium 2-methacryloyl oxyethyl sulfate or vinyl phosphonic acid were useful as sequestering agents for Ca, Fe and other ions, as additives in textile desizing, bleaching , dyeing or printing, as dispersing agents for pigments in paper coating compositions, as additives in leather manufacture for improving chrome tanning, softness, brightness etc (Krause & Klimmek, 1984).
30. Monosaccharide and oligosaccharide derivatives polymerized with ethylenically unsaturated monomers containing maleic acid salts gave polymers, which had chelating ability towards $CaCO_3$ and biodegradability, and was used as an adjuvant in dyeing of cotton (Yamaguchi, Fugii & Tsuboi, 1994).
31. Cyclodextrin vinyl diester polymerized with styrene and isoprene are useful as surfactants, thickeners, contacts (Vetter, 1994).
32. Chloromethylated polysulfone membrane treated with amino sugars was used as slow release biomembrane and dialysis membrane (Nakagawa et al., 1993).

33. Membranes prepared by binding maltamine to chloroethylated poly (g-Me L-gluctamate) are useful for resolution of optically active substance (Nakagawa et al., 1994).
34. Maltose and maltotriose containing polystyrenes had the ability to specifically interact with concanavalin (Kobayashi K., Sumitomo &Ina, 1985).
35. Isoamylene and maleic anhydride polymerized with $Me_3COO\ CMe_3$ and mixed with D-sorbitol were useful as water treating agents, scale inhibitors, detergents and the have Fe-chelating ability (Moriguchi, 1994).
36. Copolyemrs of styrene, methyl methacrylate or acrylonitrile with small amounts of vinyl sugars (with sulfate groups) had heparin like activity (Wulff, Bellmann, Schmidt & Zhu, 1998).
37. Partial deprotection of poly(1,2:5,6 di-O-isopropylidene α-D-glucofuanose) was carried out in order to get good coating and imaging properties (Havard, et al., 1999).
38. Sucrose methacrylates were polymerized to obtain hydrogels, which were further reacted with succinic anhydride and maleic anhydride to obtain adsorbers for bilirubin (Gruber & Knaus, 2000).
39. 6,1',6'-tri-O-(p-vinyl benzyl) sucrose was copolymerized with styrene or methyl methacrylate in presence of a crosslinking agent to obtain biodegradable polymers (Khan, 1976).
40. Sucrose diacids were cross-linked with diepoxide crosslinking agents to obtain sucrogels (Faulkner, 1977). Sucrogel was also prepared by transesterification processes (Carraher, et al., 1981). Sucrose was also reacted with organostannane dihalides to form crosslinked network. These gels are useful for separation of heavy metal ions (Alvarez C., et al, 1991). Sucrogel (Gruber & Greber, 1991) have applications in drug delivery systems. The sugar hydroxyls were derivatized by trimethyl silyl groups and they were incorporated into hydrophilic silicon rubbers which were useful for medical purposes (Gruber & Knaus, 2000).
41. Synthetic linear D-glucan was used as a tool to elucidate the role of the determinants present in dextran which are responsible for the antigenic

properties in dextran and it was found that three to seven units of (1→6)-α-D-glucopyranose were the antigenic determinants of dextran (Kabat, 1954, 1957 & 1960).

42. Sucrose has been extensively used in the manufacture of urethane foams (Faulkner, 1977) and in particular rigid polyurethanes. Sucrose has great advantage due to its relatively low cost and excellent physical properties of the resulting polymers. Generally sugar having a functionality of 2,3 hydroxy groups are used for manufacture of flexible foams coatings, adhesives and elastomers whereas sugars having a functionality of 4,5 hydroxyl groups are used in the manufacture of rigid polyurethane foams. Others uses include solid urethane plastics and foam coatings.

1.6. Other potential applications of polymers containing carbohydrates:

2. They are potentially processible and biodegradable polymers (Carneiro et al., 2001).
3. They are potential biocompatible polymers eg. (Chen, Dordick & Rethwisch, 1995; Patil, Dordick & Rethwisch, 1991). Poly(methyl 6- acryloyl-β-galactoside-hema) copolymer swelled in water and contained 98% water and could hold nearly 50- fold its weight in water. Such materials have potential use as biocompatible hydrogels for biomedical and membrane applications (Martin, Ampofo, Linhardt & Dordick, 1992).
4. Crosslinked poly(vinylsaccharide)s form hydrogels which can act as superabsorbers (Wulff, Schmidt & Venhoff, 1996; Patil, Dordick & Rethwisch, 1991; Zhou et al., 1999). Polymers like poly(α-methyl galactoside 6-acrylate) hydrogels have this potential (Chen, Dordick & Rethwisch, 1995; Patil, Dordick & Rethwisch, 1991).
5. Drug delivery systems (Zhou et al., 1999).
6. Chromatographic supports for isolation of proteins with specificity for different sugar residues (Zhou et al., 1999).

7. They can be used as stabilizers in dispersion polymerizations (Zhou et al., 1999).
8. They can serve as anti-inflammatory agents (Fraser & Grubbs, 1995).
9. They might enhance or trigger desirable biological responses such as for use in cancer immunotherapy (Fraser & Grubbs, 1995).
10. Biomimetic models of glycoconjugates (Nishimura et al., 1994).
11. Therapeutic and diagnostic purposes in biomedical and biochemical fields (Nishimura et al., 1994; Nishimura, Matsuoka & Kurita, 1990).
12. They are useful for elucidation of biological roles of carbohydrates and their pharmacological and physiological applications (Kobayashi K. & Sumitomo, 1980).
13. Poly(methacryloyl glucose) has the potential to be used as a technical viscosifier for special applications (Klein, Herzog & Hajibegli, 1985).
14. Since glucosamine derivatives containing alkyl chains exhibit antimicrobial properties, polymers containing glucosamine derivatives have potential anti-microbial properties (Tirkistani, 1997).
15. Poly(vinylsaccharide)s have the potential to serve as water-soluble non-ionic polymers (Wulff, Schmidt & Venhoff, 1996).
16. Poly(vinylsaccharide)s also have the potential to be used as thickeners in the tertiary oil recovery (Wulff, Schmidt & Venhoff, 1996).
17. They also have the potential to be used as stable dextran analogues for the immobilization of enzymes and as gel permeation chromatography materials for the separation of water-soluble substances (Wulff, Schmidt & Venhoff, 1996).
18. They can be used for surface modification of standard polymers (Wulff, Schmidt & Venhoff, 1996).
19. Poly(vinylsaccharide)s have the potential to be used ad flocculating agents and also as polymeric detergents (Wulff, Schmidt & Venhoff, 1996).
20. Polystyrene and polyacrylamide which contain reducing sugar moteies like glucose have been reported to interact with the glucose transporter protein of

red blood cells and hence has the potential for fixation of cells via a transporter protein (Kitugawa et al., 2001).

21. A poly(vinylsaccharide) containing glucose and adipic acid produced superoxide in aqueous solution under the influence of nitrobluetetrazolium, hence they have an important role in biological systems such as inactivation of viruses and clevage of DNA (Kitugawa et al., 2001).
22. D- glucaric derivatives are known to behave as inhibitors of β- glucuronidase and hence poly(vinylsaccharide)s containing D-glucaric acid derivatives are potentially capable of inhibiting the activity of β- glucuronidase. The inhibition of this enzyme would enhance the discharge of toxic xenobiotics (Hashimoto, Oshawa & Saito, 1999).
23. Sulfated alkyl oligosaccharide)s have exhibited high anti-AIDS virus activity and hence sulfated poly(vinylsaccharide)s have the potential for such activity (Uryu)
24. Sucrogels are potentially useful for oral drug delivery (Gruber &Greber, 1991).
25. Amphiphilic polymers with variable hydrophobic- hydrophilic balance can obtained by polymerization of N-substituted amides of sugars and 2-isocyanatoethyl methacrylate which influence the properties of the resulting polymers. Hence these polymers are potential surfactants and help to alleviate the allergic problems associated with commercial surfactants (Klein, 1990).

1.7. Conclusions:

It is thus clear that more efforts are needed to synthesize poly(vinylsaccharide)s by avoiding multi-step protection- deprotection procedures, at the same time having the ability to control the incorporation of sugars to give tailor made properties for biodegradability, hydrophilicity, solubility and physical and mechanical properties. Our group is currently working along these lines to synthesize linear poly(vinylsaccharide)s by grafting of monosaccharides and disaccharides onto functional polyolefins without having to use hydroxyl

protection group chemistry of the sugar molecule. The advantage of this procedure is that it is a single step process. Initial results have shown some promise and we plan to extend our work to oligosaccharides. Another interesting aspect of the study would be to obtain structure-property relationships by reacting the functionalized polymer with specific hydroxyls of the sugar. These sugar modified polyolefins have been found to have greatly enhanced rates of biodegradation as compared to the original functionalized polyolefins (Galgali, Varma, Gokhale, Puntambekar & Khire, 2002)

1.8. References:

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CHAPTER 2

"Towards biodegradable polyolefins : strategy of anchoring minute quantities of monosaccharides and disaccharides onto polystyrene, and their effect on polymer biodegradation rates and microorganism specificity"

(reproduced from our paper published in Chemical Communications, 2884, 2002)

Abstract:

A hypothesis was developed, and successfully tested, to greatly increase the rates of biodegradation of polyolefins, by anchoring minute quantities of glucose, sucrose, lactose, onto functionalized polystyrene (polystyrene-maleic anhydride copolymer) and measuring their rates of biodegradation.

2.1. Introduction:

The continuing growth in consumption of non-degradable polymers has led to severe problems of plastic waste disposal by land filling or incineration. One of the alternatives to overcome this problem is the development of biodegradable polymers. Natural polymers are still some way from being developed as viable alternatives to petroleum derived polymers. This realization has led to voluminous researches on additives for polyolefins that can cause degradation of these polymers (Scott and Gilead, 1995). However, these additives are toxic, can leach out, and also affect the other additives needed to process polyolefins. On the other hand, hardly any successful research has gone into designing new plastics or effecting minor chemical modifications of polyolefins or similar polymers with attachment of sugar molecules (Tokiwa, 2000; Rios and Bertorello, 1999; Alvarez, Strumia and Bertorello, 1988; Bahulekar, 1980; Lee, Pometto, Fratzke, and Bailey, 1991) that can render the polymer molecule intrinsically biodegradable or bioassimilable, and convert these large volume polymers into environmentally benign materials.

2.2. Results and Discussion:

In this paper we present our results on a new strategy to vastly improve the rates of biodegradation of polystyrene. Polystyrene, functionalized with maleic anhydride (14% by weight), was used as the base polymer onto which we chemically anchored minute quantities of various monomeric sugars like glucose, lactose and sucrose.[$]

Instead of using a mixture of several microorganisms, we chose three pure soil bacterial cultures[#] (Serratia marcescens, Pseudomonas sp., and Bacillus sp.) for studying their individual growth patterns on these new polymers in comparison to their growth in glucose solution or onto the unmodified polymer.

Figure 1 shows that the rates of increase in optical density in sugar-linked polymers (reflecting material degradation) is much higher than the starting polymer and even the glucose control. Among the three bacteria tested, the behavior of two of them Pseudomonas sp. (NCIM 2220) and Serratia marcescens (NCIM 5061) was similar in terms of preference for a particular set of polymers (Figures 1a and 1b), while that of the third, Bacillus sp. (NCIM 2812) was markedly different. (Figure1c).[†] Thus, for Pseudomonas sp. (NCIM 2220) and Serratia marcescens (NCIM 5061) the preferences are lactose-linked polymer > glucose-linked polymer > sucrose-linked polymer > starting polymer, while for Bacillus sp. (NCIM 2812) the preferences are sucrose-linked polymer > glucose-linked polymer > Control glucose > lactose-linked polymer > starting polymer. (See supplementary data).

The advantages of using pure cultures in biodegradation studies of polymers helps to identify the types of soil bacteria that preferentially attack a particular type of sugar polymer. This is useful in designing biodegradation culture media.

Overlapping FTIR spectra of poly(styrene maleic anhydride) grafted with sucrose and its biodegraded products were recorded (see supplementary data). The biodegraded products show prominent reduction in the intensity of the bands at 3200 cm^{-1} (sugar—OH), 1780 cm^{-1}(anhydride carbonyl), 1716 cm^{-1}(ester carbonyl) and 1600 cm^{-1}(polystyrene phenyl ring). Thus, not only the sugar component, but also the polystyrene component of the polymer is degraded by these microorganisms.

In conclusion, we have proved our hypothesis that anchoring of minute quantities of saccharide moieties onto polyolefins would greatly improve their rates of biodegradation that can make these polymers acceptable to a society desiring eco-friendly materials. We feel this is a general strategy that can be applied to all

polyolefins, and on-going detailed research in our laboratory in this area will be published in due course. The dramatic changes in growth pattern of individual bacteria on the unmodified polymer as well as on the saccharide modified polymers can have wide-ranging ramifications in the design of sugar based biodegradable polymers, the biodegradation media, and the mechanism of biodegradation of sugar-laced polymers by these bacteria.

2.3. Notes and references :

2.3.1. General procedure for grafting of sugars onto poly(styrene maleic anhydride):

A solution of the polymer in dry DMF was added to a solution of the sugar and 4-dimethyl amino pyridine in dry DMF slowly over a period of one hour at 47-50°C in presence of dry nitrogen. The ratios of maleic anhydride to sugar were either 1:3 in case of lactose and sucrose and 2:1in case of glucose. The reaction mixtures were stirred at 47-50°C for 18 hours. The products were precipitated in brine, washed with water several times till they were free of chloride and dried in a vacuum oven. FTIR (Shimadzu Hyper 8300) in nujol: 1710-1716 cm^{-1}(—**CO**OR—), ~3400 cm^{-1} (sugar —**OH**), reduction in the intensity of band at 1780 & 1857 cm^{-1} (anhydride >**C=O**). NMR (^{1}H and ^{13}C) (Bruker) showed only traces of sugar in the polymer molecule. Quantification of the sugar content of the polymer by NMR was therefore very difficult. Thermogravimetric analysis (Perkin Elmer) of some of these polymers was carried out under nitrogen atmosphere. This data, as well as the FTIR data, however, proves the chemical linking of the sugars onto the polymer .

All the organisms listed in the tables were obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune. Cultures were routinely maintained in Nutrient agar slopes (Beef extract, 1.0%; NaCl, 0.5%; peptone, 1.0% and Agar 2.0%). Test organisms used were: Bacillus sp. NCIM 2812, Pseudomonas sp. NCIM 2220 and Serratia marcescens NCIM 5061.

2.3.2. Determination of biodegradability of polymers using aerobic microorganisms

Testing of the samples: Cultures were grown in Minimal medium containing (g/l): $(NH_4)_2SO_4$, 2.0; K_2HPO_4, 14.0; KH_2PO_4, 6.0; Trisodium citrate, 1.0; $MgSO_4.7H_2O$, 0.2. The pH of the medium was adjusted to 7.0 prior to sterilization. The medium was sterilized at 121°C for 20 min. The test samples were surface sterilized with 70% ethanol at a concentration of 0.5% for 2 h and then added separately to the sterilized medium. The cells of the cultures grown in 10 mL Nutrient broth for 24 h at 30 ^oC were suspended in 10 ml of saline and this suspension was used as an inoculum. Approximately 0.1 mL (~10^8 cells) were inoculated into 20 ml of the minimal medium in 100 ml conical flasks. The flasks were incubated at 28°C with shaking at 180 RPM. The growth (optical density) was monitored over a period of four weeks using Systronics 117 spectrophotometer.the polymer was separated from the cells by filtration using whatman filter paper. The residue was further washed many times with water followed by washing with 70% ethanol. It was dried at 50°C and analysed by IR spectroscopy and the percentage weight losses of the polymers were recorded. For recording the weight losses, thick films of the sugar-grafted poly(styrene maleic anhydride) were made and treated with the bacteria in the same manner.

2.4. References:

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G. Scott and D.Gilead,; Editors (UK). 1995 (Chapman & Hall: London, UK), 416 pp.

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Figures 1: Growth patterns of different bacteria in presence of carbohydrate- linked polystyrenes: Curve A= Control 1: Control without any source of carbon; Curve B= Control 2: Control with glucose as the sole carbon source; Curve C= Control 3: Unmodified poly(styrene maleic anhydride); Curve D= Lactose- linked polystyrene; Curve E= Glucose- linked polystyrene; Curve F= Sucrose- linked polystyrene

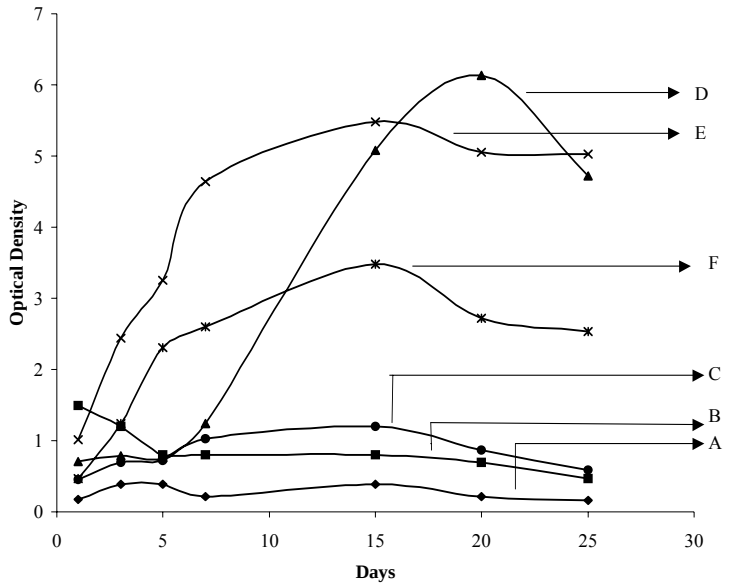


Figure 1a: Growth pattern of Pseudomonas sp. (NCIM 2220) in presence of carbohydrate- linked polystyrenes

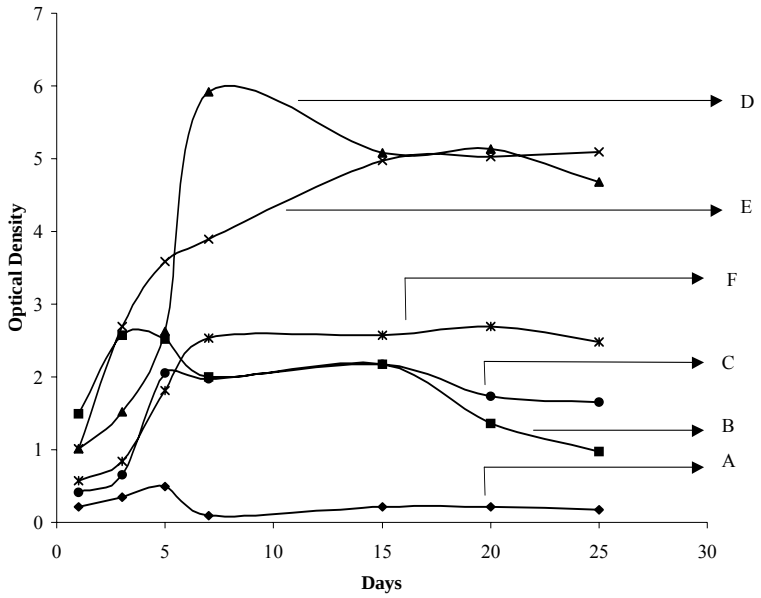


Figure 1b: Growth pattern of Serratia sp. (NCIM 5061) in presence of carbohydrate- linked polystyrenes

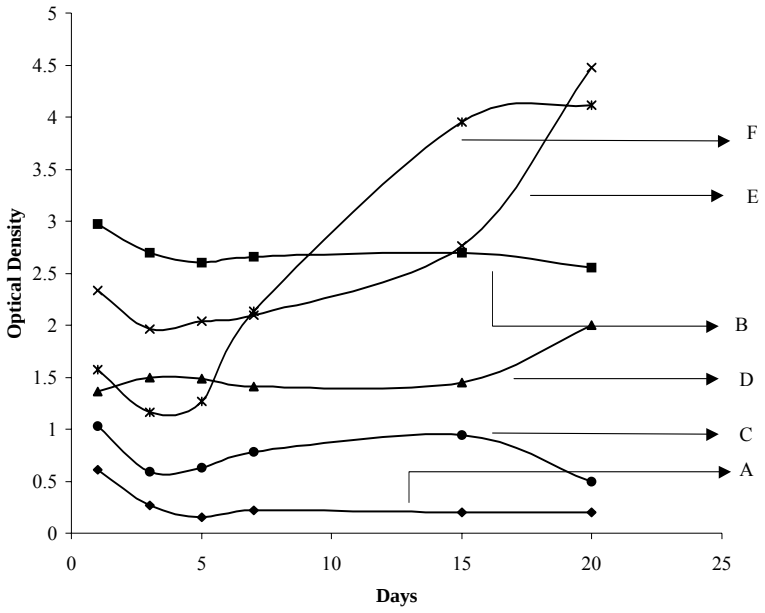


Figure 1c: Growth pattern of Bacillus sp. (NCIM 2812) in presence of carbohydrate- linked polystyrenes

2.5. Supplementary Data:

2.5.1. Weight loss data:

These were carried out for some representative samples. Due to adherence of bacterial cells onto the polymer, the weight losses are lesser than expected. It is reported that in many such cases, weight loss data are inconclusive due to cell wall accumulation (Ref. Byungate Lee et al., Applied and Environmental Microbiology, pg. 678-685, Mar. 1991).

Table 1: Weight loss data of sugar- linked poly(styrene maleic anhydride) polymers degraded by bacteria

Sample	**Initial weight (mg)**	**Final weight (mg)**	**% weight loss**
PSMAH graft lactose degraded by Serratia sp.	250	222	11.2
PSMAH graft lactose degraded by Bacillus sp.	250	231	7.6
PSMAH graft sucrose degraded by Bacillus sp.	250	220	12
PSMAH degraded by Serratia sp.	250	250	0
PSMAH degraded by Bacillus sp.	250	244	2.4

PSMAH= poly(styrene-co-maleic anhydride)

2.5.2. FTIR Spectral Data:

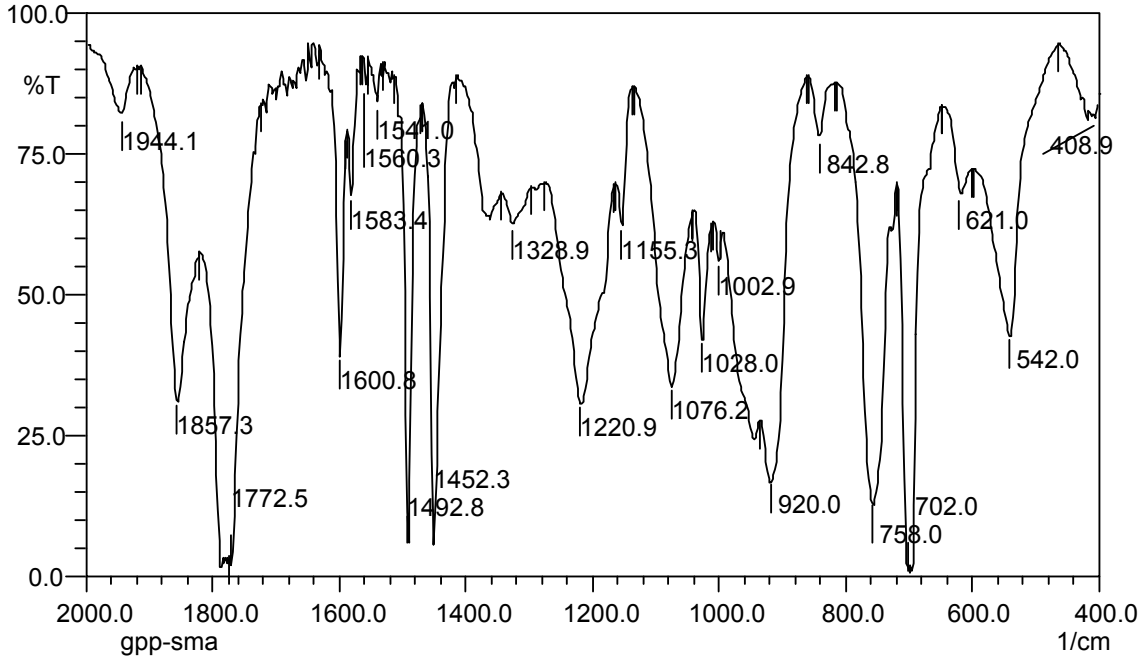


Figure 2a: FTIR spectrum of poly(styrene-co-maleic anhydride) contg. 14 wt% maleic anhydride (Aldrich) (Film)

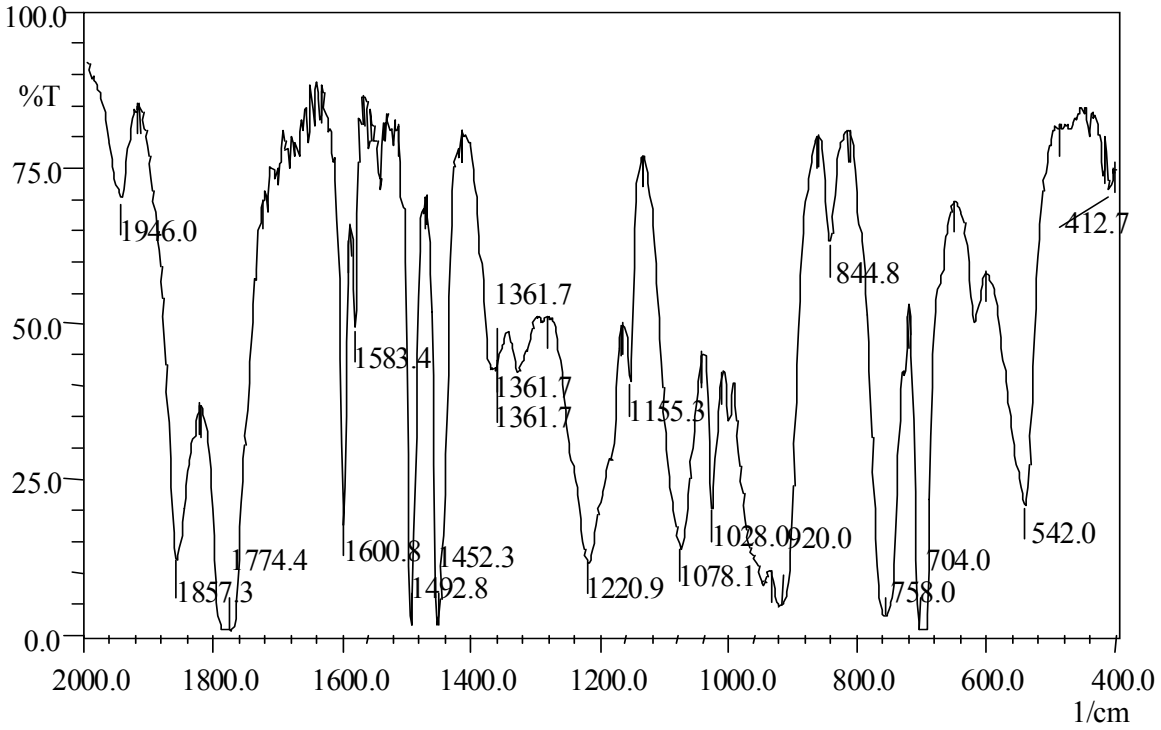


Figure 2b: FTIR spectrum of poly(styrene-co-maleic anhydride) degraded by *Bacillus* sp. (Film)

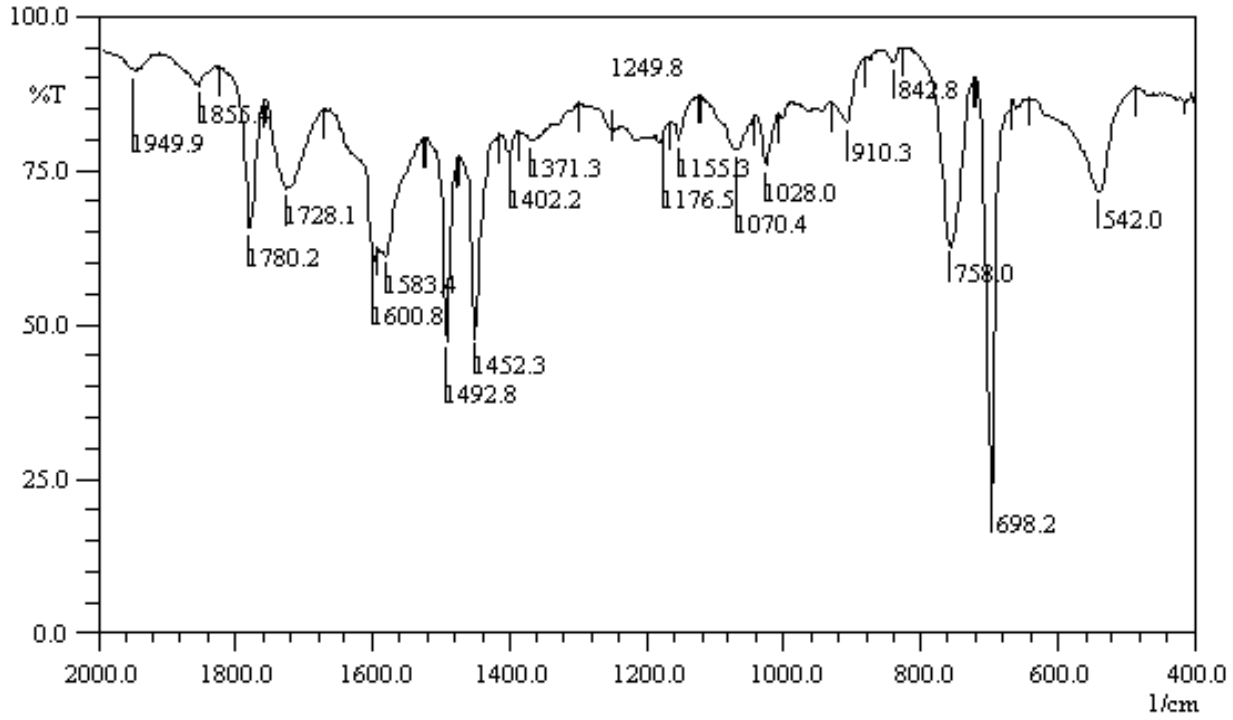


Figure 3a: FTIR spectrum of poly(styrene-co-maleic anhydride) graft Sucrose (MAH: Sucrose 1:3) (KBr)

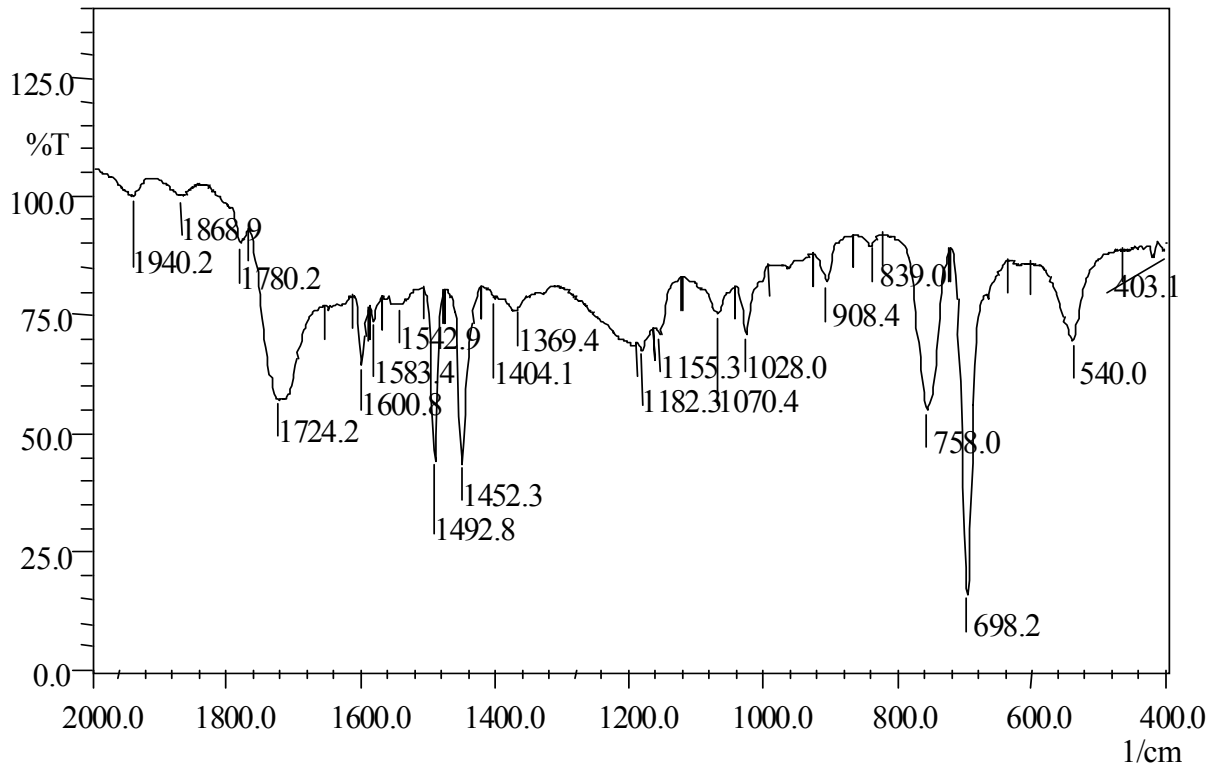


Figure 3b: FTIR spectrum of poly(styrene-co-maleic anhydride) graft sucrose degraded by Serratia marscecens (KBr)

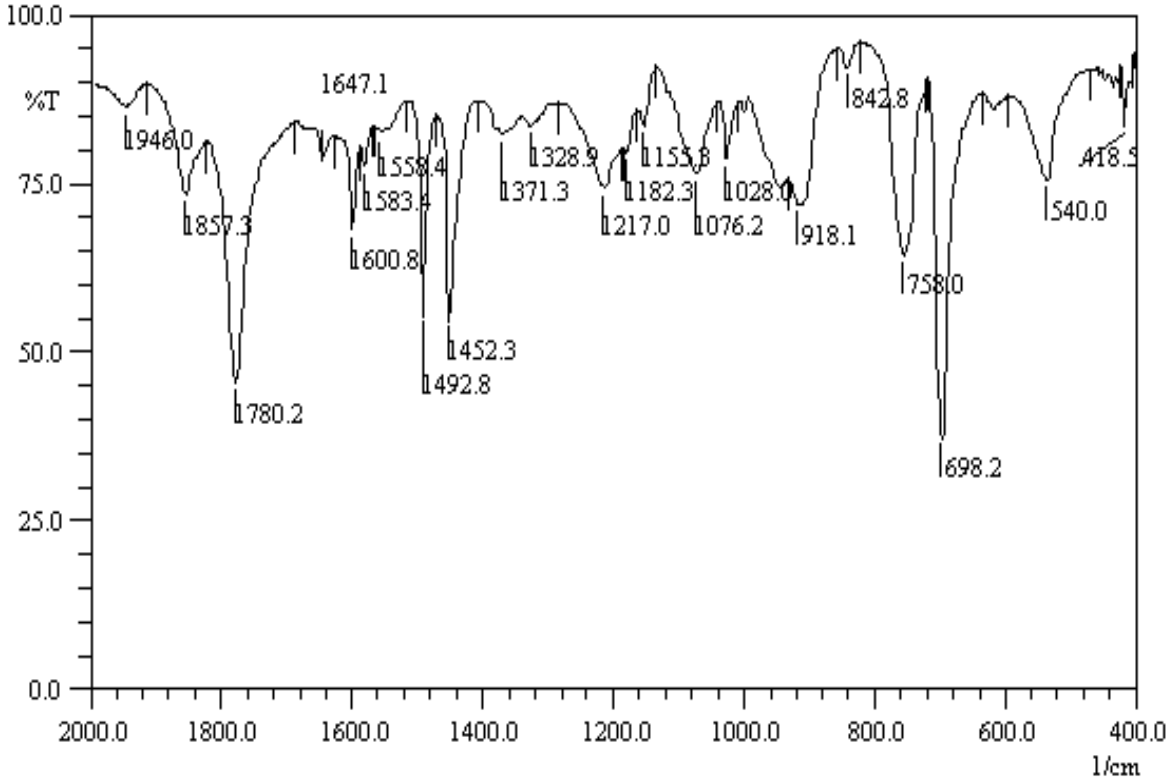


Figure 4: FTIR spectrum of blank Reaction of poly(styrene-co-maleic anhydride) (KBr)

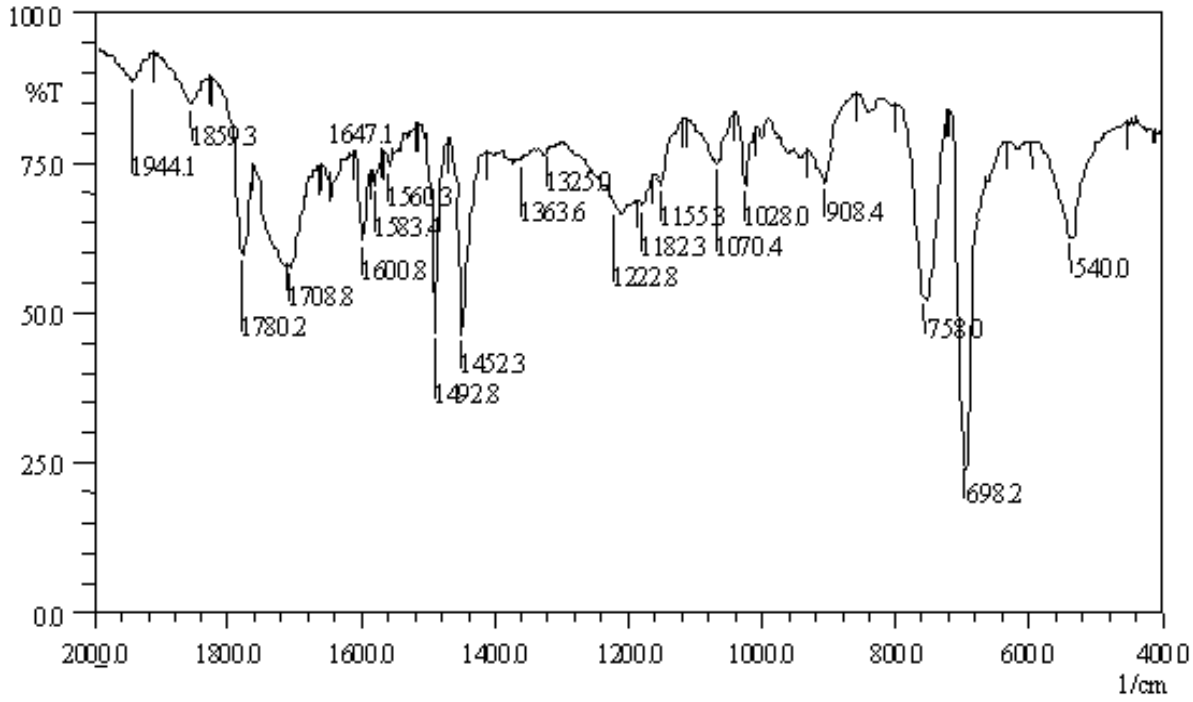


Figure 5: FTIR spectrum of hydrolyzed poly(styrene-co-maleic anhydride) (KBr)

2.5.3. Use of colorimetry for determination of the sugar content in the poly(styrene maleic anhydride) linked with glucose: The phenol- sulfuric acid reaction method.

(Ref: Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A.,Smith F., Anal. Chem., 28(3), (1956).

Glucose and glucose-linked poly(styrene maleic anhydride) polymers were dried overnight in an vacuum oven at ~60°C. A standard plot for glucose was plotted using different concentrations (0.0175mg/ml to 0.07mg/ml) of glucose in water. To 2ml of each glucose solution was added 1ml phenol and 5ml concentrated sulfuric acid was added at a fast rate. In a similar way a blank was prepared using distilled water instead of sugar solution. The absorbances were recorded at 490λ and a standard plot was plotted. Each of the accurately weighed samples (glucose-linked poly(styrene maleic anhydride)) in the range of 25mg was taken in a 50ml beaker to which were added 4ml water, 2ml 5% phenol solution in water and 10ml concentrated sulfuric acid was poured at a fast rate into the above solution. The solutions were placed in a water-bath at 25-30°C for 10 minutes. The solutions were filtered through a sintered disc to remove the undissolved polymeric backbone. The absorbances were recorded at 490λ and the unknown concentrations of the sugars were calculated from the standard plot. Using these concentrations the mole percentages and the weight percentages of the sugars in the polymers. The range of sugar content in the polymer was found to be between 0.1 – 3.7 weight %.

2.5.4. Quantification of carbohydrate groups linked to poly(styrene-maleic anhydride) by silylation of the carbohydrate hydroxyl's and NMR anlysis of the spectrum :

As an illustration we report here a silylation reaction on poly(styrene-co-maleic anhydride) chemically linked with glucose and its NMR. Glucose linked poly(styrene-co-maleic anhydride) was dissolved in dry pyridine, flushed with argon and reacted with trimethylsilyl chloride in presence of argon. The reaction was stirred at room temperature for 1hour and 30minutes. The reaction mixture was filtered, precipitated in n-hexane, washed with hexane and dried in a vacuum dessicator. The NMR of the product was recorded in d6-acetone solvent on a 200 MHz Bruker instrument. The NMR showed peaks at 0δ (0.26 H) and at 6.3-7.4δ (5 aromatic H). Integration of the peaks show that the weight percentage of the carbohydrate in the polymer is 1.11.

2.5.5. Molecular weight decrease after biodegradation by GPC :

The reduction in the molecular weight of the degraded sample was also confirmed by GPC. The samples were dissolved in tetrahydrofuran, and passed through a ultrastyragel column of 500A°-10^5A°. The instrument was equipped with a Waters 590 HPLC Pump, RI Waters dectector 410, Waters injecting system U6k. The biodegraded lactose linked-poly(styrene-co-maleic anhydride) sample was not fully soluble in tetrahydrofuran.

Only the soluble fraction was used for injection. This was a general difficulty for all the degraded samples, and reflects changes in the material after subjecting to biodegradation.

The figure below shows GPC Curves of starting Poly(styrene-maleic anhydride) (Curve A) , Poly(styrene-maleic anhydride) degraded by *Bacillus* sp. (Curve B), and Poly(styrene-maleic anhydride-linked lactose) degraded by *Serratia marscecens*

(Curve C). Notice the shift in the peak maximum to the right, indicating lower peak molecular weights.

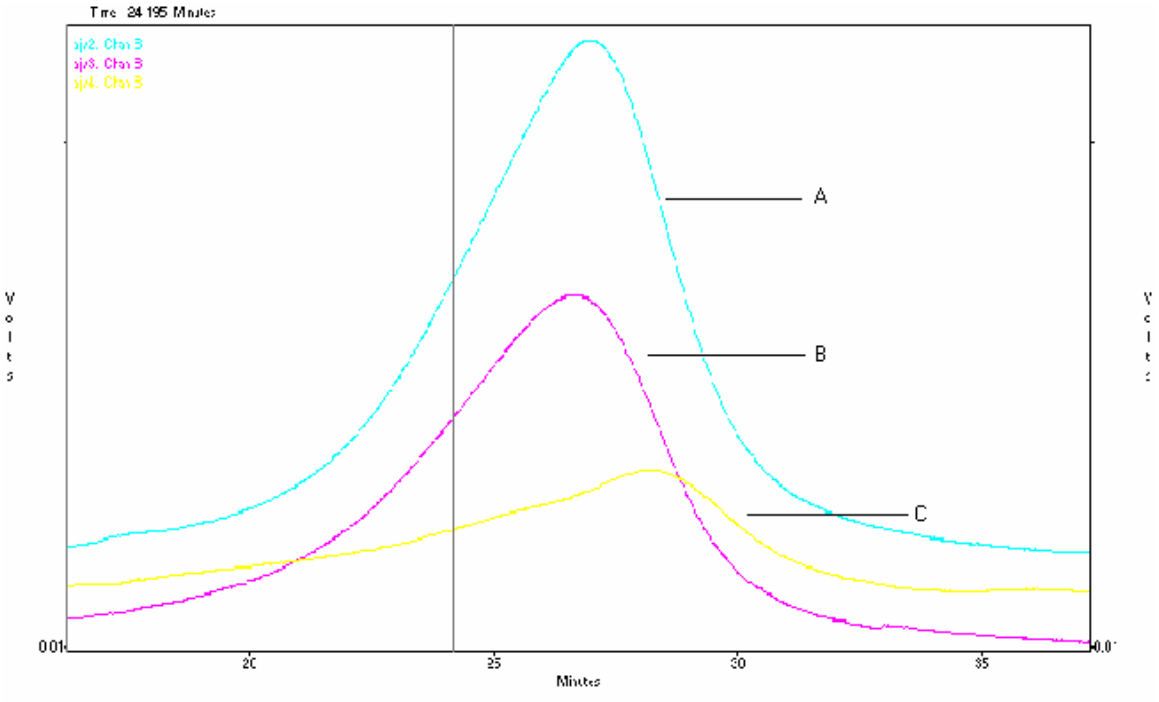
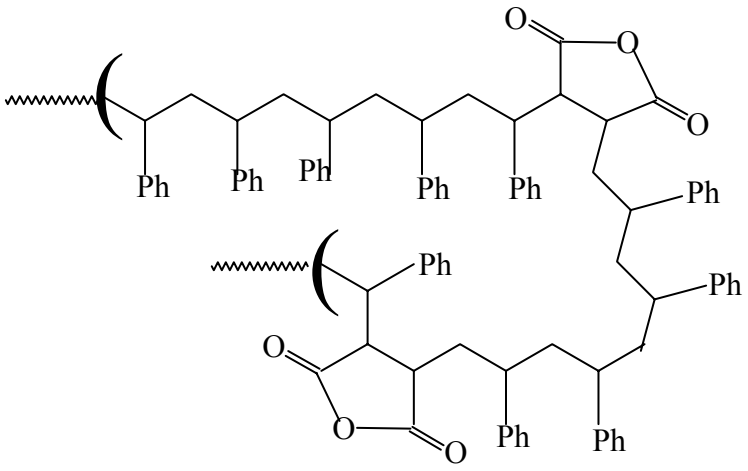


Figure 6: GPC curves of poly(styrene maleic anhydride) (PSMAH), PSMAH degraded and lactose- linked PSMAH degraded.

2.6. Appendix 1:

Mechanism of reaction of poly(styrene maleic anhydride) with the sugar

The structure of poly(styrene maleic anhydride) is as follows:

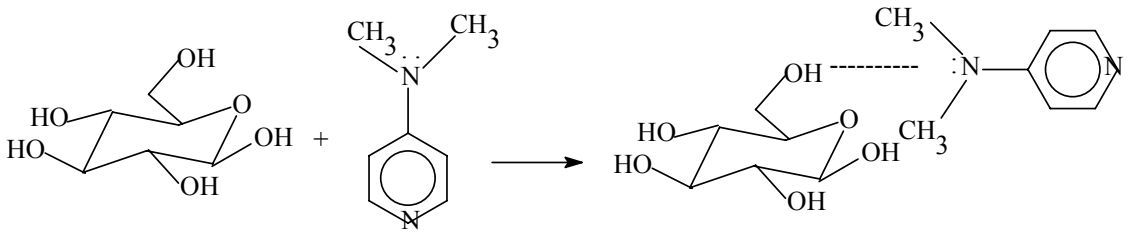


Poly(styrene maleic anhydride)

Reaction mechanism: the reaction of poly(styrene maleic anhydride) with the sugar can theoretically follow two different pathways:

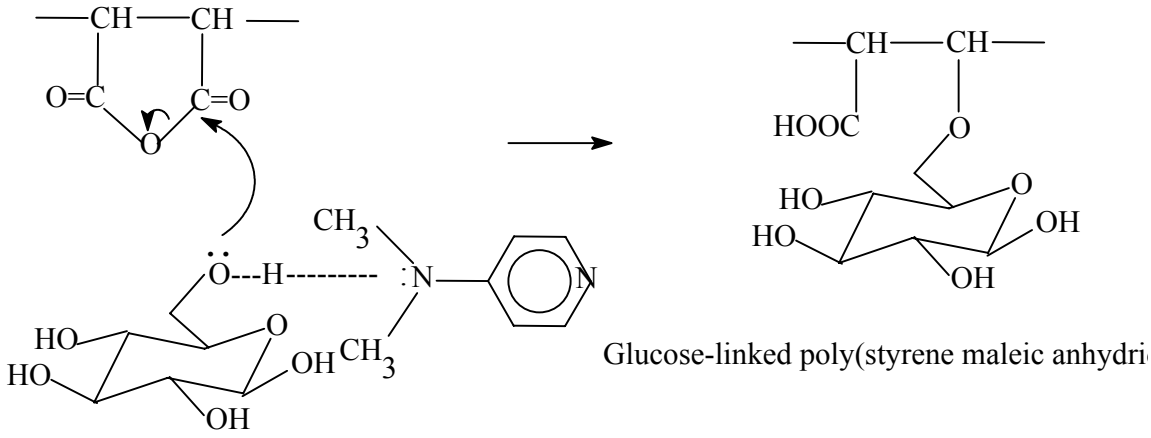
<u>Route 1:</u> formation of a complex between D-glucose and N,N-dimethylaminopyridine, rendering the glucose hyroxyl nucleophilic, followed by the nucleophilic attack of the glucose hydroxyl on the anhydride carbonyl.

Step 1:



D-Glucose 4-Dimethylaminopyridine Complex

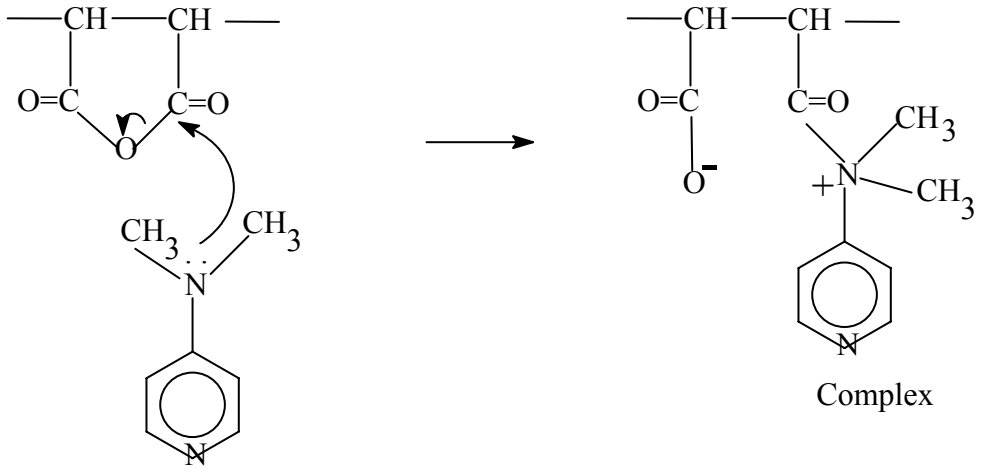
Step 2:



Glucose-linked poly(styrene maleic anhydri

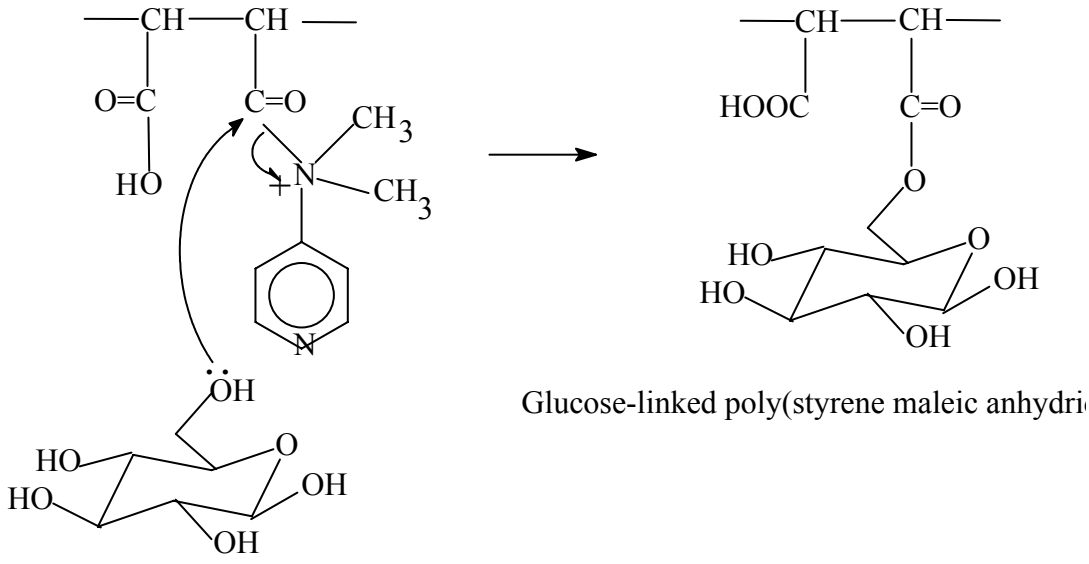
Route 2: attack of the nucleophile viz. 4-dimethylaminopyridine on the anhydride carbonyl resulting in the clevage of the C-O-C bond, followed by the attack of the sugar hydroxyl on the electrophilic carbonyl carbon of the anhydride.

Step 1:



Complex

Step 2:



Glucose-linked poly(styrene maleic anhydri

Referring to literature on the kinetics of the reaction of poly(styrene maleic anhydride) with different alcohols using 4-DMAP as the catalyst, it is anticipated that the reaction follows the second route (Martinez F., Neculqueo G., Torres M. and Olea A., *Bol. Soc. Chil. Quim.,* vol. 46(2),2001; Hu G.H. and Lindt J.T., *J. Polym. Sci., Part A: Poly. Chem. Ed.,* 31, 691, 1993).

2.7. Appendix 2:

Scanning electron micrographs of the polymers before and after bacterial degradation:

The SEMs presented in this appendix are not necessarily the SEMs of the samples included in chapter 2 or Chem. Commun. Representative samples were chosen. All the samples were in the form of thick films. In some cases the surfaces were smooth and in some cases they were rough. After subjecting the polymers to biodegradtion, the films were thoroughly washed with water and suspended in 70% ethanol overnight and then dried. The cases in which, SEMs were recorded before the treatment, have been specified.

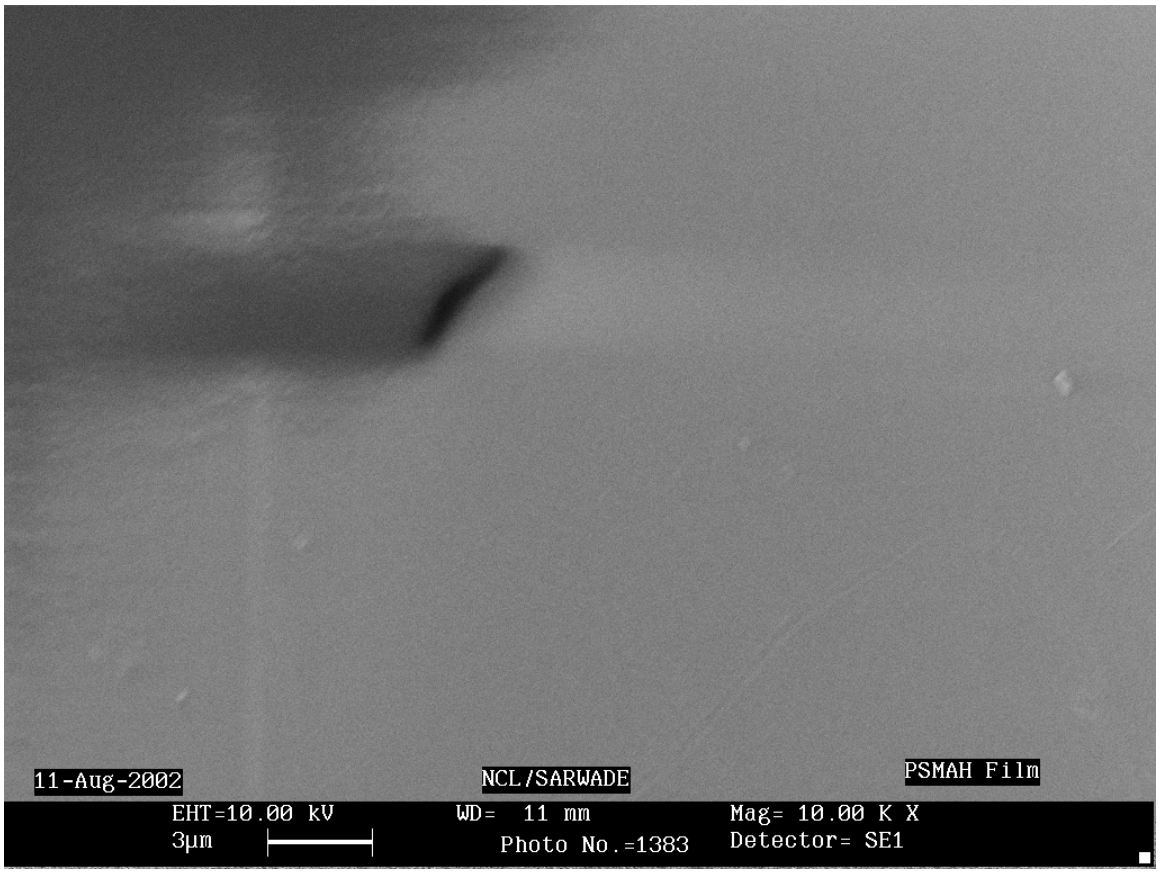


Figure 1: SEM of poly(styrene maleic anhydride) before subjecting to biodegradation

The SEM shows the surface of the polymer to be smooth.

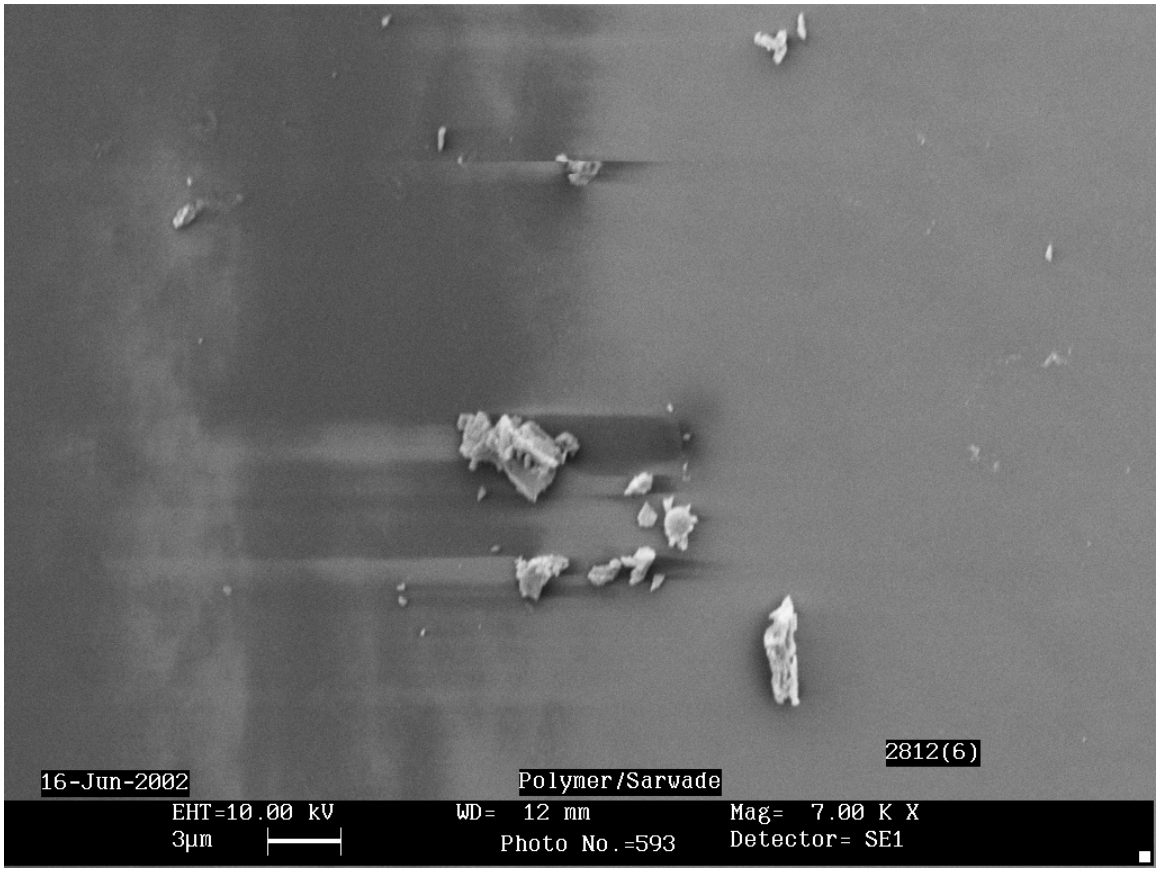


Figure 2: SEM of poly(styrene maleic anhydride) after degradation by Bacillus sp. (NCIM 2812)

Practically there seem to be no changes in the morphology of the polymer as compared to the morphology of the polymer before degradation. The surface of the polymer shows some residual debris.

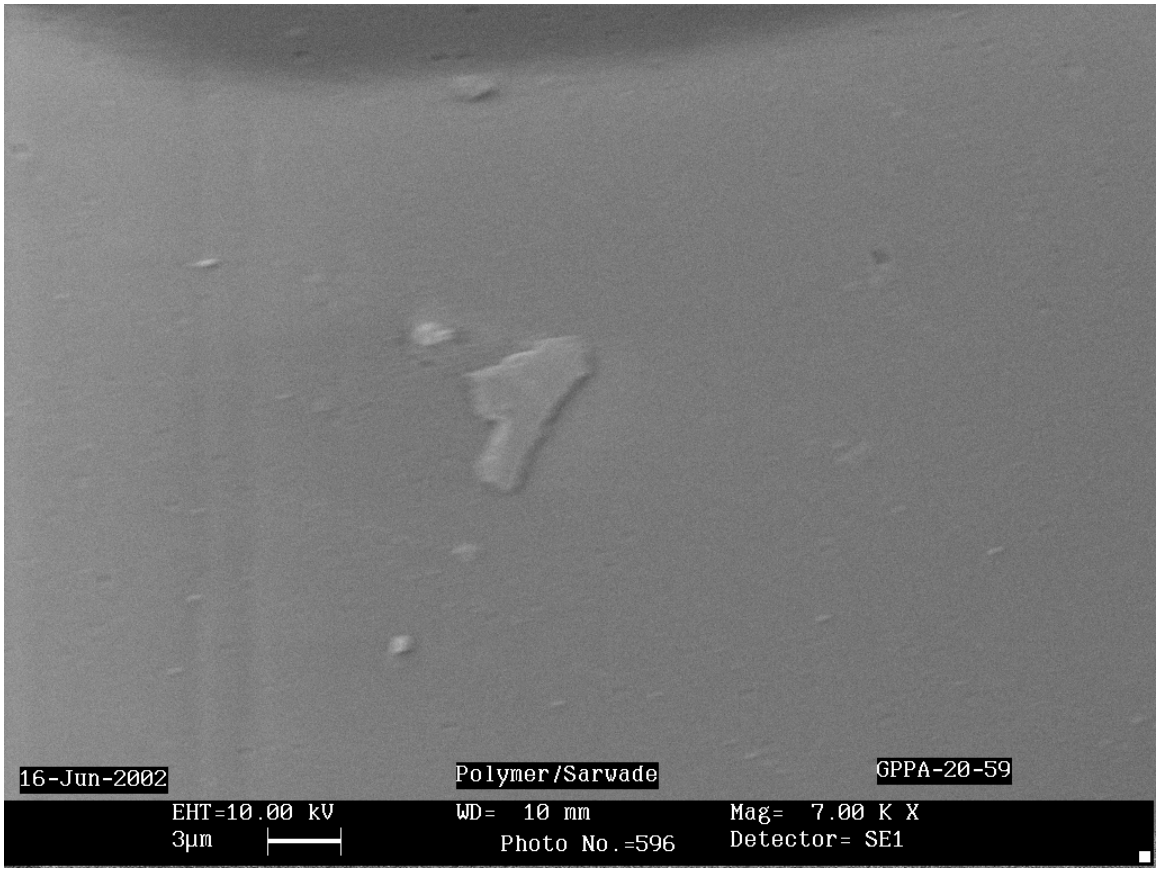


Figure 3: SEM of lactose- linked poly(styrene maleic anhydride) before subjecting to biodegradation

From figure 3, it is seen that the surface of the polymer in general is smooth.

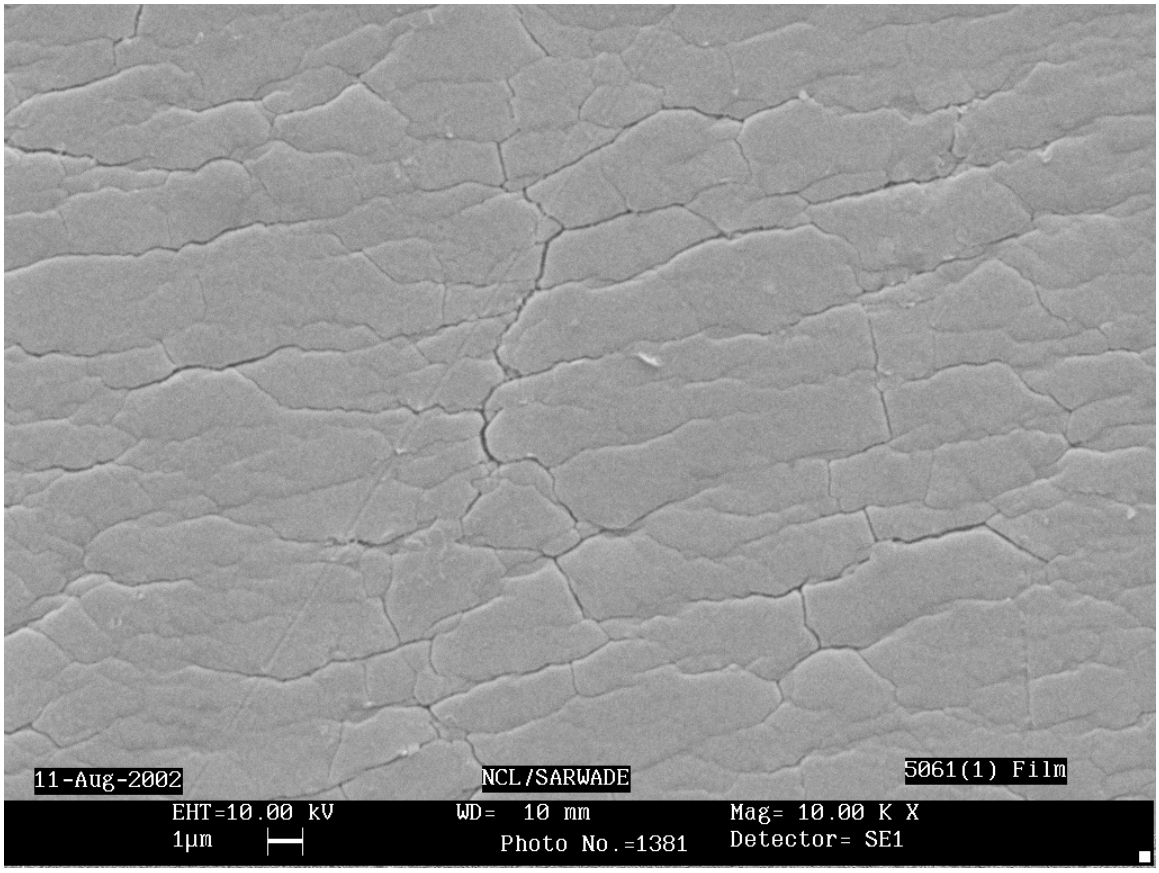


Figure 4: SEM of lactose- linked poly(styrene maleic anhydride) after degradation by Serratia marscecens (NCIM 5061)

The SEM clearly shows the formation of cracks on the surface of the polymer caused due to the degradation by Serratia marscecens

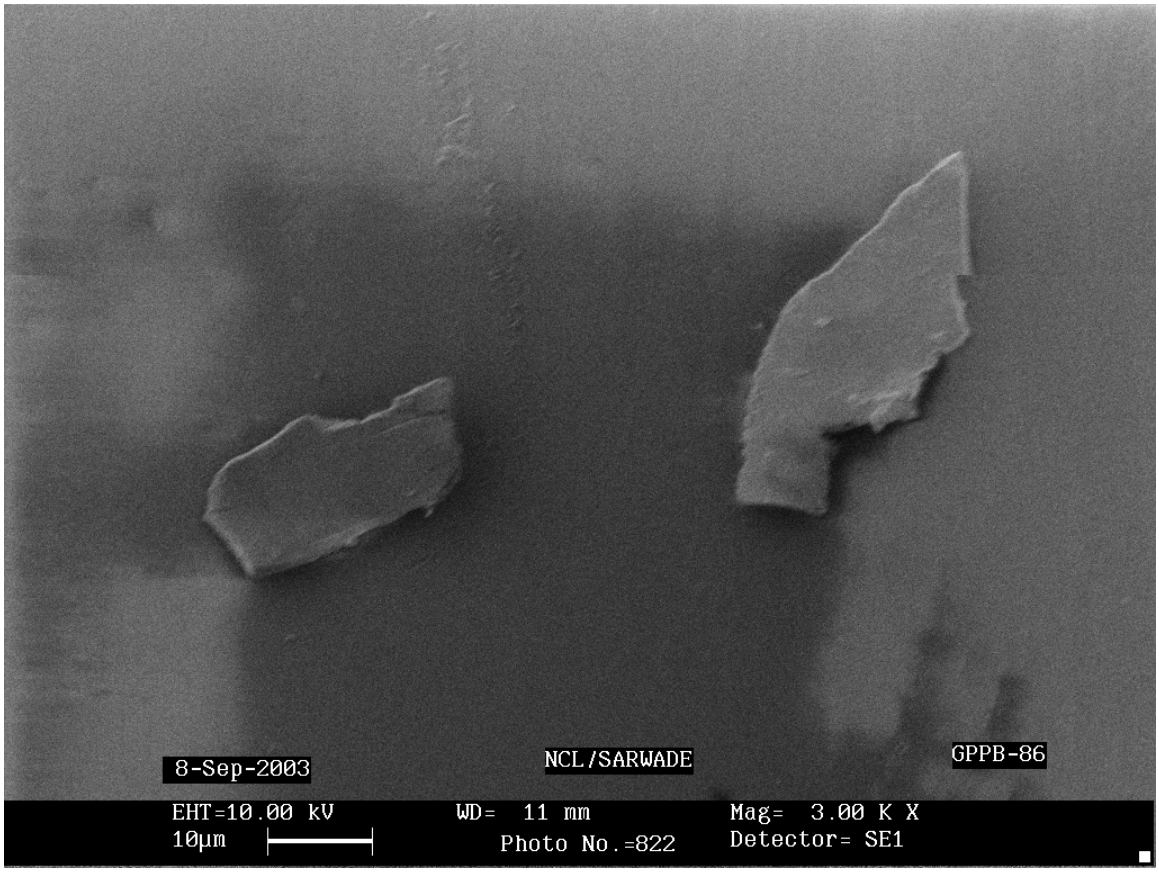


Figure 5: SEM of sucrose- linked poly(styrene maleic anhydride) before subjecting to biodegradation

Similar to the other sugar- linked poly(styrene maleic anhydride) polymers, the surface of this polymer also shows a smooth surface with perhaps some uneven folds in the macrophase after casting.

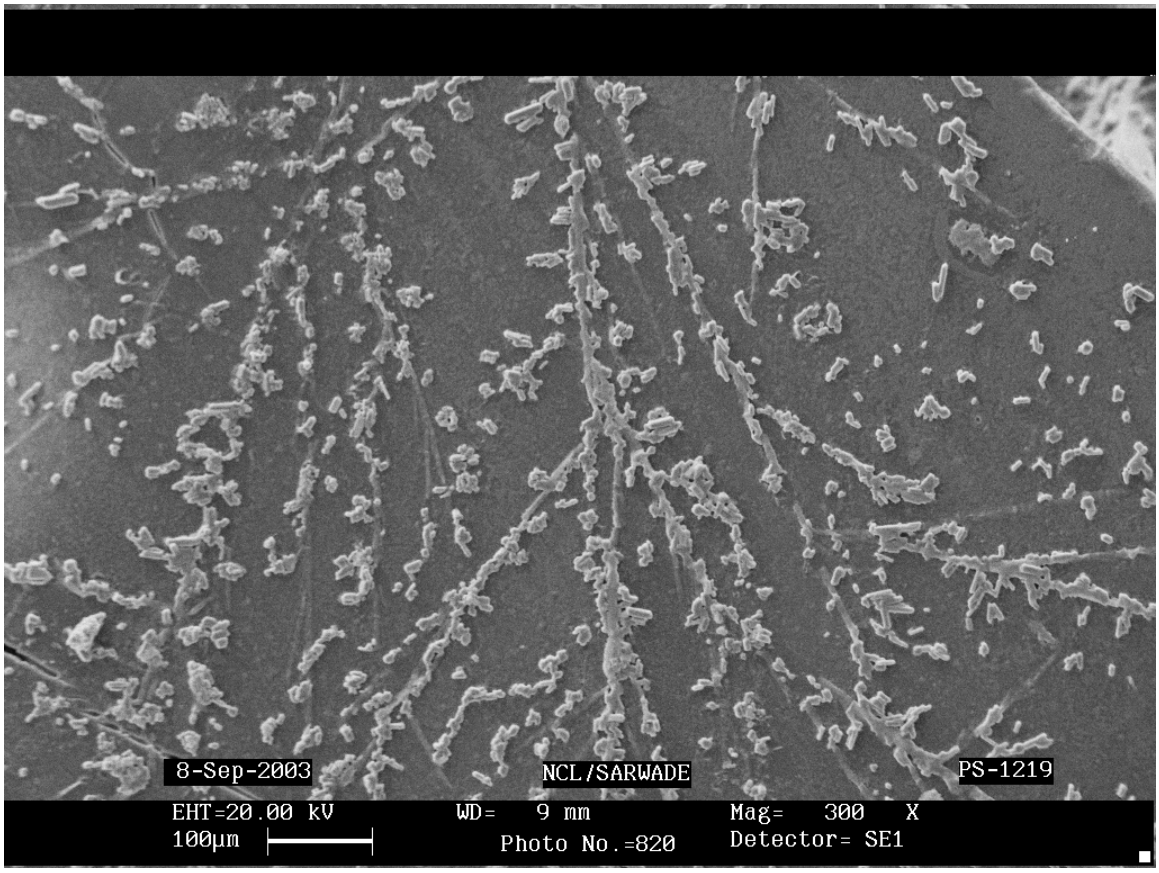


Figure 6: SEM of sucrose- linked poly(styrene maleic anhydride) degraded by Serratia marscecens (NCIM 5061) before washing and treatment with ethanol.

The figure shows the adherence of colonies of bacteria in a structured fashion

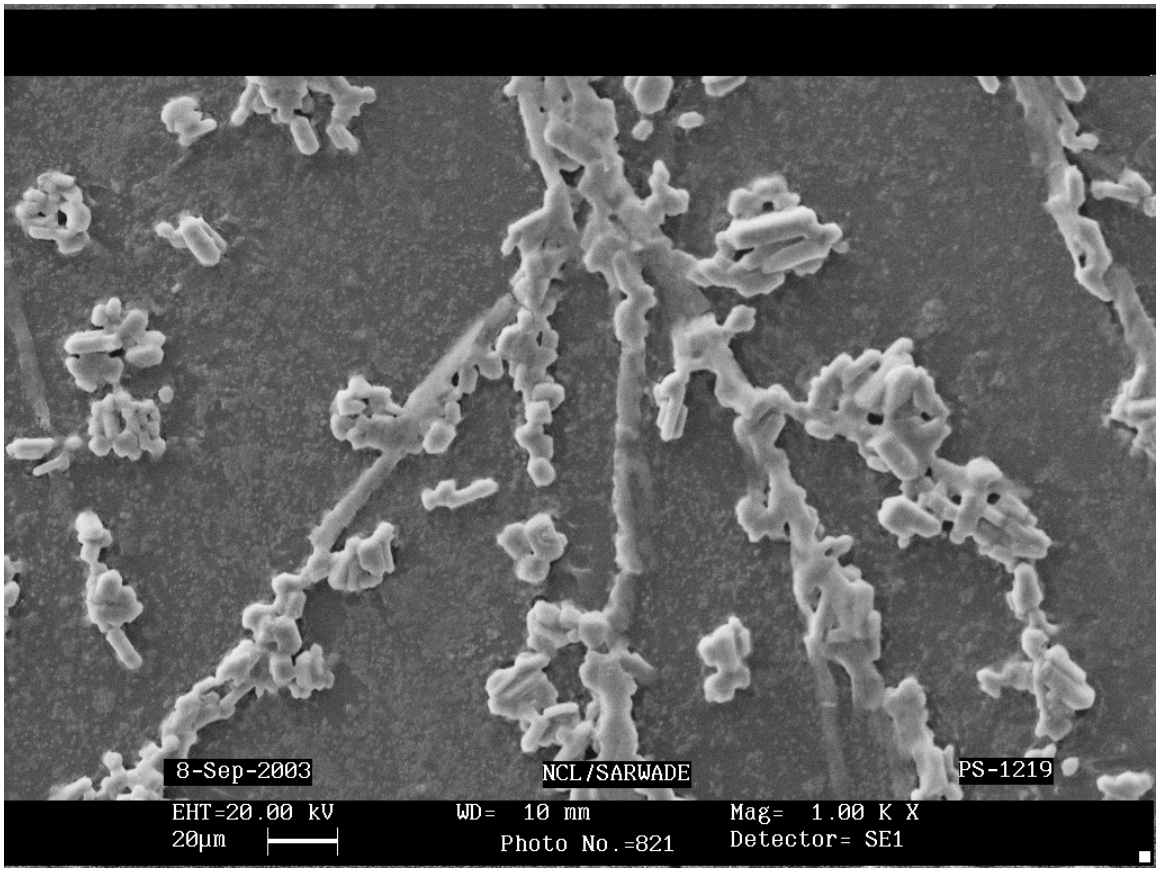


Figure 7: SEM of sucrose- linked poly(styrene maleic anhydride) degraded by Serratia marscecens (NCIM 5061) before washing and treatment with ethanol.

The growth pattern of the bacteria is better viewed at a higher magnification

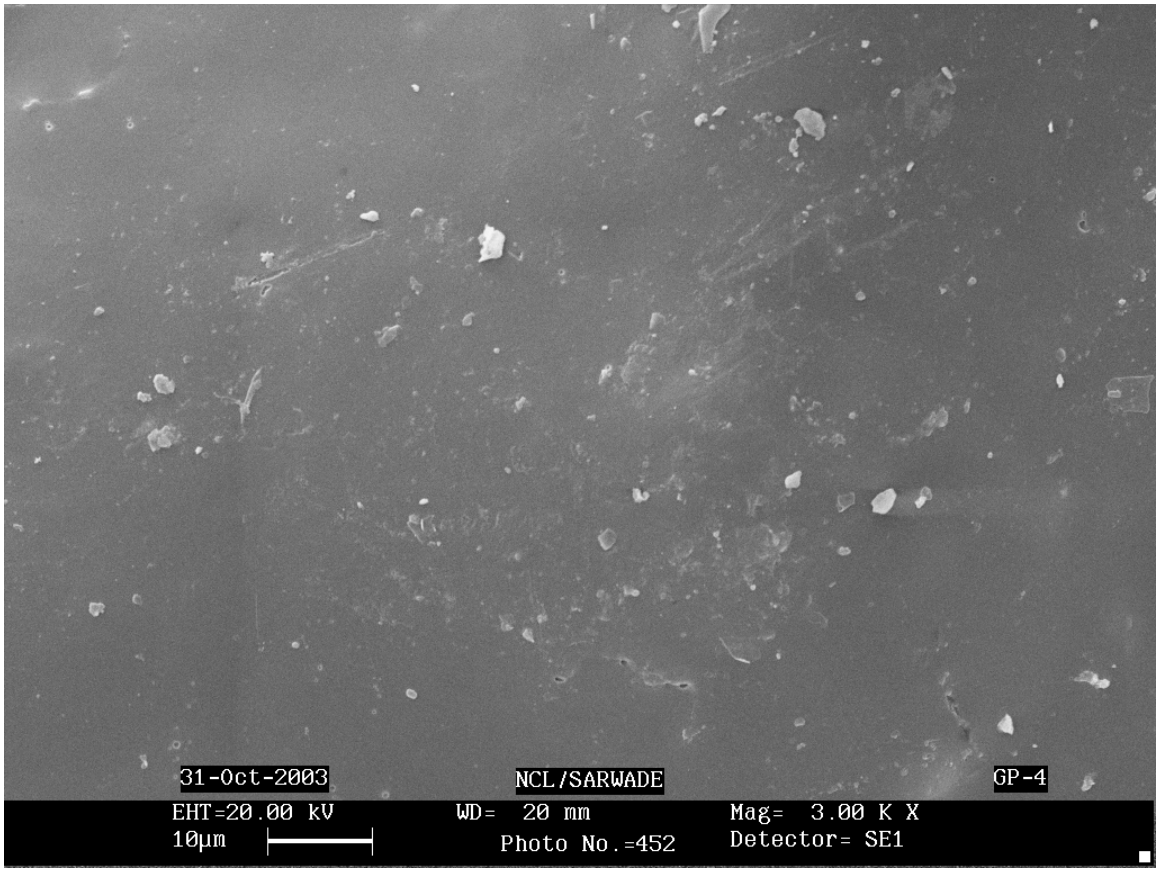


Figure 8: SEM of glucose- linked poly(styrene maleic anhydride) before subjecting to biodegradation

This film did not dissolve completely, and the film was cast from a gelled solution. This could be the reason for the slight un-evenness of the surface

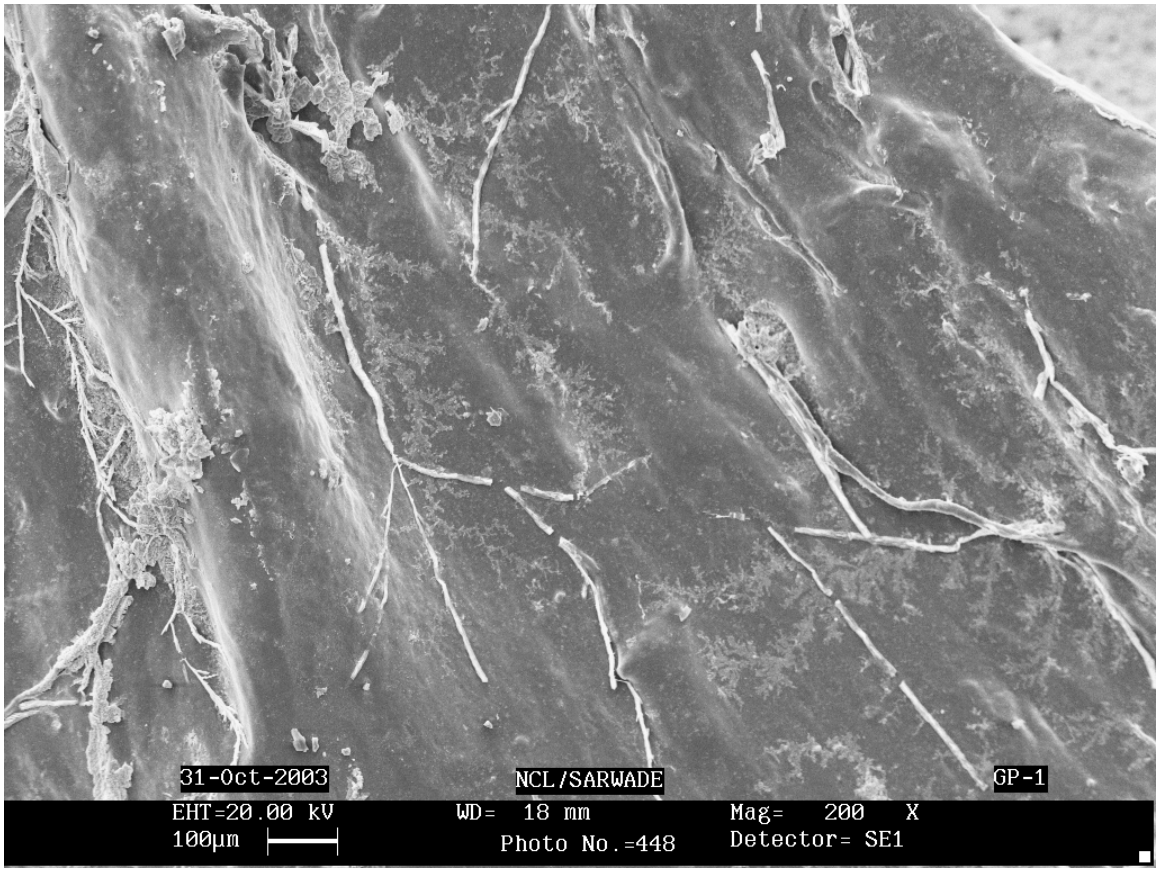


Figure 9: SEM of glucose- linked poly(styrene maleic anhydride) degraded by Serratia marscecens (NCIM 5061) before washing off bacterial cells and treatment with ethanol.

The SEM shows the adherence of bacterial colonies in definite patterns.

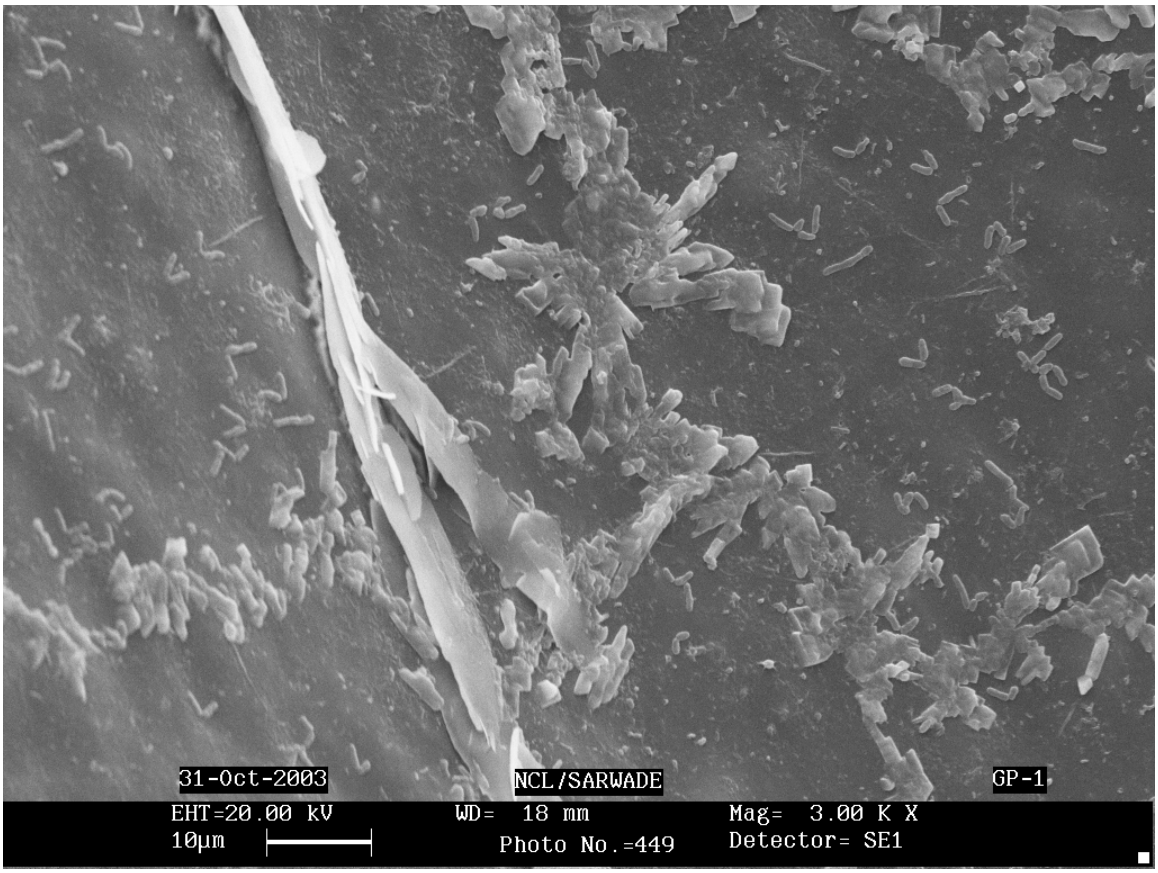


Figure 10: SEM of glucose- linked poly(styrene maleic anhydride) degraded by Serratia marscecens (NCIM 5061) before washing off bacterial cells and treatment with ethanol.

This is same as that of figure 9, recorded at a higher magnification.

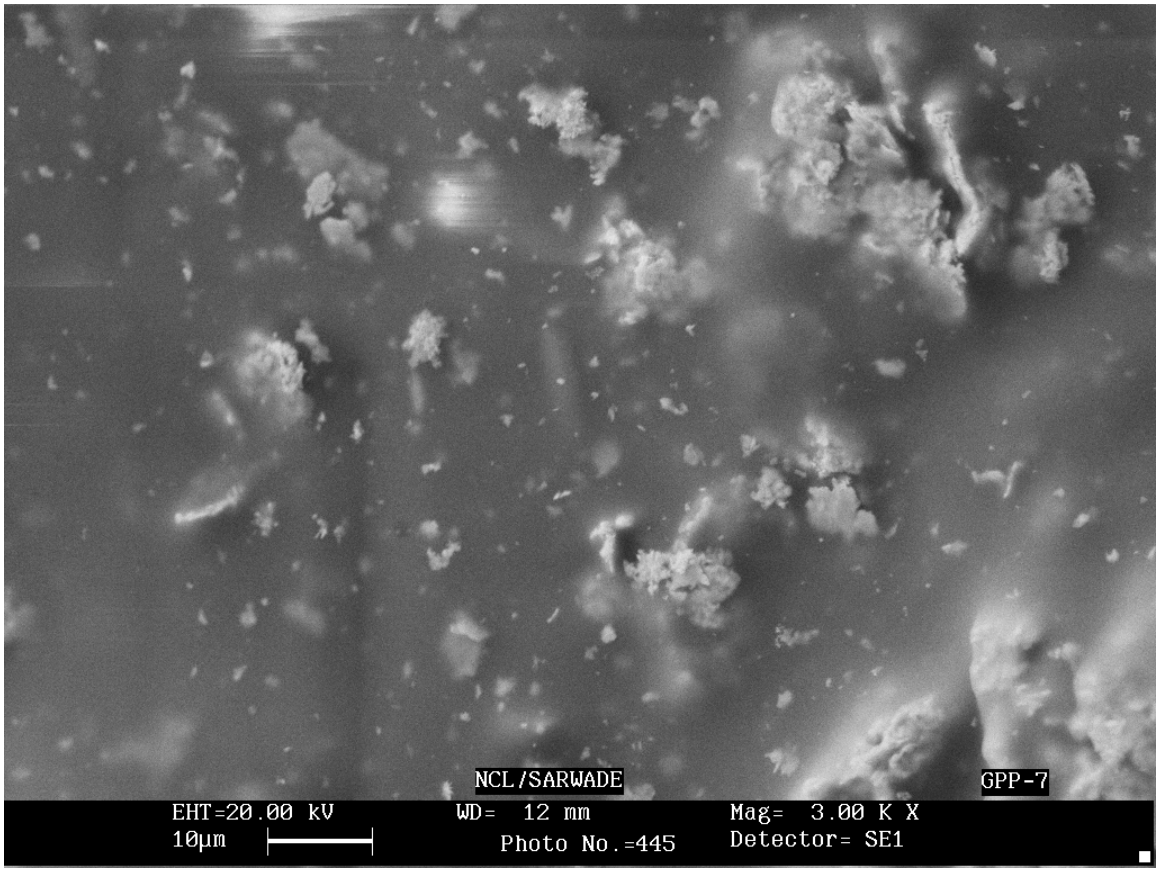


Figure 11: SEM of glucose- linked poly(styrene maleic anhydride) degraded by Serratia marscecens (NCIM 5061) after washing off bacterial cells and treatment with ethanol.

After washing, the initial un-evenness has increased considerably, showing degradation.

CHAPTER 3

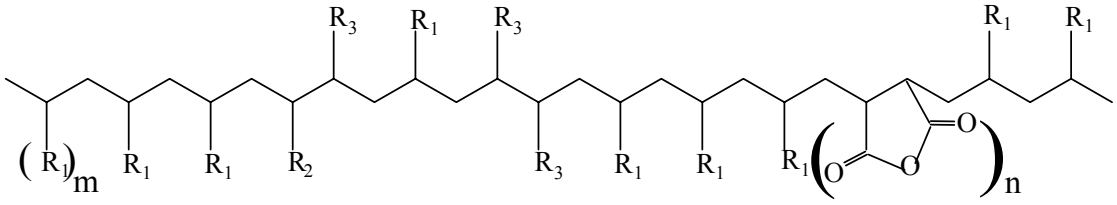
Process for the preparation of biodegradable synthetic polymers

(This is a patent application which is pending with the patent authorities)

Abstract:

Carbohydrate molecules were linked chemically onto functionalized synthetic polymers to greatly improve their biodegradability without affecting their key processing properties such as thermal properties, thereby ensuring they can be processed easily. These sugar-linked polymers have been found to have greatly enhanced rates of biodegradation over the unmodified polymers. These carbohydrate modified synthetic polymers are expected to find increased acceptability in an environment conscious world.

3.1. Formula of the product:



Formula (I)

Wherein

R_1 = H /Ph

R_2 = COO R_4

R_3 = COOH

R_4 = Carbohydrate

m = 50 - 98 mole %

n = 2 - 50 mole %

The present invention relates to the said products and the process for the preparation thereof through introduction of carbohydrate molecules. More particularly it relates to developing and synthesizing biodegradable polymers based on synthetic polymers,

particularly polyolefins and their copolymers, and any other chemical modifications of polyolefins, and chemically linking carbohydrate molecules onto these base synthetic polymers.

3.2. Introduction:

Most of the earlier work on synthesis of polymers with synthetic backbone and pendant carbohydrate units was based on either homopolymerization of vinylsaccharide monomers, or their copolymerization with other vinyl monomers (Patil D.R., Dordick J.S., Rethwisch D.G., *Macromolecules*, 24, (1991), 2462-2463; Klien J., *BMFT-Forshungsbericht (ET 1077A),* 1982; Klien J., Herzog D., Hajibegli A., *Makromol. Chem. Rapid Commun*.6, (1985), 675; Kimura S., Imoto M., *Makromol. Chem.,* 50, (1961), 155; Kimura S., Hirai K., *Makromol. Chem.,* 58, (1962), 232; Black W.A.P., Dewar E., Rutherford D., *J. Chem. Soc*., (1963), 4433). Synthesis of vinylsaccharide monomers had many disadvantages for commercial exploitation such as multi-step synthesis, including protection and deprotection of the carbohydrate monomers, isolation of intermediates (Gruber H., Knaus S., *Macromol. Symp.,* 152, (2000), 95-105), use of a large inventory of specialized chemicals, and so on. Copolymerization of vinyl monomers with vinylsaccharides is limited by the reactivity ratios, in addition to the usual other disadvantages mentioned for homolpolymers of vinyl carbohydrates. Therefore these methods are unsuitable and economically unviable, and inspite of several years of development have not been commercialized for use as bulk plastics.

Due to the excellent processing properties, polyolefins have occupied a special status as commodity plastics. However the major drawback of polyolefins is that they are non- biodegradable and hence pose severe problems of their disposal after their useful life. With the aim of developing biodegradable polymers based on polyolefins, blending of starch with polyolefins (particularly with polyethylene) has been much explored and also put in practice in a limited way (Griffin G.J.L., Adv. Chem. Ser., 134, (1974), 159; Griffin G.J.L., US Patent 4,016,117 (1977); Griffin G.J.L., UK

Patent 1,485,833 (1977); Griffin G.J.L., UK Patent 1,487,050 (1977); Otey F.H. Westhoff R.P., Doane W.M., *Ind. Eng. Chem. Res*., 26, (1987), 1659). The intention of such blending procedures was that after disposal, degradation of starch in the blend would create voids and weaken the integrity of the polyethylene and result in its degradation. However the main drawback of this methodology is that attainment of such properties demands larger volumes of starch (in the range of 30% or higher) due to which the physical properties of the polyethylene have to be compromised (Lenz R.W.*, Advances in Polymer Sciences,* 107, Ed. Peppas, N.A. and Langer R.S., (1993), 31).

Polystyrenes with pendant carbohydrate residues have been synthesized by polymerization of vinyl benzyl sugars. Polystyrene derivatives with maltose, lactose and maltotriose substituents on each phenyl ring were synthesized by coupling the corresponding oligosaccharide lactones with p-vinylbenzyl amines followed by radical polymerization (Kobayashi K., Sumitomo H., Ina Y., *Polymer J.,* 17, (1985), 567-575; Kobayashi K., Sumitomo H., Ina Y., *Polymer J.,* 15, (1983), 667-671; Kobayashi K., Sumitomo H., Kobayashi A., Akaike T., *J. Macromol. Sci.-Chem.,* A25(5-7), (1998), 655-667; Kobayashi K., Tsuchida A., Usui T., Akaike T., *Macromolecules*, 30, (1997), 2016-2020). These polymers are water soluble, and are potential biomedical materials wherein the oligosaccharide moieties are used as recognition signals. However, there is no scope to develop these into bulk plastics as substitutes for polyolefins. Carbohydrates have also been incorporated into silicone rubbers in order to improve their wettability and biocompatibility. Allyl ethers of the protected carbohydrates were used as additives in the crosslinking of H-Si-polysiloxanes and vinyl-Si- polysiloxanes followed by deprotection of the protecting groups (Gruber H., Knaus S., *Macromol. Symp.,* 152, (2000), 95-105). The synthesis, products and the applications are quite different from the current patent proposal. Inert polyolefins such as polypropylene were hydrophilized with carbohydrate (carbohydrate azides) using UV radiations in acetone solvent. This procedure involved only the surface modification to make the surface hydrophilic so as to facilitate cell adhesion, to improve the dyeability, printability and biocompatability of

the polymers (Gruber H., Knaus S., *Macromol. Symp.,* 152, (2000), 95-105). The synthesis, products and the applications are quite different from the current patent proposal.

Composites of polypropylene (PP) and cellulose were synthesized by kneading the two components in presence of a compatibilizer viz. polypropylene-maleic anhydride in an extruder in an attempt to obtain potential biodegradable polymers (Felix J.M., Gatenholm P., *J. Appl. Polym. Sci.,* 42, (1991), 609-620). The synthesis and structure of these polymers are quite different from the current patent proposal. A biodegradable polyolefin based on carbohydrates has been synthesized wherein the synthetic polymeric backbone is poly(vinyl alcohol) and the carbohydrate component is glucose. The glucose is linked to the polymer via a spacer viz. adipic acid. The monomer, viz. the vinyl carbohydrate, was synthesized enzymatically whereas the polymerization was carried out by chemical means. The polymers with lower molecular weights (Mn 3600 and 7000) were degraded to 70% in 28 days whereas polymers with high molecular weights (Mn 12900 and 34400) degraded less. Polyvinyl alcohol degrading bacteria such as Pseudomonas, Bacillus megaterium and Alcaligenes faecalis were used for the biodegradation tests (Tokiwa y., Fan H., Hiraguri Y., Kurane R., Kitagawa M., Shibatani S., Maekawa Y., *Macromolecules,* 33, (2000), 1636-1639). It is well known that poly(vinly alcohol) itself is a biodegradable polymer, and the synthesis, products and the applications of this system are quite different from the current patent proposal.

Glucosamine hydrochloride and galactosamine hydrochloride were grafted onto polyacryloyl chloride by polymer analogous reactions in carbonate buffer (Bahulekar R., Tokiwa t., Kano J., Matsumura T., Kojima I., Kodama M., *Carbohydrate Polymers,* 37, (1998), 71-78). The synthesis, products and the applications are quite different from the current patent proposal. The aim was not to develop processable polyolefins for any usual applications of polyolefins, and the methods were not tailored for developing such methods. Other reports of linking carbohydrates onto synthetic polymer backbones have also been found in literature. Sucrose was attached

to low molecular weight carboxylated polybutadiene and utilized for synthesis of polyurethanes (Alvarez C., Strumia M., and Bertorello H., *Polym. Bull.* 19, (1988), 521-526). In another study, surface modification of PVC films was carried out with sucrose to make the surface of the polymer wettable and as a carbon source for microorganisms (Rios P., Berterello H., *J. Appl. Polym. Sci.,*64, (1997), 1195-1201). This is quite different from making the entire polymer a carbon source by modifying with carbohydrates (as in the case of our report) rather than just modifying the surface of the polymer.

Polyethylene containing prooxidant and 6% starch was reported to be biodegradable. Pure cultures of Streptomyces baduis, S virido sporus and S. Setonii were used for the biodegradation tests. The films were either chemically disinfected or irradiated by UV or were thermally treated prior to the biodegradation tests (Lee B., Pometto III A.L., Fratzke A., Bailey T.B., *Applied and Environmental Microbiology*, (1991), 678-685). However, these are blends of polymers and not discrete carbohydrate linked polymer chains.

Carbohydrate based copolymers are also reported (Pinilla I.M., Martinez M.B., Mata F.Z., Galbis J.A., *Macromolecules, 35(8),* (2002), 2977-2984). Copoly(ester amide)s were prepared by random copolymerization of protected carbohydrates. A very recent comprehensive review of biodegradable polymers in one of the world's best known journals (Gross R.A., Kalra B., *Science,* 297, (2002), 803-807) also does not mention research results of the type mentioned in this patent proposal.

3.3. Objective of the present invention:

The present invention, which relates to developing and synthesizing biodegradable polymers based on synthetic polymers, particularly polyolefins and their copolymers, and any other chemical modifications of polyolefins, by chemically linking carbohydrate molecules onto these synthetic polymers has many advantages over the earlier methods described above. In the present invention no protection of the

carbohydrates is required. The reaction conditions are mild. Even few number of carbohydrate molecules being attached to the synthetic polymers (less than 5% by weight) brings about greatly enhanced rates of biodegradation. It is not at all obvious that presence of a few molecules (as low as 5% by weight) can cause a significant increase in the rates of biodegradation of these polymers. The further advantage of having very low number of carbohydrate molecules attached to the polymer chain is that the physical /mechanical properties of the base synthetic polymer are not greatly affected. Further, the advantage of less number of synthetic steps and mild reaction conditions is ease of technology development and subsequent implementation.

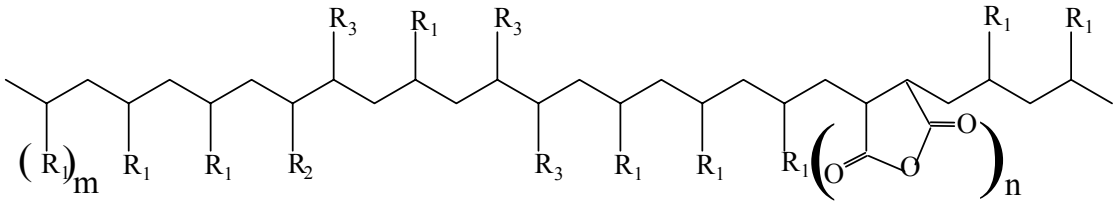
The main objective of the present invention therefore is to provide biodegradable polymers and the preparation thereof by covalently linking minute quantities of carbohydrate molecules onto base synthetic polymers, in particular polyolefins and their copolymers.

Another object is to use unprotected carbohydrate residues thereby avoiding tedious multi-step synthesis of the carbohydrate monomers.

Yet another object is to utilize very mild reaction conditions to obtain the above mentioned biodegradable polymers.

Still another object is to render non-biodegradable polyolefins biodegradable by anchoring minute quantities of carbohydrates onto the polymers.

Accordingly the present invention provides biodegradable synthetic polymers having a
formula



Formula (I)

Wherein

R_1 = H /Ph

R_2 = COO R_4

R_3 = COOH

R_4 = Carbohydrate

m = 50 - 98 mole %

n = 2 - 50 mole %

The present invention also provides a process for the preparation of biodegradable synthetic polymers of formula (I), which comprises drying a base synthetic polymer and a carbohydrate in vacuum at a temperature ranging between 55 to 60ºC for a period of 17 to 19 hours, preparing separately the solutions of the base synthetic polymer in dry organic solvent, preparing a solution of a carbohydrate and a catalyst in dry organic solvent, adding the solution of the base synthetic polymer into the solution of carbohydrate and the catalyst under agitation, heating the reaction mixture to a temperature ranging between 25 to 110ºC for 2- 48 hours under nitrogen and agitation, cooling the reaction mixture to room temperature, precipitating the product using a solution of an inorganic salt in a non-solvent, washing the product with a solvent till the product is free from the salt to obtain the product.

3.4. Preferred Embodiments:

In one of the embodiments of the present invention the base synthetic polymer may be a polyolefin and more preferably polystyrene, functionalized with reactive groups such as carboxyl, hydroxyl, amino, alkene, halide, ester, acid chloride and preferably anhydride.

In another embodiment the carbohydrate may be monosaccharides, and disaccharides: both reducing and non-reducing and oligosaccharides, wherein none of the hydroxyls are protected, or carbohydrates in which at least one but not all hydroxyls have been protected.

In another embodiment the dry organic solvent used for the preparation of the polymer solution may be dry N,N'-dimethylformamide, dry pyridine or dry toluene and their mixtures.

In still another embodiment the catalyst may be pyridine, 4-dimethylaminopyridine, para-toluenesulfonic acid and carbodiimide catalysts.

In still another embodiment the dry organic solvent used for the preparation of solution of carbohydrate and the catalyst may be dry N,N'- dimethylformamide or dry pyridine and their mixtures.

In yet another embodiment the molar ratio of base synthetic polymer to the carbohydrate in the reaction mixture may be 1: 0.01 or 1:1 or 1:2 or 1: 6, but preferably 1: 0.5 or more preferably 1: 3.

In yet another embodiment the molar ratio of the functional groups on the base synthetic polymer and the catalyst may be 1: 0.01 upto 1: 2, but preferably 1: 0.14.

In still another embodiment the non-solvents used for the preparation of the salt solution may be water, ethanol, methanol or acetone and their mixtures.

In yet another embodiment the salts used for the preparation of the salt solution may be sodium or potassium salts, preferably sodium chloride, potassium chloride, sodium bromide, potassium bromide and most preferably sodium chloride.

In still another embodiment the solvents used for washing the product to make it free from salt may be water, ethanol, methanol or acetone and their mixtures.

In a feature of the present invention the process for rendering polystyrene biodegradable comprises of reacting functionalized polystyrene with monosaccharides, disaccharides and oligosaccharides without the protection of the hydroxyl groups, in dry organic solvents in presence of catalysts and in inert atmosphere.

In further feature in case of functionalized polystyrene eg. polystyrene functionalized with maleic anhydride, dissolved in dry N,N'-dimethylformamide, was added to a stirred solution of the carbohydrate eg. glucose, sucrose, lactose, methyl glucoside, etc. and the catalyst viz. 4-dimethylaminopyridine in a three-necked round bottom flask provided with a magnetic stir bar, thermowell, addition funnel and a dry nitrogen balloon at a temperature 45-65°C over a period of 1/2 -1 hour. The mole ratio of the maleic anhydride content of the polystyrene-maleic acid copolymer versus the carbohydrate was either 1: 3 or 2:1. Stirring was done for 4 - 18 hours at temperatures of 45 - 65°C. The amount of carbohydrate incorporated into the polymer by this method was generally between 0.5 to 5% by weight. The reaction mixtures were precipitated in brine, washed several times with water till free of chloride and dried in a vacuum oven.

These polymers were found to be degraded by both bacterial a well as fungal cultures.
Samples of polymers were taken with pure bacterial cultures such as *Pseudomonas sp., Bacillus sp.,* etc. in flasks containing salts and no other source of carbon. The growth of the microorganisms was monitored by optical density measurements. The degradation was evidenced by weight losses and was also supported by spectral methods (IR spectoscopy). Similarly, the fungal cultures consisted of *Aspergillius niger, P. ochro-chloron, Trichoderma sp., Pullularia pullulans,* etc.

The present invention is illustrated using polystyrene-co-maleic anhydride (PSMAH) as the base synthetic polymer; lactose, D-glucose, sucrose and methyl glucoside as the carbohydrates; and 4- N,N'- dimethylaminopyridine (4-DMAP) as the catalyst. It is pertinent to note that the working of the present invention is not limited to the base synthetic polymer exemplified below but is also applicable to other polyolefins and their chemical modifications as base synthetic polymers. It is also not limited to the carbohydrates exemplified below, but may include all variations of monosaccharides, disaccharides and oligosaccharides and their derivatives. The invention is also not limited to the catalysts exemplified, but includes their derivatives and other catalysts.

3.5. Experimental/ Examples:

The following examples are given by way of illustration and therefore should not be construed to limit the scope of the present invention.

Example 1

Poly(styrene-co-maleic anhydride) (referred to as PSMAH) containing 14-wt % maleic anhydride (PSMAH) and lactose were dried overnight in a vacuum oven at ~ 60°C. PSMAH (10g) dissolved in dry N,N'-Dimethylformamide (DMF) (125mL) was added to a stirred solution of lactose (15.44g) and 4- N,N'-dimethylaminopyridine (4 - DMAP) (250mg) in dry DMF (200mL) over a period of 30 minutes. The mole ratio of the base synthetic polymer to the carbohydrate was 1:3. The reaction mixture was stirred at 60-65°C in presence of dry nitrogen for 4 hours. The reaction mixture was precipitated in brine, filtered, washed with water several times till free of chloride, dried to obtain the product.

Example 2

Poly(styrene-co-maleic anhydride) (PSMAH) and D-glucose were dried overnight at ~60°C in a vacuum oven. PSMAH (10g) dissolved in dry N,N'-dimethylformamide (DMF) (150mL) and was added to a solution of D-glucose (7.72g) and 4- N,N'-dimethylaminopyridine (4- DMAP) (250mg) in dry DMF (150mL) over a period of 1 hour. The mole ratio of the base synthetic polymer to the carbohydrate was 1:3. The reaction mixture was stirred at 60°C for 4 h in presence of dry nitrogen. The reaction mixture was precipitated in brine, filtered, washed with water several times till free of chloride and dried to obtain the product.

Example 3

Poly(styrene-co-maleic anhydride) (PSMAH) and sucrose were dried overnight in a vacuum oven at ~60°C. PSMAH (10g) dissolved in dry N,N'-dimethylformamide (DMF) (150mL) and was added to a solution of sucrose (14.67g) and 4- N,N'-dimethylaminopyridine (4 - DMAP) (250mg) in dry DMF (150mL) over a period of 1 hour. The mole ratio of the base synthetic polymer to the carbohydrate was 1:3. The reaction mixture was stirred at 48-50°C for 18 h in presence of dry nitrogen. The reaction mixture was precipitated in brine, filtered, washed with water several times till free of chloride and the dried to obtain the product.

Example 4

Poly(styrene-co-maleic anhydride) (PSMAH) and D-glucose were dried overnight at ~60°C in a vacuum oven. PSMAH (10g) dissolved in dry N,N'-Dimethylformamide (DMF) (150mL) and was added to a solution of D-glucose (7.72g) and 4- N,N'-dimethylaminopyridine (4 - DMAP) (250mg) in dry DMF (150mL) over a period of 1 hour. The mole ratio of the base synthetic polymer to the carbohydrate was 1:3. The reaction mixture was stirred at 47- 48°C for 18 h in presence of dry nitrogen. The reaction mixture was precipitated in brine, filtered, washed with water several times till free of chloride and dried.

Example 5

Poly(styrene-co-maleic anhydride) (PSMAH) and D-glucose were dried overnight at ~60°C in a vacuum oven. PSMAH (10g) dissolved in dry N,N'-dimethylformamide (DMF) (150mL) and was added to a solution of D-glucose (1.29g) and 4- N,N'-dimethylaminopyridine (4 - DMAP) (250mg) in dry DMF (150mL) over a period of 1 hour. The mole ratio of the base synthetic polymer to the carbohydrate was 2:1. The reaction mixture was stirred at 50°C for 18 h in presence of dry nitrogen. The reaction mixture was precipitated in brine, filtered, washed with water several times till free of chloride and dried.

Example 6

Poly(styrene-co-maleic anhydride) (PSMAH) and sucrose were dried in a vacuum oven at ~60°C. PSMAH (10g) dissolved in dry N,N'-dimethylformamide (DMF) (150mL) and was added to a solution of sucrose (2.5g) and 4- N,N'-dimethylaminopyridine (4 - DMAP) (250mg) in dry DMF (150mL) over a period of 1 hour. The mole ratio of the base synthetic polymer to the carbohydrate was 2:1. The reaction mixture was stirred at 50°C for 18 h in presence of dry nitrogen. The reaction mixture was precipitated in brine, filtered, washed with water several times till free of chloride and dried.

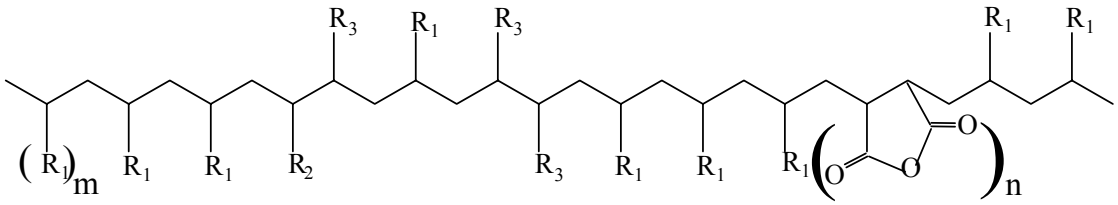
Example 7

Poly(styrene-co-maleic anhydride) (PSMAH) and methyl glucoside were dried in a vacuum oven at ~60 °C. PSMAH (10g) dissolved in dry N,N'-dimethylformamide (DMF) (150mL) and was added to a solution of methyl glucoside (8.3g) and 4- N,N'-dimethylaminopyridine (4 - DMAP) (250mg) in dry DMF (150mL) over a period of 1 hour at 50-55 °C under argon. The mole ratio of the base synthetic polymer to the

carbohydrate was 1:3. The reaction mixture was stirred at 50-51°C for 18 h in presence of argon. The reaction mixture was precipitated, filtered, washed with water several times till free of chloride and dried.

3.6. Claims:

1. Biodegradable synthetic polymers having a formula



Formula (I)

Wherein,

R_1 = H /Ph

R_2 = COOR_4

R_3 = COOH

R_4 = Carbohydrate

m = 50 - 98 mole %

n = 2 - 50 mole %

2. A process of the preparation of biodegradable synthetic polymers of formula (I), which comprises drying a base synthetic polymer and a carbohydrate in vacuum at a temperature ranging between 55 to 60 ºC for a period of 17 to 19 hrs., preparing separately the solutions of the base synthetic polymer in dry organic solvent and a solution of a carbohydrate and a catalyst in dry organic solvent, adding the solution of the base synthetic polymer into the solution of carbohydrate and the catalyst under agitation, heating the reaction mixture to a temperature ranging between 25 to 110ºC for 2 - 48 hours under nitrogen and agitation, cooling the reaction mixture to room temperature, precipitating the product using a solution of an inorganic salt in a non-solvent, washing the product with a solvent, till the product is free from the salt to obtain the product.
3. A process as claimed in claim (2) wherein the preferred base synthetic polymer is a polyolefin, which includes polystyrene with reactive functional groups like carboxyl, hydroxyl, amino, alkene, halide, ester and anhydride.
4. A process as claimed in claim (2) wherein the carbohydrates include monosaccharides, and disaccharides: both reducing and non-reducing, and oligosaccharides, wherein none of the hydroxyls are protected, and carbohydrates in which at least one but not all hydroxyls are protected.
5. A process as claimed in claim (2) wherein the dry organic solvents used for the preparation of the base synthetic polymer are dry N,N'-dimethylformamide, dry pyridine, dry toluene and their mixtures.
6. A process as claimed in claim (2) wherein the catalysts are pyridine, 4-dimethylaminopyridine, para-toluenesulfonic acid and carbodiimide catalysts.
7. A process as claimed in claim (2) wherein the dry organic solvent used for the preparation of solution of carbohydrate and the catalyst may be dry N,N'-dimethylformamide or dry pyridine and their mixtures.
8. A process as claimed in claim (2) wherein the molar ratio of base synthetic polymer to the carbohydrate may be 1:0.01 or 1:1 or 1:2 or 1:6, but preferably 1: 0.5 or more preferably 1:3.

9. A process as claimed in claim (2) wherein the molar ratio of the functional groups of the base synthetic polymer and the catalyst may be 1: 0.01 upto 1:2, but preferably 1:0.14.
10. A process as claimed in claim (2) wherein the non- solvent for the preparation of the salt solution may be water, methanol, ethanol, acetone and their mixtures.
11. A process as claimed in claim (2) wherein the salt used for the preparation of the salt solution are sodium and potassium salts, which include sodium chloride, potassium chloride, sodium bromide, potassium bromide and their mixtures.
12. A process as claimed in claim (2) wherein the solvents used for washing the product to make it free from salt are water, ethanol, methanol, acetone and their mixtures.
13. Biodegradable synthetic polymers and process for preparation thereof substantially as herein described with reference to the examples and drawings accompanying this specification.

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3.7. *Conclusion:*

The main advantages of the present invention are :

1. *Development of new polymers based on synthetic polymers, especially polyolefins, by functionalizing them and linking small quantities of monosaccharides, disaccharides, and oligosaccharides in a facile manner by polymer analogous reactions.*
2. *The basic physical properties of the modified synthetic polymers prepared as in #1 are not changed substantially by linking a few carbohydrate molecules, thereby extending their applications for legally and socially acceptable disposable products.*
3. *The process is economic and simple.*

4. *The products are environment friendly due to their biodegradability and substantially as herein described with reference to the examples accompanying this specification.*

CHAPTER 4

Fungal degradation of carbohydrate-linked polystyrenes

(reproduced from our paper published in Carbohydrate Polymers, 55, 393-399, 2004)

Abstract:

A series of carbohydrate molecules like glucose, sucrose and lactose were linked to maleic anhydride functionalized polystyrene by polymer analogous reactions to produce biodegradable polymers. Pure culture system was used for evaluating the biodegradability of these sugar linked polystyrene-maleic anhydride copolymers by known fungal test organisms. Weight loss measurements after fungal treatment confirmed the biodegradability of the carbohydrate-linked polymers, and it was observed that the degree of susceptibility to degradation varied with the type of test organism as well as with the type of sugar. FTIR spectra confirmed the degradation of the polymer. A photograph of fungal degradation is presented to show the increased fungal growth in lactose- linked PSMAH with Trichoderma sp., as compared to the fungal growth in the unmodified PSMAH and the control solution.

4.1. Introduction

Due to the excellent processing properties, polyolefins have occupied a special status as commodity plastics. However the major drawback of polyolefins is that they are non- biodegradable and hence pose severe problems of their disposal after their useful life. With the aim of developing biodegradable polymers based on polyolefins, blending of starch with polyolefins (particularly with polyethylene) has been much explored and also put in practice in a limited way (1-5). The intention of such blending procedures was that after disposal, degradation of starch in the blend would create voids and weaken the integrity of the polyethylene and result in its degradation. However the main drawback of this methodology is that attainment of such properties demands larger volumes of starch (in the range of 30% or higher) due to which the physical properties of the polyethylene have to be compromised (6).

There have been several reports of chemically modifying polystyrenes with non-polymeric sugars. Polystyrenes with pendant sugar residues have been synthesized by polymerization of vinyl benzyl sugars. Polystyrene derivatives with maltose, lactose and maltotriose substituents on each phenyl ring were synthesized by coupling the corresponding oligosaccharide lactones with p-vinylbenzyl amines followed by

radical polymerization (7-10). These polymers were water soluble, and are potential biomedical materials wherein the oligosaccharide moieties are used as recognition signals. However, there is no scope to develop these into bulk plastics as substitutes for polyolefins. Sugars have also been incorporated into silicone rubbers in order to improve their wettability and biocompatibility.

The present paper pertains to synthesizing biodegradable polymers based on functionalized polystyrene, by chemically linking carbohydrate molecules onto the polymer, by polymer analogous reactions, and testing their biodegradation by using pure fungal cultures.

4.2. Experimental.

4.2.1. Materials :

Poly(styrene-co-maleic anhydride) (PS-MAH) (maleic anhydride content 14% by weight) was obtained from Aldrich, USA. All solvents were obtained from SD Fine Chemicals, pune, India, and were distilled and dried before use. The microorganisms used *Aspergillus niger* NCIM 1025 (ATCC 9642), *Trichoderma* sp. NCIM 1297 (ATCC 9645) and *Pullularia pullulans* NCIM 1049 (ATCC 9348) were obtained from the National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, India.

4.2.2. Synthesis of sugar linked PS-MAH (General Procedure) :

PS-MAH, dissolved in anhydrous N,N'-dimethyl formamide, was added to a stirred solution of the sugar eg. glucose, sucrose, or lactose, etc and the catalyst viz. 4-dimethylaminopyridine taken in a three-necked round bottom flask provided with a magnetic stir bar, thermowell, addition funnel and a dry nitrogen balloon at a temperature 45-65°C over a period of 1/2 -1 hour. The mole ratio of the maleic anhydride content of the polystyrene-maleic anhydride copolymer versus the sugar

was either 1:3 or 2:1. In case of lactose, only a 1:3 ratio was investigated; the lactose sugar was not completely soluble in DMF under the reaction conditions. Stirring was done for 4 - 18 hours at temperatures of 45 - 65°C. The amount of sugar incorporated into the polymer by this method was generally between 0.2-3.5 by weight. The reaction mixtures were precipitated in brine, washed several times with water till free of chloride and dried in a vacuum oven. These polymers were found to be degraded by both bacterial a well as fungal cultures.

Use of colorimetry for quantification of the sugar content in the poly(styrene maleic anhydride) linked with glucose, sucrose, and lactose: The phenol- sulfuric acid reaction method.

The sugar content of the polymer was found by phenol sulfuric acid assay (11). Glucose, sucrose, lactose, and the sugar-linked poly(styrene maleic anhydride) polymers were dried overnight in an vacuum oven at ~60°C. A standard plot for glucose/ sucrose/ lactose was plotted using different concentrations (0.01mg/ml to 0.1mg/ml) of the respective sugar in water. The solutions of different concentrations were prepared as follows:

Table 1: Preparation of sugar solutions of different concentrations using a stock solution of 0.1 mg/mL

Sr.no.	Solution	Amount of solution 1 added (mL)	Amount of distilled water added (mL)	Final concentration (mg/mL)
1	Solution 1[a]	25	-	0.10
2	Solution 2	20	5	0.08
3	Solution 3	15	10	0.06
4	Solution 4	10	15	0.04

5	Solution 5	5	20	0.02
6	Solution 6	2.5	22.5	0.01
7	Blank Solution	-	25	0

[a] = solution 1 was prepared by dissolving 10 mg of the sugar (glucose/ sucrose/ lactose)

in 100 mL water

To 2ml of each sugar solution was added 1ml of 5% aqueous phenol followed by a rapid addition of 5ml concentrated sulfuric acid. In a similar way a blank was prepared using distilled water instead of sugar solution. The solutions were placed in a water-bath at 35-40°C for 30 minutes. The absorbency of the sugar solutions was recorded at 490λ and a standard plot was plotted. Each of the accurately weighed samples (glucose/ sucrose/ lactose-linked poly(styrene maleic anhydride)) in the range of 15 - 25mg was taken in a 50ml beaker to which were added 4ml water and 2ml 5% aqueous phenol solution followed by a rapid addition of 10ml concentrated sulfuric acid. The solutions were placed in a water-bath at 35-40°C for 30 minutes. The solutions were filtered through a sintered disc to remove the undissolved polymeric backbone. The absorbency of these solutions was recorded at 490λ and the unknown concentrations of the sugars were calculated from the standard plot. Using these concentrations the mole percentages and the weight percentages of the sugars in the polymers. The range of sugar content in the polymer was found to be between 0.2- 3.5 weight %.

Table 2: Table showing number of moles of the sugars in the polymers (weight percentages are in brackets) calculated by colorimetric experiments

Polymer	Moles and weight percentages of sugars			
	1	**2**	**3**	**Average**
PSMAH linked to glucose	0.198 (0.338)	0.15 (0.26)	0.116 (0.198)	0.155 (0.265)
PSMAH linked to sucrose	0.952 (3.05)	0.811 (2.6)	0.818 (2.63)	0.860 (2.76)
PSMAH linked to lactose	0.351 (1.18)	-	-	0.351 (1.18)

PSMAH = poly(styrene maleic anhydride)

4.2.3. FTIR Spectra :

FTIR spectra were recorded on a Shimadzu 8300 spectrometer. For PS-MAH, its fungal treated product and sucrose linked PS-MAH, transparent films were cast from chloroform or tetrohydrofuran solution, and were used to record the spectra. For biodegraded products of sucrose linked PSMAH, films could not be cast, and their spectra were recorded in KBr.

4.2.4. Test microorganisms:

The selected microorganisms *Aspergillus niger* NCIM 1025 (ATCC 9642), *Trichoderma* sp. NCIM 1297 (ATCC 9645) and *Pullularia pullulans* NCIM 1049 (ATCC 9348) were standard fungal strains (ASTM Standards, G-21, 1980) and *Penicillium ocro-chloron* NCIM 1219 (IS 9000, Part X, 1979) recommended for testing the fungal resistance. All the microorganisms used for the test were obtained

from our culture collection, National Collection of Industrial Microorganisms (NCIM). These cultures were routinely maintained on Potato Dextrose Agar (PDA) slopes.

4.2.5. Testing of the samples:

The cultures were grown in minimal medium (ASTM Nutrient Salts Medium) containing (g/l): KH_2PO_4 0.7; K_2HPO_4 0.7; $MgSO_4.7H_2O$ 0.7; NH_4NO_3 1.0; NaCl 0.005; $FeSO_4.7H_2O$ 0.002.; $ZnSO_4.7H_2O$ 0.002.; $MnSO_4.H_2O$ 0.001. The pH of the medium was adjusted to 6.5 ± 0.2 and sterilized at 121° C for 20 minutes. The test samples (250mg/ 50 ml) were surface sterilized with 70% ethanol for 2 h and added aseptically to the sterilized medium.

The inoculum was prepared by suspending the spores from fully grown cultures on PDA slopes in 10 ml of sterile saline. This suspension (1.0 ml) was added to 50ml of minimal medium (MM) in 250mL Erlenmeyer conical flasks. Flasks were incubated at 30°C for 10 weeks under stationary condition.

Test samples were harvested, washed in sterile distilled water several times to remove mycelia and spores followed by three washings with 70% ethanol and drying at 37°C for 48h.

4.3. Results and Discussion:

Test organisms used in the present study were standard cultures used for testing samples for their fungal resistance. Table 3 shows that the percent weight loss (indicating biodegradability) of the sugar linked polystyrene-maleic anhydride (PS-MAH) copolymer varies with the type of mono- or disaccharide attached. Thus, we observed that the PS-MAH without any sugar linked to it was not degraded by any of the four fungal species used in this study. Lactose linked PS-MAH showed maximum degradation with *Penicillium ochro-cloron* NCIM 1219 (12%), and the least with

Aspergillus niger NCIM 1025 (2.8%). In the case of glucose linked PS-MAH, the maximum weight loss was with *Aspergillus niger* NCIM 1025 (20.4%), and the lowest with *Trichoderma* sp. NCIM 1297 (2.8%), while for the sucrose linked PS-MAH, the greatest degradation was caused by *Trichoderma* sp. NCIM 1297 (weight loss 19.6%), while *Aspergillus niger* NCIM 1025 caused no degradation at all. Thus, each fungal microorganism has specific preference for different saccharide types linked to PS-MAH.

The test cultures used in the present study are organisms widely found in the environment. However, use of pure culture system offers the advantage of reproducibility of results, and would aid in designing biodegradation systems for different polymers after their disposal as waste materials.

Figures 1 and 3 show the FTIR spectra of PS-MAH and its fungal treated (*Aspergillus niger* NCIM 1025) product. There is no change in the spectra, indicating no chemical changes. This is supported by the fact that no weight loss was observed. Figure 2 shows the FTIR spectrum of sucrose linked PS-MAH, while Figures 4 and 5 are the spectra of the latter biodegraded by *Pullularia pullulans* NCIM 1049 and *Trichoderma* sp. NCIM 1297, respectively. Comparing Fig. 2 with Figs. 4 and 5, we find that the fungal treated polymer samples have greatly reduced intensity of the 1780 cm^{-1} anhydride peak and an increase in the carbonyl peak at ~ 1724 cm^{-1} due to ring opening of the maleic anhydride group. The carboxyl region at 2500-2900 cm^{-1} and the hydroxyl region beyond 3200 cm^{-1} also show broadening and increased intensity. Changes are also seen in the 1000-1400 cm^{-1} region. The spectrum shows an additional peak at 1660 cm^{-1} and at 1387 cm^{-1}. These clearly show that the chemical structures of the sucrose linked PS-MAH are changed after fungal treatment. This is supported by weight loss data. The other carbohydrate-linked polymers also showed very similar results and their spectra are therefore not presented here. The overlapping spectra in the expanded form (1000 – 2000) are also provided for comparison, which clearly depict the changes mentioned.

Table 3: Weight loss of different sugar linked polystyrene - maleic anhydride (PSMAH) copolymers in presence of different fungal strains

Sample	% weight loss by different strains			
	Aspergillus niger NCIM 1025	*Penicillium ochro-cloron* NCIM 1219	*Pullularia pullulans* NCIM 1049	*Trichoderma* sp. NCIM 1297
Control (without any carbon source)	-	-	-	-
PS-MAH	0.0	0.0	0.0	0.0
Lactose linked PS-MAH	2.8	12.0	9.2	6.4
Glucose linked PS-MAH	20.4	9.6	5.2	2.8
Sucrose linked PS-MAH	0.0	10.0	14.0	19.6

4.4. *Conclusions :*

It has been shown in this study that otherwise non-biodegradable synthetic polymers can be incorporated with structural features which can induce biodegradability. Thus, chemically linking small amounts of carbohydrate molecules with synthetic polymers like polystyrene-maleic anhydride leads to new polymers that are partially degraded by fungal cultures. The role of the carbohydrate molecule is the key to the further biodegradation of the synthetic polymer. The differences in the weight losses of the different types of carbohydrate-linked polymers, as well as the type of fungal culture used will throw useful new light on the design of new biodegradable polymer systems. More work is needed to fully biodegrade the modified synthetic polymer, and work in this direction is proceeding in our laboratory.

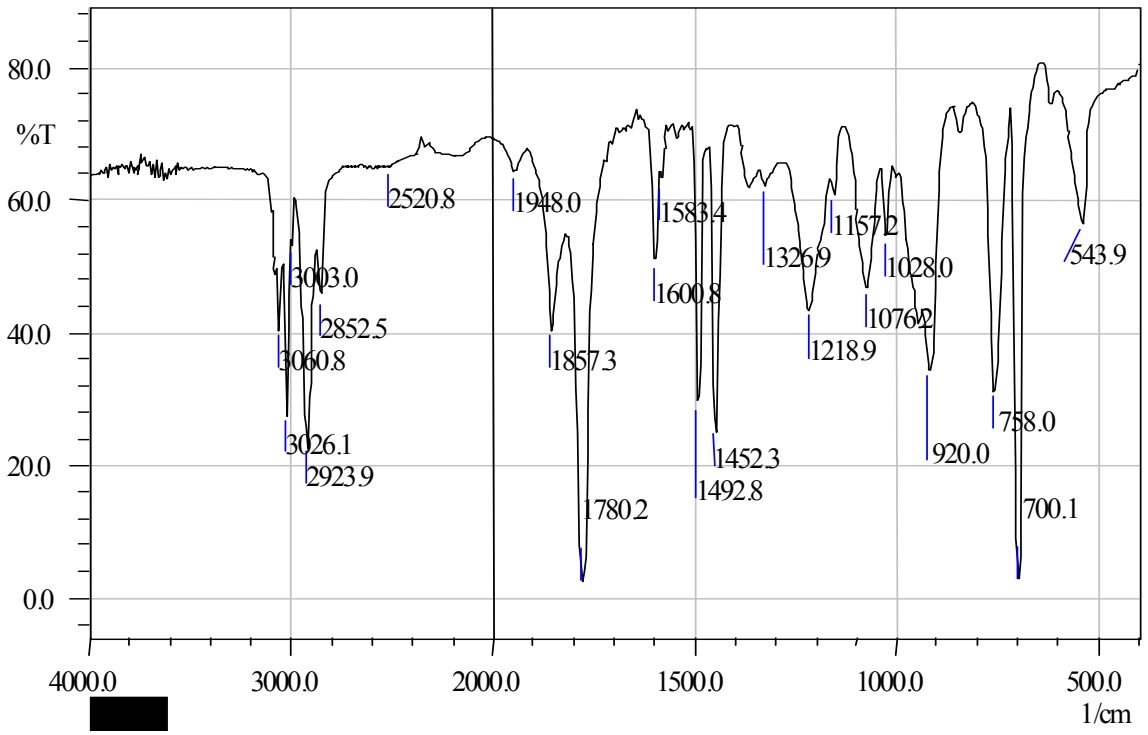

Figure 1: FTIR spectrum of poly(styrene maleic anhydride)

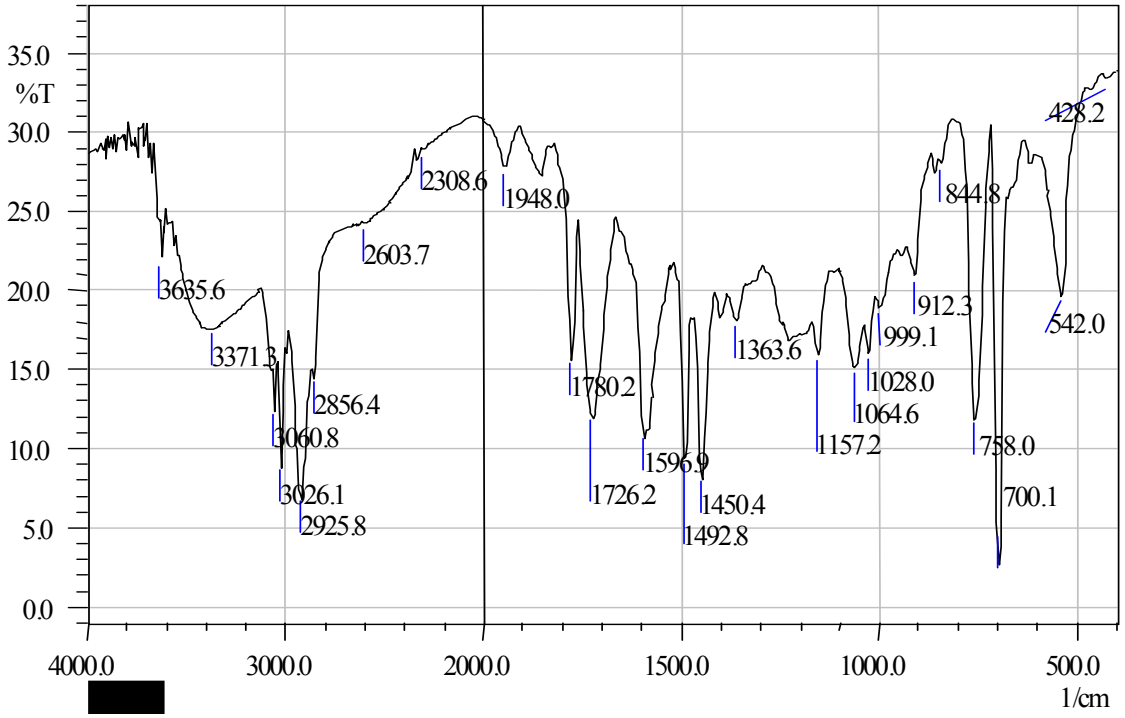

Figure 2: FTIR spectrum of sucrose linked to poly(styrene maleic anhydride)

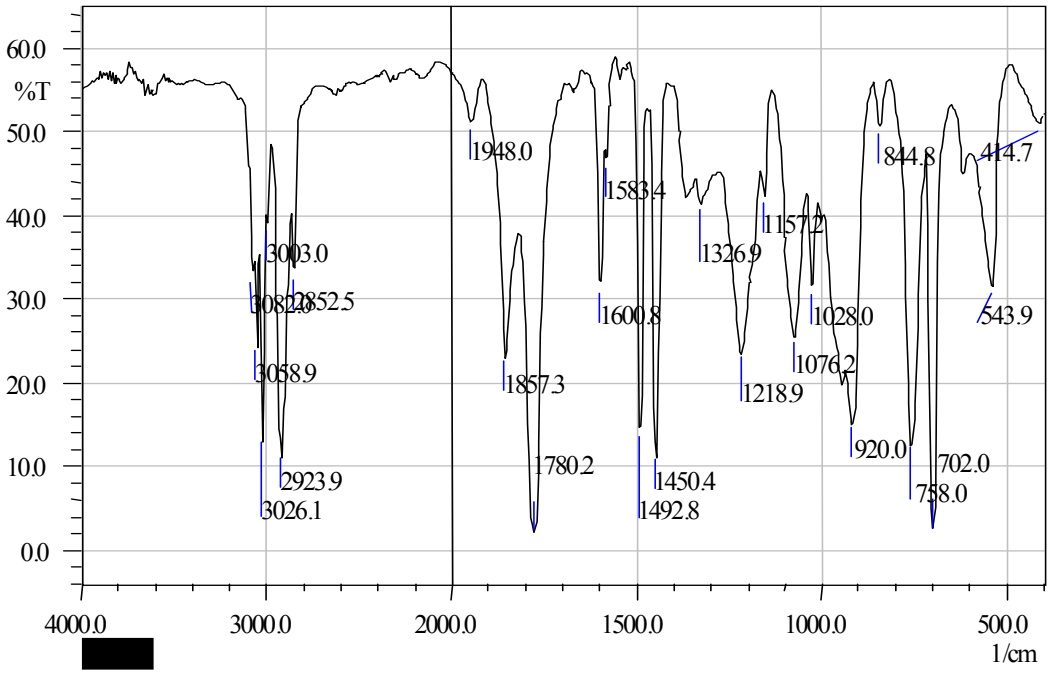

Figure 3: FTIR spectrum of poly(styrene maleic anhydride) degraded by *Aspergillus niger* NCIM 1025

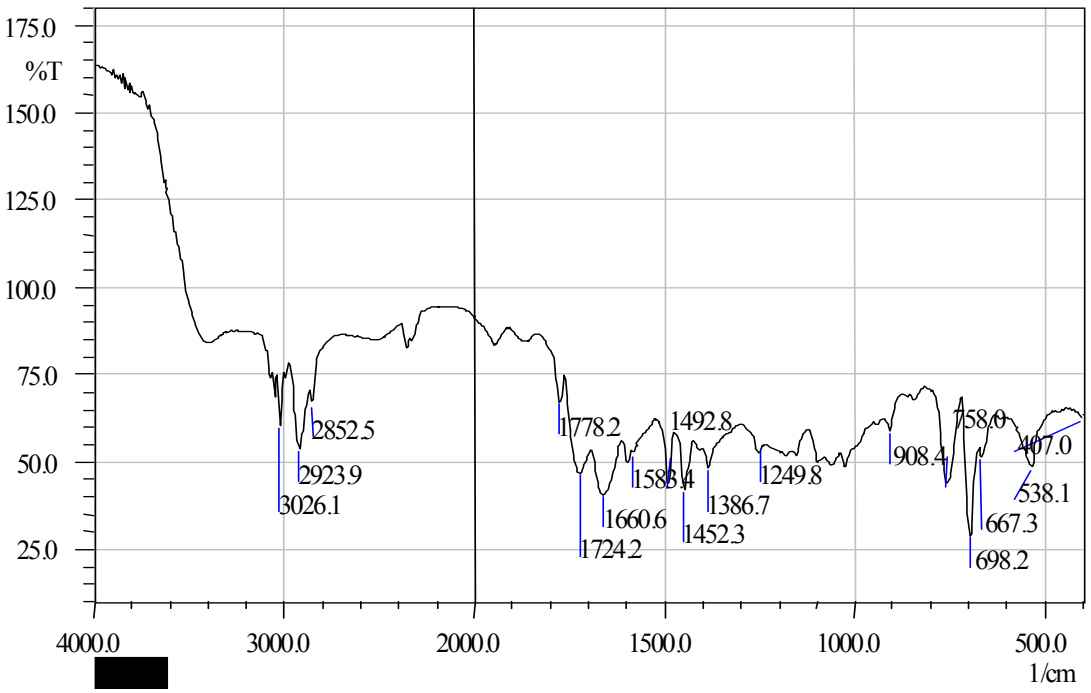

Figure 4: FTIR spectrum of poly(styrene maleic anhydride) linked by sucrose degraded by *Pullularia pullulans* NCIM 1049

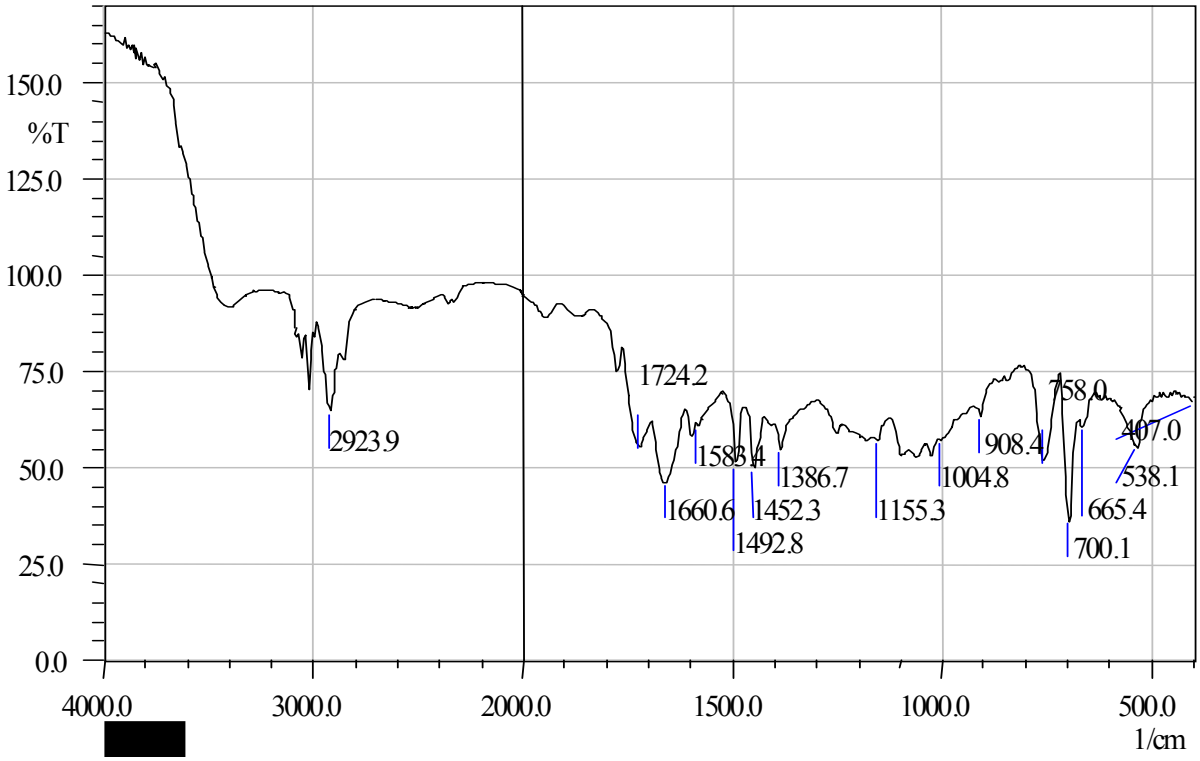

Figure 5: FTIR spectrum of poly(styrene maleic anhydride) linked by sucrose degraded by *Trichoderma sp.* NCIM 1297

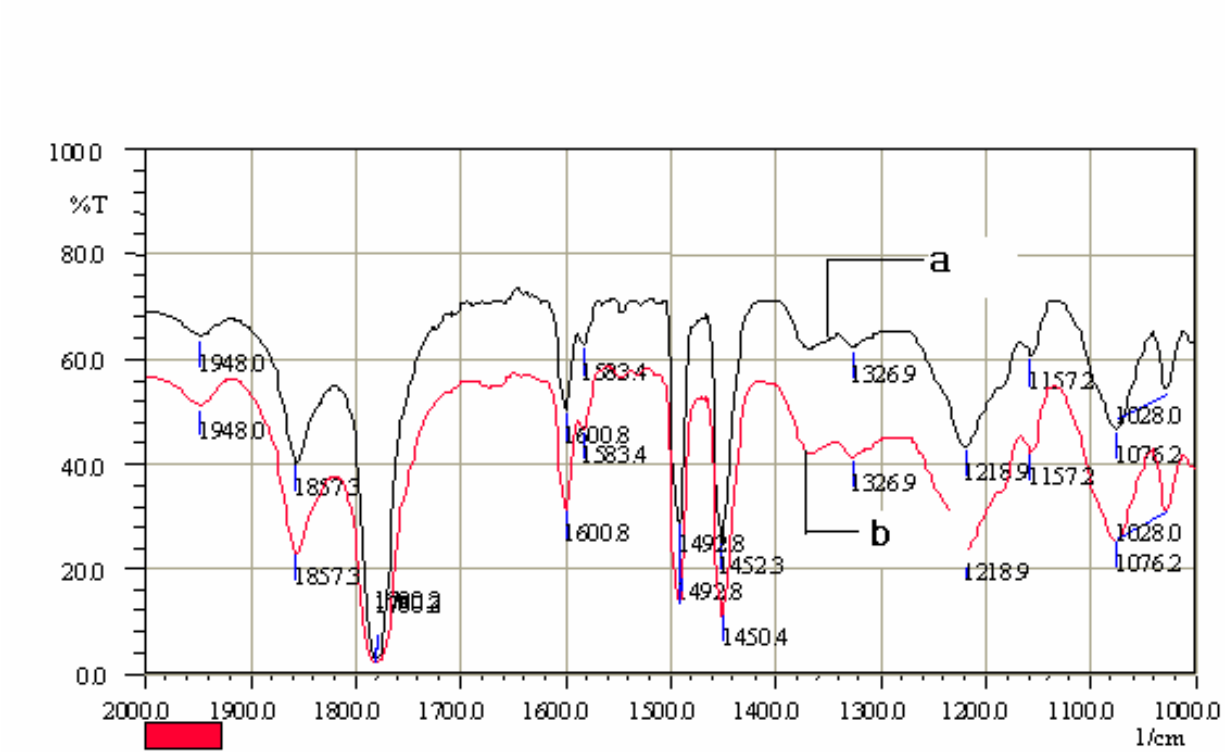

Figure 6: Overlapping FTIR spectra of poly(styrene maleic anhydride) and its degraded product by *Aspergillus niger* NCIM 1025

a= undegraded poly(styrene maleic anhydride)

b= poly(styrene maleic anhydride) degraded by *Aspergillus niger* NCIM 1025

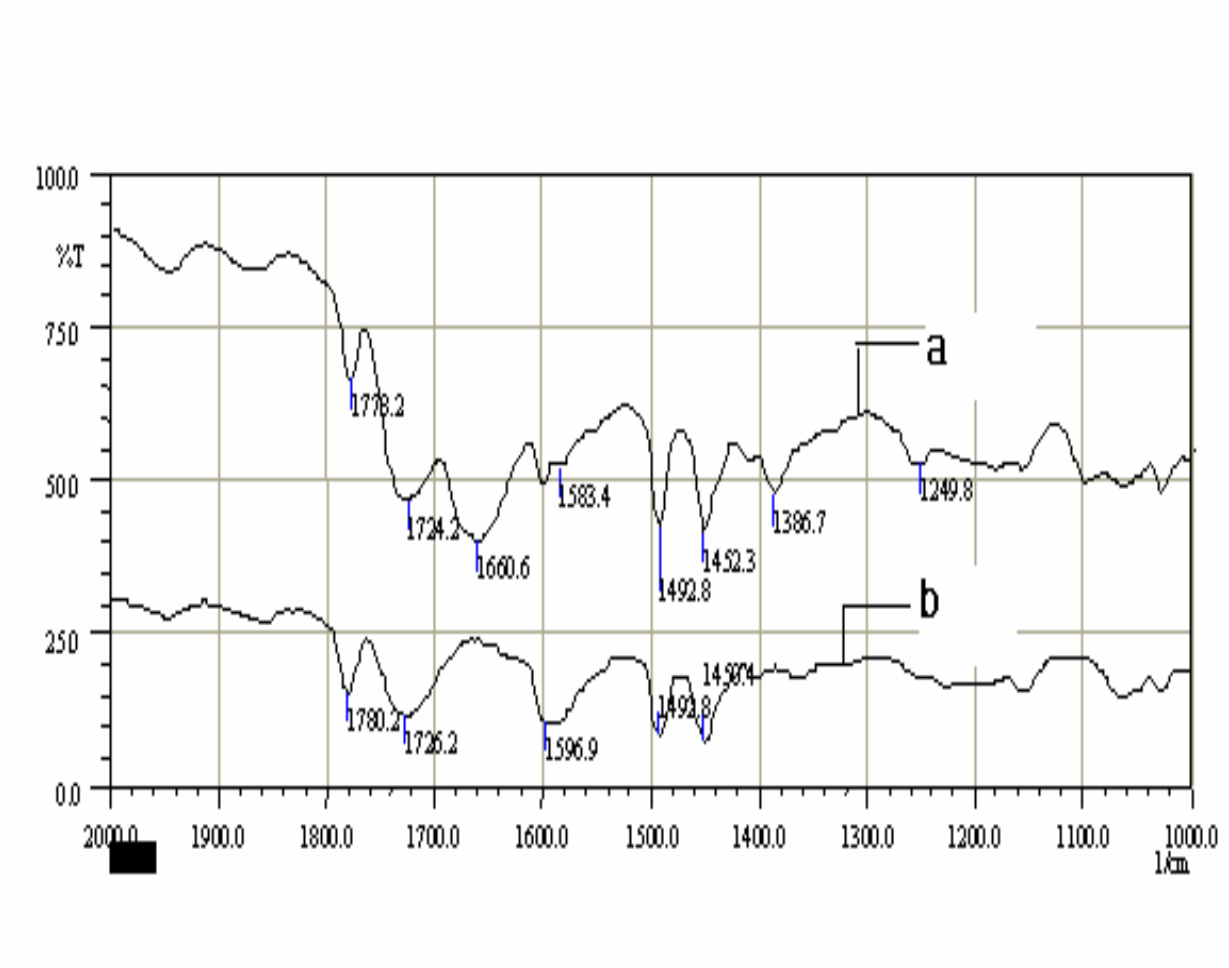

Figure 7: Overlapping FTIR spectra of poly(styrene maleic anhydride) and its degraded product by *Pullularia pullulans* NCIM 1049

a= poly(styrene maleic anhydride) degraded by *Pullularia pullulans* NCIM 1049

b= undegraded poly(styrene maleic anhydride)

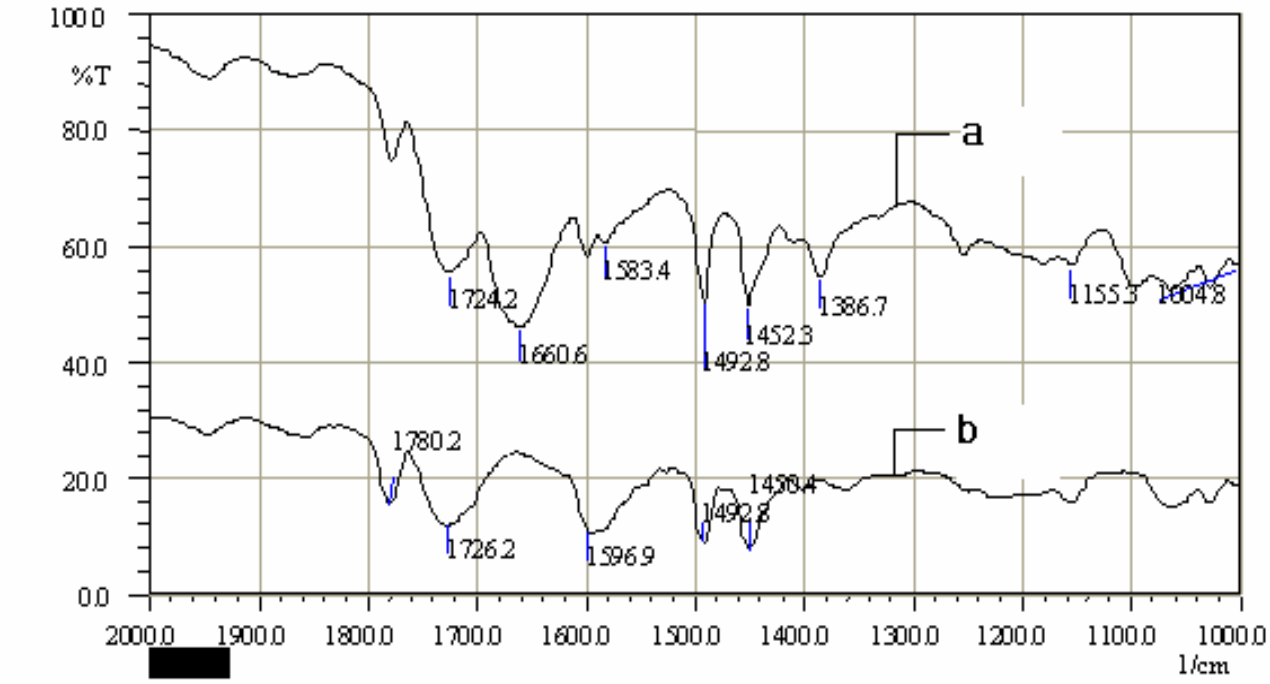

Figure 8: Overlapping FTIR spectra of poly(styrene maleic anhydride) linked by sucrose and its degraded product by *Trichoderma sp.* NCIM 1297

a= poly(styrene maleic anhydride) linked by sucrose degraded product by *Trichoderma sp.* NCIM 1297

b= undegraded poly(styrene maleic anhydride) linked by sucrose

4.5. References :

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4.6. Appendix 3:

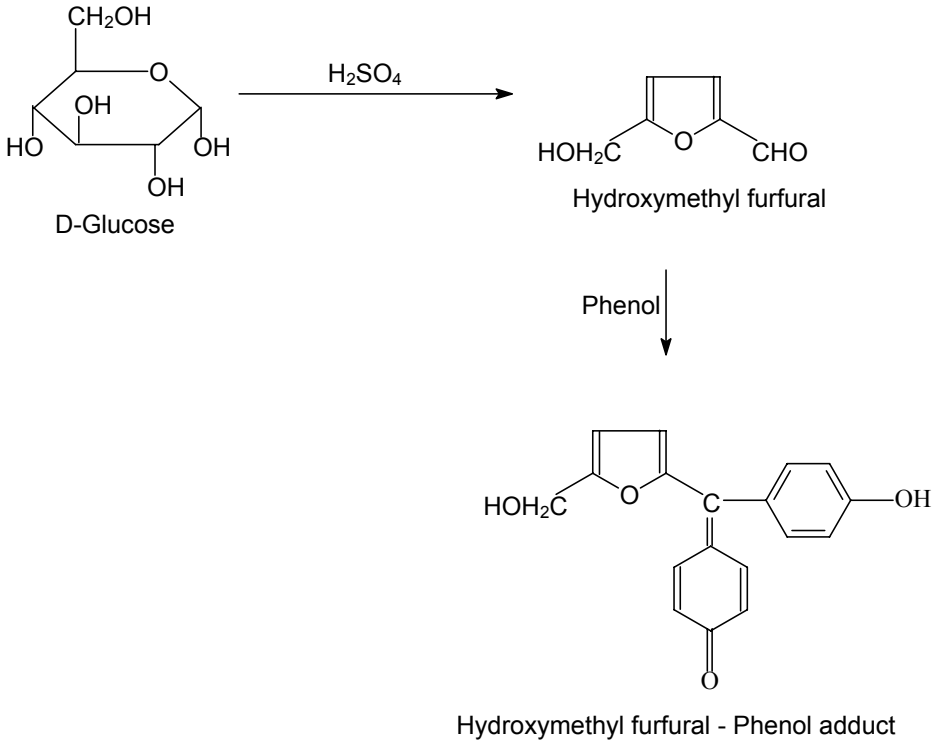
Principle, mechanism of the reaction involved and detailed calculations of a representative sample by colorimetric methods (Phenol- Sulfuric acid Method):

(Ref: Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A.,Smith F., Anal. Chem., 28(3), (1956).

This method is based on the following principle:

Concentrated sulfuric acid is used to break the bond between the polymer and the sugar. In case of disaccharides, it also breaks down the disaccharides into monosaccharides. The sugars are then dehydrated to furfural and hydroxymethyl furfural. The furfural compound then further reacts with phenol to produce a furan derivative that has a stable yellow-gold color. The concentration of carbohydrate is then determined by measuring the absorbance and comparing to a standard curve.

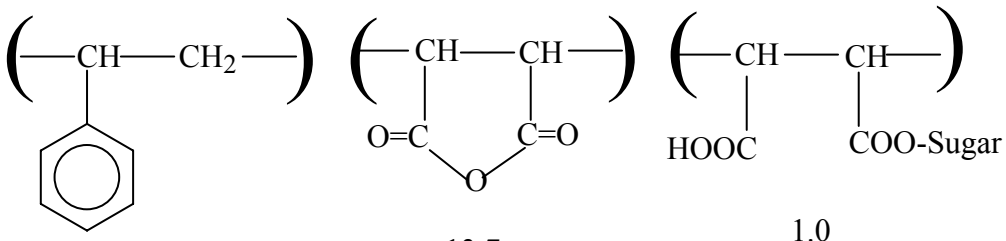
Reaction Mechanism:



Calculations (representative):

For sucrose linked to poly(styrene maleic anhydride)

Weight of the polymer taken = 20.9 mg
Concentration of the polymer = 20.9 mg in 4mL water = 5.225 mg/mL
Absorbance = 1.729
Concentration of the sugar (from the standard plot) = 0.136 mg/mL
Assuming that 1 mole of 14.7 moles of maleic anhydride has reacted



85.3 13.7 1.0

The molecular weights would be as follows:

$(85.3 \times 104) + (13.7 \times 98) + (99 \times 1) + (179 \times 1)$

$= 8871.2 + 1342.6 + 99 + 342$

$= 10654.8$

The weight percentages would be

$(8871.2 / 10654.8) + (1342.6 / 10654.8) + (99 / 10654.8) + (342 / 10654.8)$

$84.55 + 12.79 + 0.944 + 3.21$

Therefore we have the relation that for one mole of the sugar linked to the polymer

100g polymer will contain 3.21 g sugar, or

100mg polymer → 3.21mg sugar

Now if we assume that one mole of sucrose has been incorporated into the polymer

100mg polymer → 3.21mg sucrose

5.225 mg of the polymer → (3.21 x 5.225) / 100 = 0.1677 mg of sucrose

However, from the standard plot the concentration of sucrose was found to be 0.136 mg.

0.1677 mg → 1 mole

Therefore, 0.136 mg → 0.136 / 0.1677 = 0.811 moles

Thus the polymer contains 0.811 moles of sucrose.

No. of moles of sucrose in the polymer = 0.811

Weight percent of sucrose in the polymer = 2.6

The weight percent of sucrose in the polymer would be 0.811 x 3.21 = 2.6

4.7. Appendix 4:

Scanning electron micrographs of the polymers before and after fungal degradation:

In some cases the surfaces were smooth and in some cases they were rough. After subjecting the polymers to biodegradtion, the films were thoroughly washed with water and suspended in 70% ethanol overnight and then dried. The cases in which, SEMs were recorded before the treatment, have been specified.

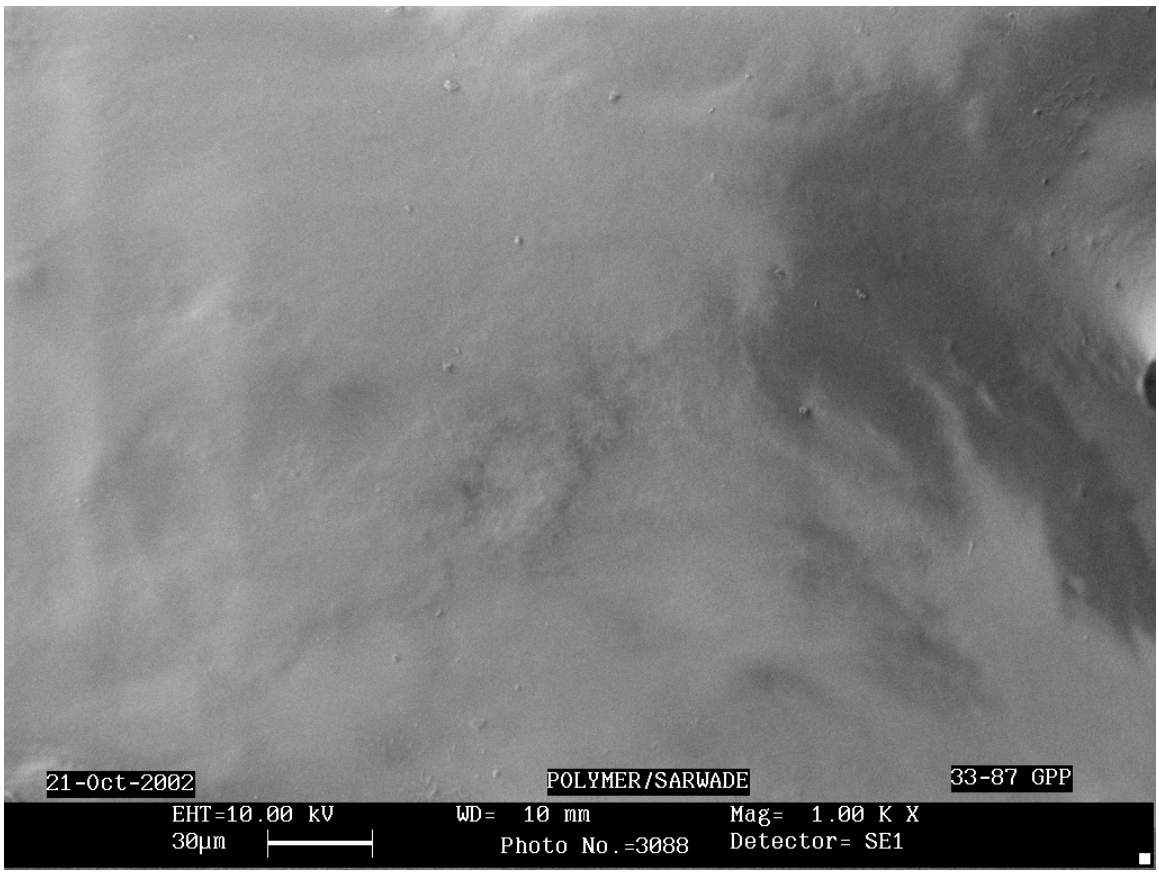


Figure 1: SEM of sucrose- linked poly(styrene maleic anhydride) before subjecting to biodegradation

The surface of the polymer appears smooth on the whole before subjecting to biodegradation.

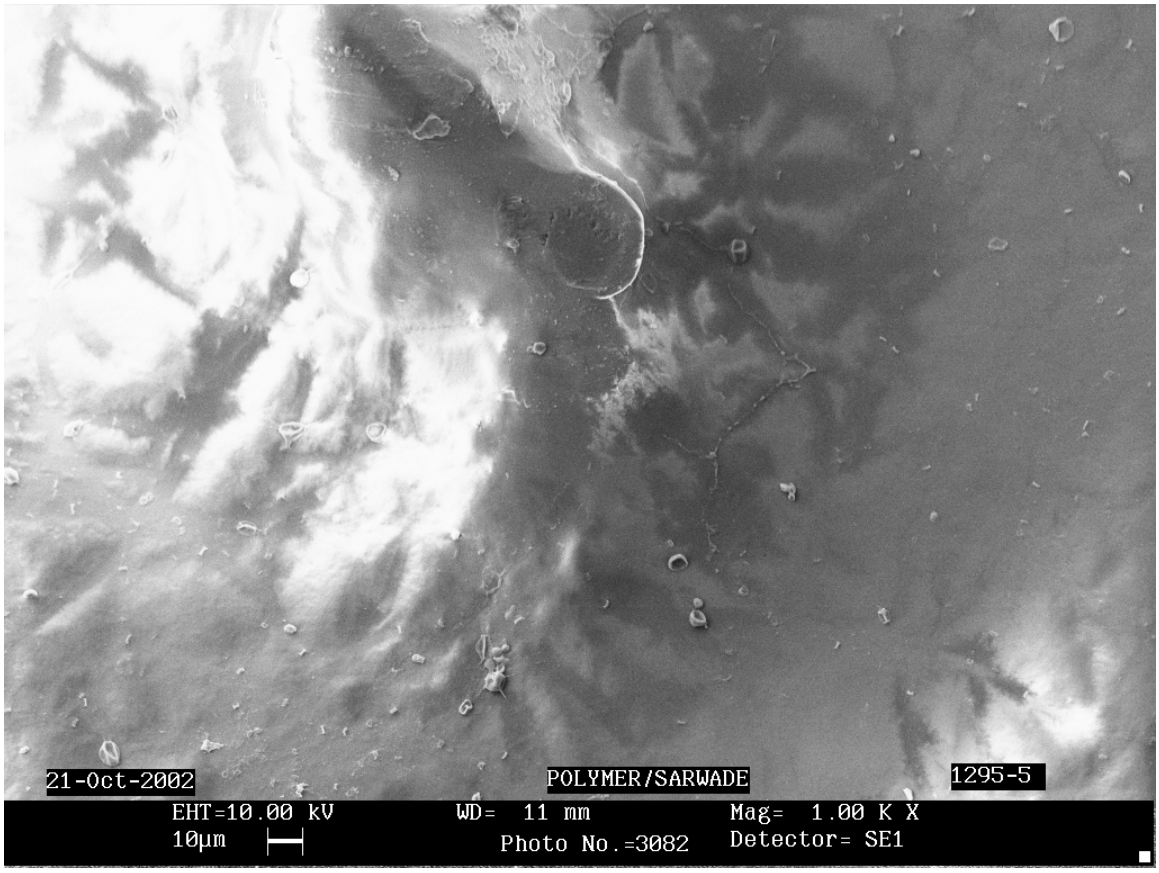


Figure 2: SEM of sucrose- linked poly(styrene maleic anhydride) after subjecting to biodegradation by Trichoderma sp. (NCIM 1297)

The above figure shows changes in the morphology of the polymer after the fungal degradation

The visual growth pattern of Trichoderma sp. (NCIM 1297) on lactose- linked PSMAH is depicted below.

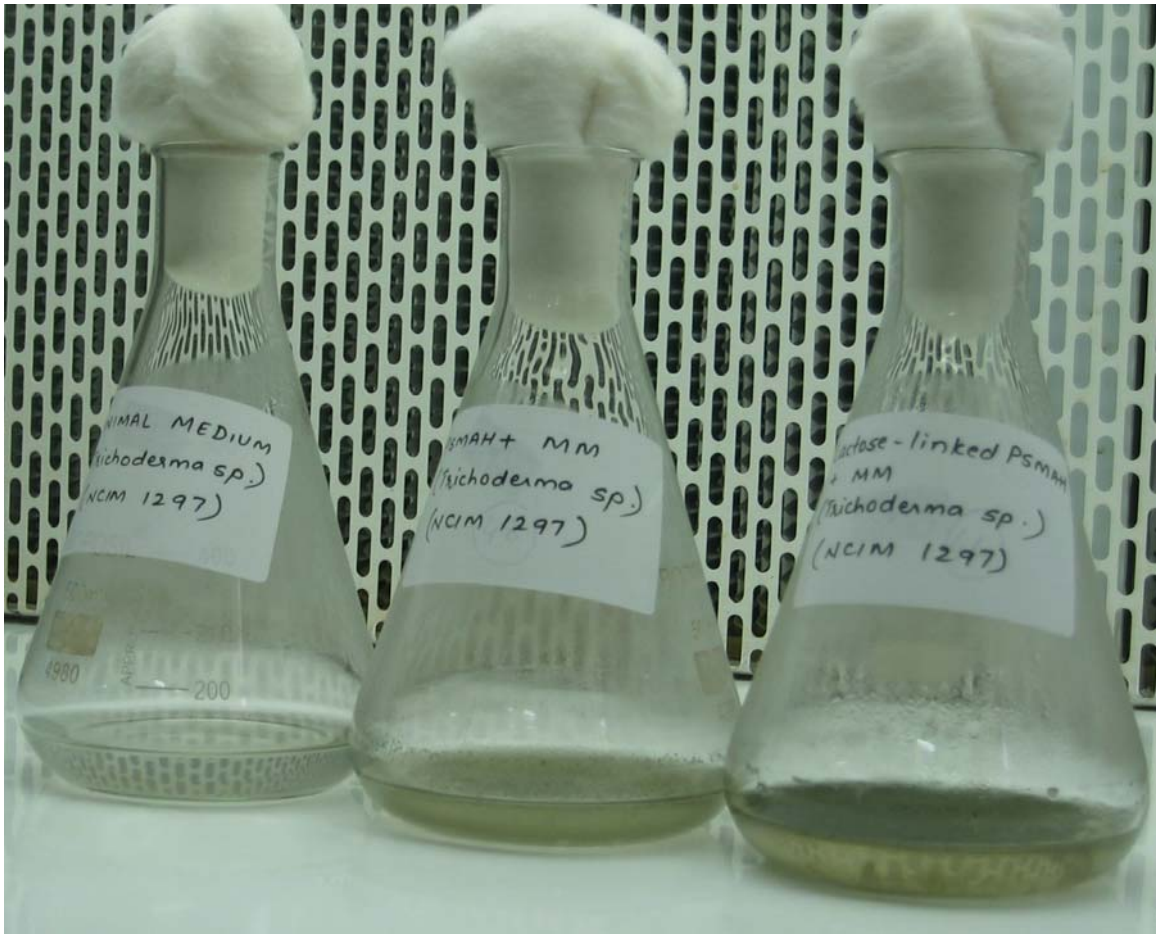


Figure 3: Photograph of the growth pattern of Trichoderma sp. on minimal medium (left), PSMAH (middle) and lactose- linked PSMAH (right)

CHAPTER 5

Elucidation of the ester peak positions and reactivities of the different glucose hydroxyls : An FTIR study of the reaction of glucose and glucose derivatives with poly(styrene maleic anhydride)

Abstract:

This chapter presents a detailed FTIR characterization of sugar- linked poly(styrene maleic anhydride) (PSMAH) polymers. An attempt was made to identify the peak positions of the ester bonds formed by the primary hydroxyl, secondary hydroxyls, as well as the hydroxyl group on the anomeric carbon of the glucose molecule with the maleic anhydride moeity of PSMAH. We also attempted to obtain a qualititative idea of the relative reactivities of the different hydroxyl groups for this reaction. To ascertain this, the reaction products of the following compounds with PSMAH were evaluated by FTIR : D-glucose, 6-O-trityl glucose, methyl glucoside, 1,2-5,6 diisopropylidene D-glucose (Diacetone D-glucose), and 1,2,3,4-tetraacetyl D-glucose. Expectedly, the primary hydroxyl of glucose was found to be the most reactive. The carbonyl peak was deconvoluted to find the positions of the different esters formed in the reaction. From the results, the peak at ~1725 cm^{-1} was assigned to the ester carbonyl formed by the reaction of the primary hydroxyl of glucose, whereas, the peak at 1716-1718 cm^{-1} was assigned to the one formed by the reaction of the hydroxyl group on the anomeric carbon, while the peaks between 1730 and 1740 cm^{-1} were assigned to the esters formed by the reaction of the secondary hydroxyls of the glucose molecule (C2, C3, and C4). The ratio of the peak intensities of the residual anhydride carbonyl at 1780 cm^{-1} and the polystyrene peak at 1492 cm^{-1} was helpful in elucidating the reactivities of the different hydroxyl groups in the esterification reaction.

5.1. Introduction:

In Chapters 2 and 4 of this thesis, we have reported our results on the biodegradation of carbohydrate-linked polystyrenes, prepared by reacting poly(styrene maleic anhydride) with unprotected sugars by polymer analogous reactions. The most striking observation of this study was that incorporation of even minute quantities of

sugar brought about substantial enhancement in the rates of biodegradation of these polymers. Since the grafting percentages were very low (typically less than 3% by weight), NMRs of these polymers did not show any sugar peaks. In this context, FTIR spectroscopy proved to be a useful tool to characterize these polymers. The FTIR spectra show changes in the absorption bands in the region 3650 to 2500 cm^{-1} due to hydrogen bonded hydroxyl groups and H-bonding involved in case of carboxylic acids (Wang M., Zhu X., Wang S. and Zhang L., Polymer, 40, 1999, 7387- 7396; Zhbankov R.G., translated by Densham A.B., "Infrared spectra of Cellulose & its Derivatives", Consultants Bureau, New York, 1966) The reduction in the intensity of the carbonyl stretching bond of the anhydride group, with occurrence of a new broad band at ~1730 cm^{-1} suggests opening of some of the anhydride rings with the formation of acid and ester groups. The FTIR spectrum of glucose- linked poly(styrene maleic anhydride) did not give any information regarding which of the glucose hydroxyl groups has been esterified. In order to elucidate the reactivity of the different glucose hydroxyls, different glucose derivatives, with specific hydroxyls protected, were grafted onto poly(styrene maleic anhydride), and were characterized by FTIR spectroscopy. In this chapter we are reporting a detailed FTIR study of these polymers.

5.2. Experimental:

The glucose derivatives viz. 6-O-trityl D- glucose 1,2-5,6 diisopropylidene D-glucose and 1,2,3,4- tetraacetyl D-glucose were synthesized by standard methods (Methods in Carbohydrate Chemistry, vol. 6, pg. 411). 1,2-5,6 Diisopropylidene D-glucose (Diacetone D-glucose) and poly(styrene maleic anhydride) containing 14 weight percent of maleic anhydride were obtained from Aldrich Co. as a standard. The general procedure for esterification of poly(styrene maleic anhydride) with different sugars has been reported elsewhere (Galgali P., Varma A.J., Puntambekar U.S., Gokhale D.V., *Chem. Commun.,* . 2002, 2884-2885).

The infrared spectra of poly(styrene maleic anhydride) and sugar grafted poly(styrene maleic anhydride) were all recorded as films cast from tetrahydrofuran (THF), N, N'-dimethylformamide (DMF) or chloroform solutions. The spectra were recorded on Perkin Elmer Spectru 1 instrument at a resolution of 4 cm^{-1} and the numbers of scans were 200. Fourier deconvolution was used for assignment of the different ester carbonyls formed due to the reaction of the different hydroxyls of glucose with the maleic anhydride component of poly(styrene maleic anhydride).The gamma values (1.5- 2.3) and the smoothening points (58- 83) used for the deconvolution were within the acceptable limits.

Synthesis of glucose derivatives (see Appendix 3)

5.3. Results and Discussion:

The FTIR spectrum of poly(styrene maleic anhydride) was recorded, and peak assignments are based on reported spectral assignments of polystyrene and poly (styrene maleic anhydrdide) (Figure1 and Table 1).

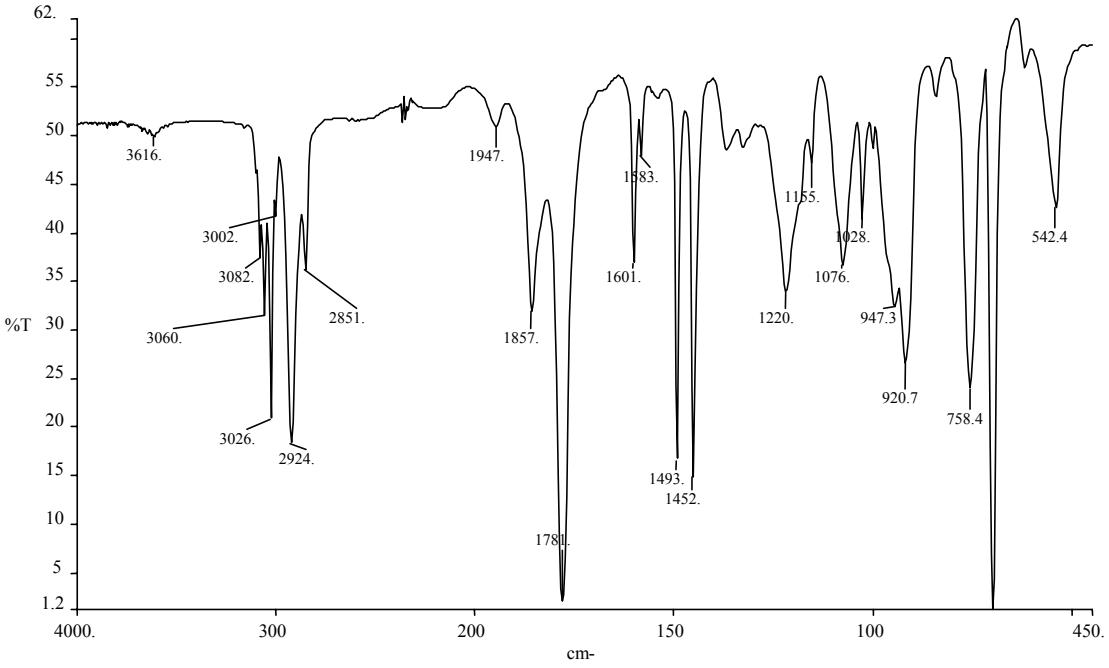


Figure 1: FTIR spectrum of unmodified poly(styrene maleic anhydride)

Table 1: Peak assignments for poly(styrene maleic anhydride) used in our study

Peak at (cm^{-1})	**Assignment**	**Reference**
3060 & 3026	Aromatic C- H stretching	A
2923 & 2852	Aliphatic C-H stretching	A
1857	Asymmetric >C=O frequency of the anhydride	C
1780	Symmetric >C=O frequency of the anhydride	C
1601, 1493 & 1453	In plane bond- stretching phenyl ring	A
1076	B_1 ring vibration in mono-substituted benzenes	B
756	Out of plane H	A
700	Out of plane phenyl ring	A

A: *Encyclopedia of Polymer Science & Engineering*, Mark H.F. and Bikales N.M., vol.8, John Wiley & Sons, New York, 1987, page 152.

B: Painter P.C. and Koeing J.L., *J. Polymer Science, Phys. Edn.,* 15, 1885, (1977).

C: "The Infrared Spectra of Complex Molecules", L.J. Bellamy, volume 1, Chapman & Hall, 1975

In addition to the C=C stretching frequencies of aromatic compounds present in the spectrum of polystyrene, the FTIR spectrum of poly(styrene maleic anhydride) shows bands at 1781 and 1858 cm^{-1} due to the symmetric and asymmetric carbonyl stretching frequencies of the anhydride group and also a band at 1220 cm^{-1} which is attributed to a five membered cyclic anhydride ("The Infrared Spectra of Complex Molecules", L.J. Bellamy, volume 1, Chapman & Hall, 1975).

The FTIR spectrum of the sugar linked poly(styrene maleic anhydride) polymers show reduction in the intensities of the anhydride carbonyl bands at 1781 and 1858 cm^{-1} with appearance of a new carbonyl band at ~1730 cm^{-1} assigned to ester and

carboxylic acid carbonyl. There is also a reduction in the intensity of the peak at 1220 cm^{-1} with a simultaneous appearance of broad peak between 1160 to 1215 cm^{-1} due to C—O stretching frequencies of the ester and carboxylic acid. Hydrolysis of poly(styrene maleic anhydride) or in other words, opening of the anhydride group would result in formation of a diacid. The FTIR spectrum of the hydrolyzed product would show a band in the carbonyl region. To find the exact position of this carbonyl an FTIR spectrum of hydrolyzed poly(styrene maleic anhydride) was recorded (Figure 2).

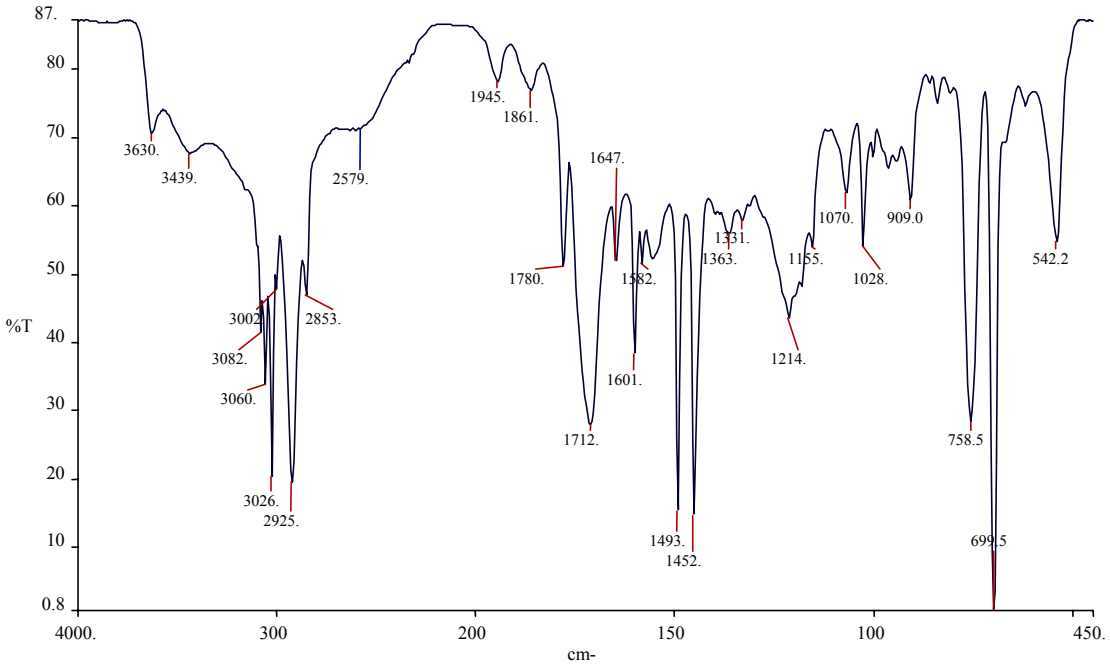


Figure 2: FTIR spectrum of hydrolyzed product of poly(styrene maleic anhydride)

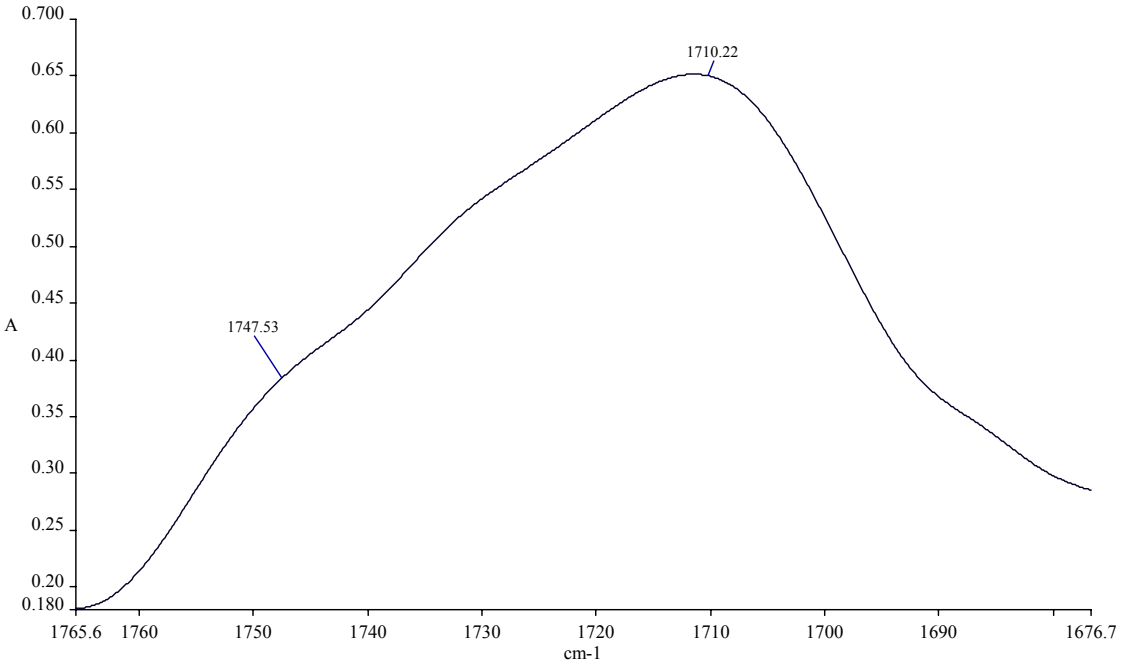


Figure 3: Deconvoluted carbonyl region of the hydrolyzed product of poly(styrene maleic anhydride)

The FTIR spectrum of the hydrolysis product of poly(styrene maleic anhydride) shows a carbonyl band at 1712 cm^{-1}, which is consistent with the reported value (Yan & Zhu, J. Appl. Polym. Sci., 74, 97-103, 1999; Bruch et al., J. Polym. Sci., 38, 1222-1231, 2000). The carbonyl bands in the hydrolyzed sample (see Figure 3) appeared at 1710 and 1747 cm^{-1} after deconvolution. A blank reaction of poly(styrene maleic anhydride) was also carried out under the same experimental conditions without the sugar in order to check whether the anhydride ring opens under these experimental conditions to diacid, however no peaks appeared in its FTIR spectrum at 1710 and 1747 cm^{-1} (Figure 4).

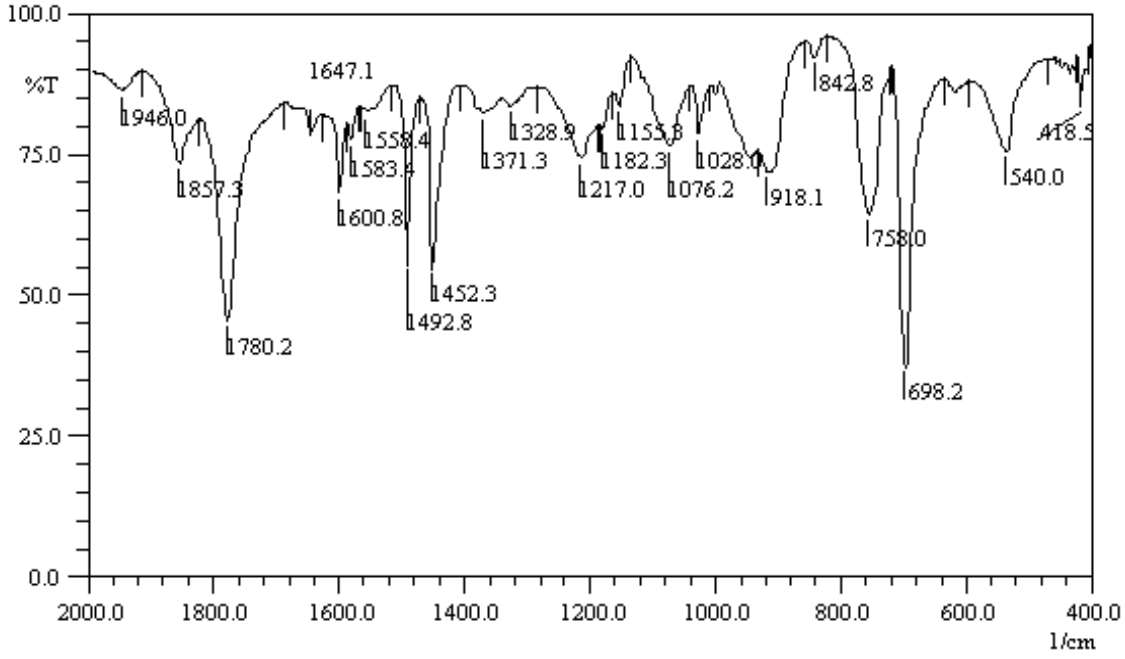
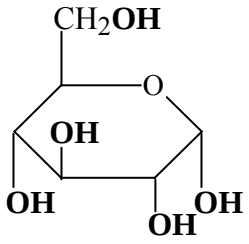
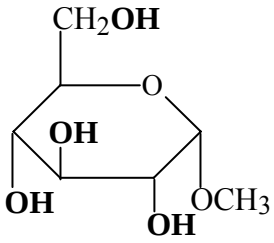


Figure 4: FTIR of blank reaction product of poly(styrene maleic anhydride)

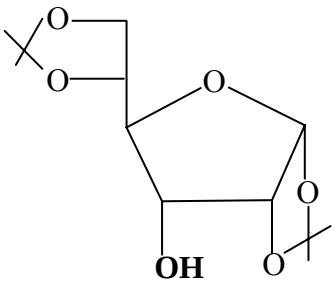
The structures of the glucose derivatives used in the reactions are shown in Figure 5



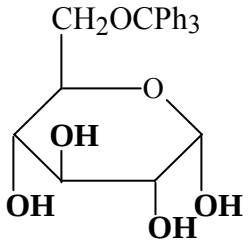
D-Glucose



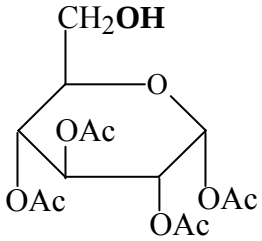
Methyl glucoside



Diacetone D- glucose



6-O-Trityl D-glucose



1,2,3,4- Tetraacetyl D-glucose

Figure 5: Structures of different glucose derivatives used in the reaction

On reaction of poly(styrene maleic anhydride) with an alcohol (in this case a sugar), the anhydride opens up into an ester and an acid. The carbonyl band (overlapping ester and carboxylic acid) appeared at 1732 cm^{-1}.

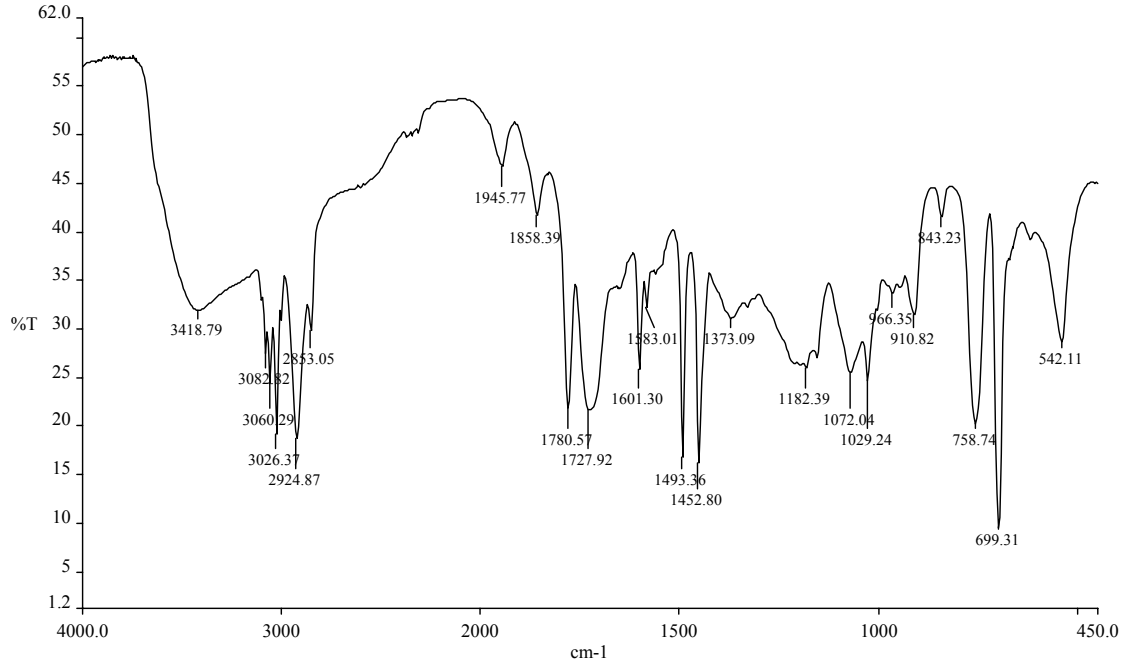


Figure 6: FTIR spectrum of glucose linked poly(styrene maleic anhydride).

Figure 6 shows the FTIR spectrum of D-glucose linked to poly(styrene maleic anhydride). The FTIR spectrum shows reduction in the intensity of the anhydride carbonyl and appearance of a new peak at 1728 cm^{-1} due to merging of the ester and carboxylic acid carbonyls. It also shows replacement of a comparatively sharp peak at 1220 cm^{-1} by a broad peak between 1155- 1260 cm^{-1} due to merging of C-O stretching frequencies of the ester, anhydride and the carboxylic acid groups. The carbonyl band was broad and asymmetric in nature as seen in Figure 7. Hence this carbonyl bond was deconvoluted. The deconvoluted carbonyl now showed six peaks at 1709, 1716, 1724, 1731, 1739 and 1747.5 cm^{-1} (see Figure 8)

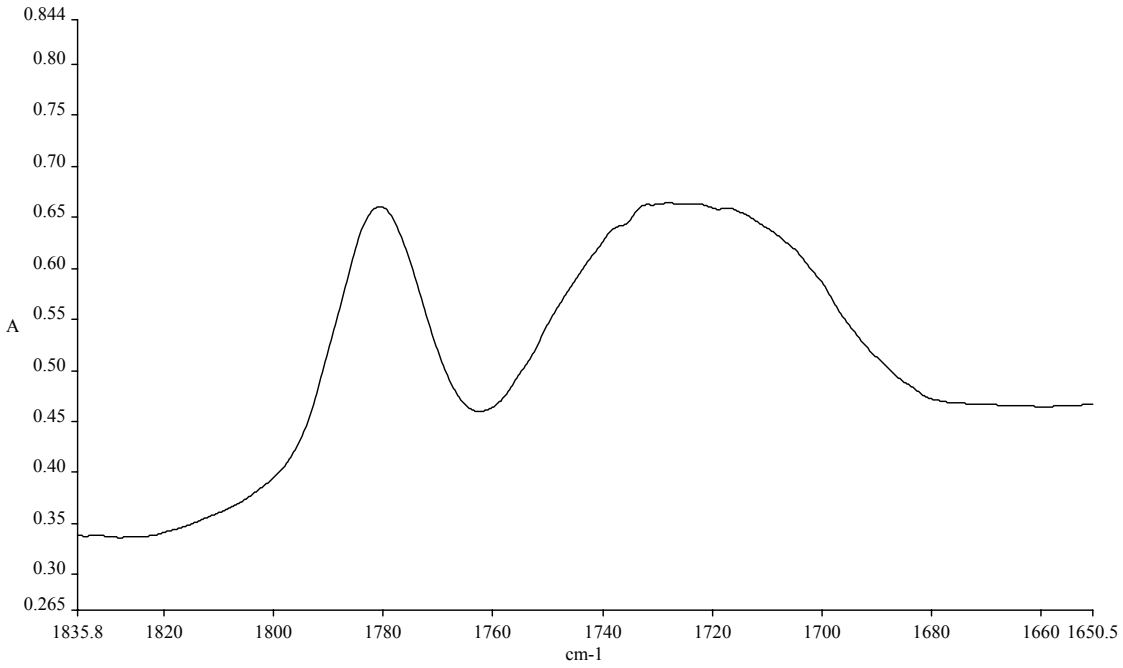


Figure 7: Carbonyl region of glucose- linked poly(styrene maleic anhydride)

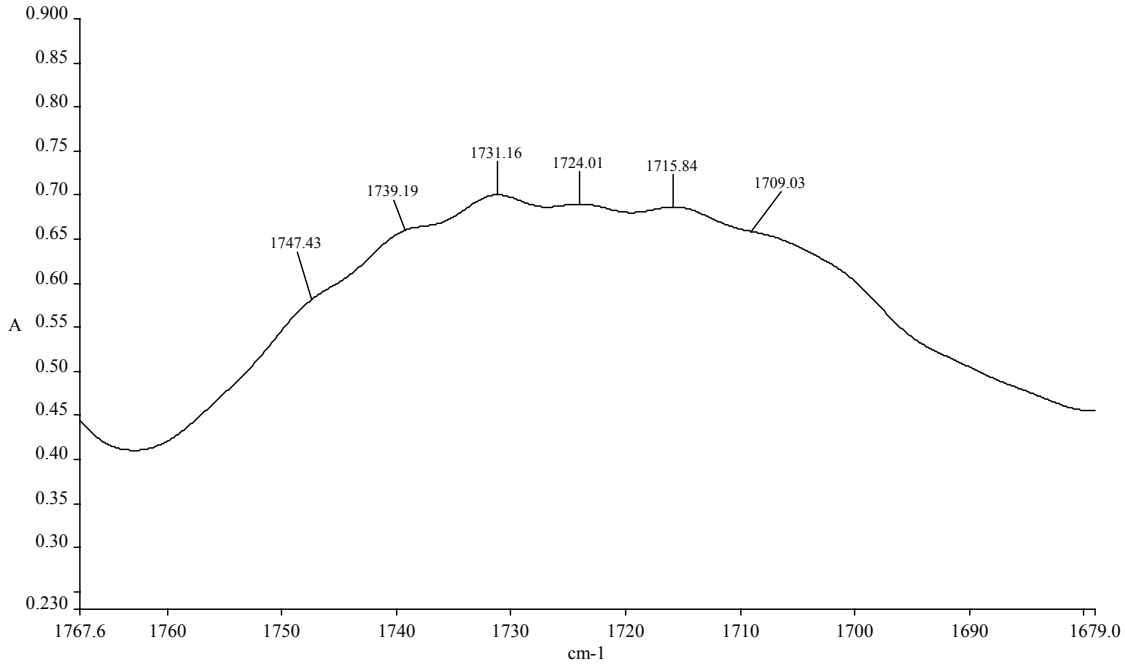


Figure 8: Deconvolution of the carbonyl band of glucose-linked poly(styrene maleic anhydride)

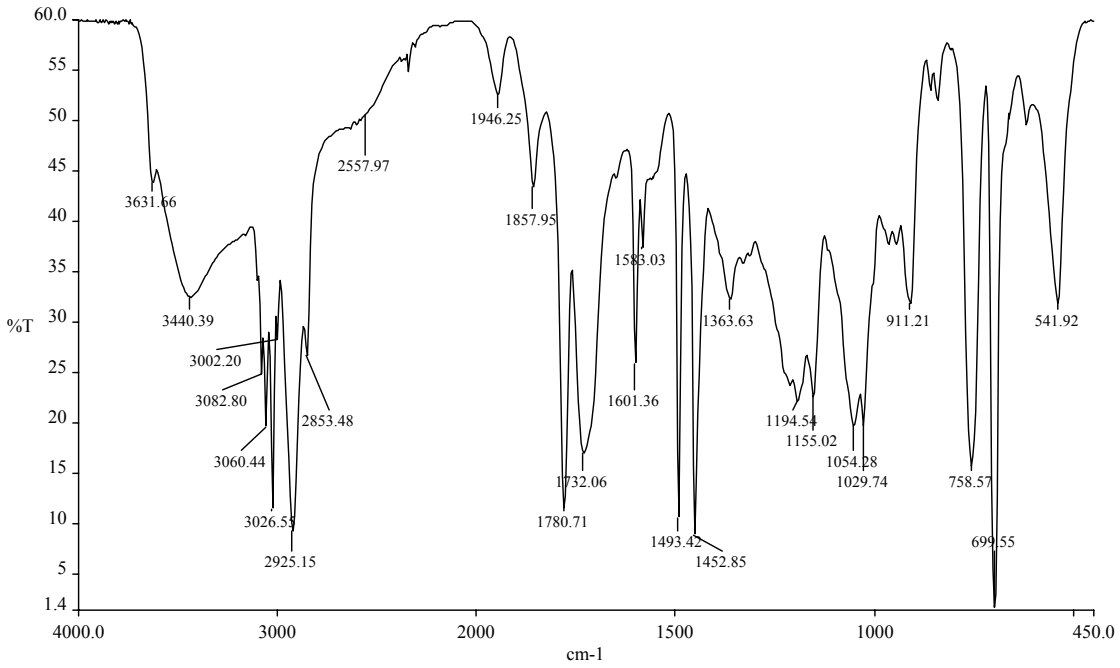


Figure 9: FTIR spectrum of methyl glucoside linked to poly(styrene maleic anhydride)

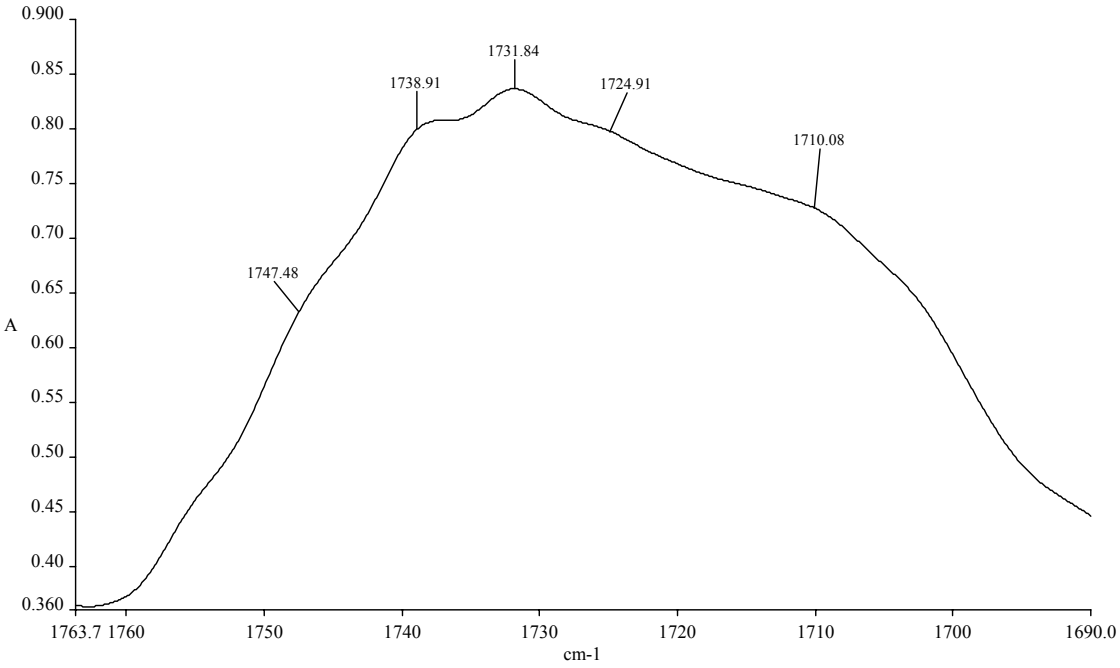


Figure 10: Deconvolution of carbonyl band of methyl glucoside- linked to poly(styrene maleic anhydride)

Figure 9 shows the FTIR spectrum of methyl glucoside linked to poly(styrene maleic anhydride). The FTIR spectrum shows changes similar to that of the glucose- linked counterpart.

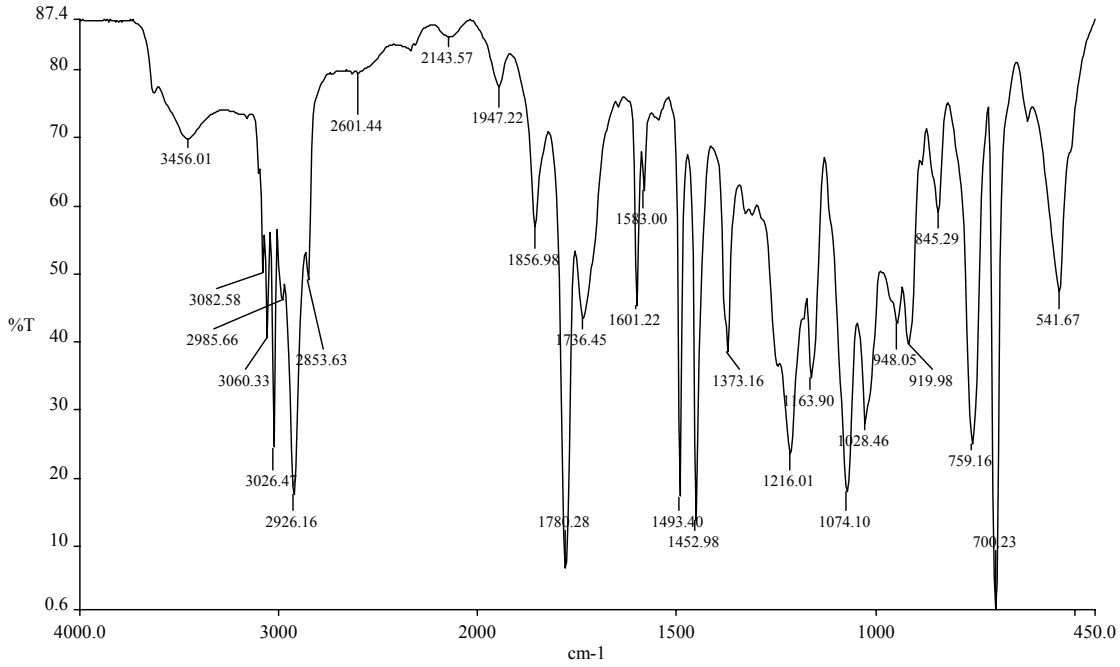


Figure 11: FTIR spectrum of 1,2-5,6 Diacetone D-glucose linked to poly(styrene maleic anhydride)

Figure 11 shows the FTIR spectrum of diacetone D-glucose linked to poly(styrene maleic anhydride). The spectrum shows reduction in the intensity of the anhydride band at 1781 and 1857 cm^{-1} and appearance a new carbonyl band at 1730 cm^{-1}. A peak also appears at 1373 cm^{-1}, which is a characteristic of gem dimethyl group present in diacetone D-glucose (Spectrometric Identification of Organic Compounds, Silverstein R.M.,& Webster F.X.,sixth edn., John- Wiley & Sons, New York, 1998; Kobayashi K. & Sumitomo H., *Macromolecules*, 1980, 13, 234- 239). The peak at 1220 cm^{-1} present in poly(styrene maleic anhydride) is replaced by a new slightly broader peak at 1216 cm^{-1}, which has broadened due to C-O stretching frequency of the carboxylic acid and a small peak at 1164 cm^{-1} due to the C-O frequency of the ester. The deconvolution of the carbonyl band shows splitting of the peak into two peaks, one at 1736 cm^{-1} (ester carbonyl) and 1710 cm^{-1} (carboxylic acid) (Figure 12).

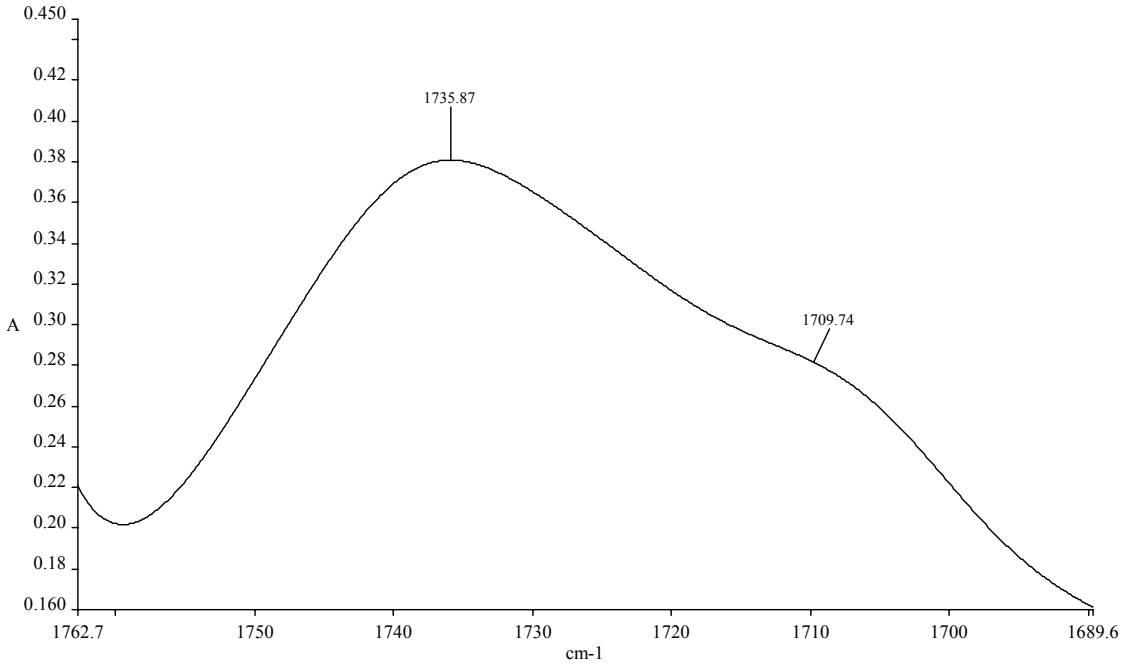


Figure 12: Deconvoluted carbonyl band of diacetone- D- glucose linked to poly(styrene maleic anhydride)

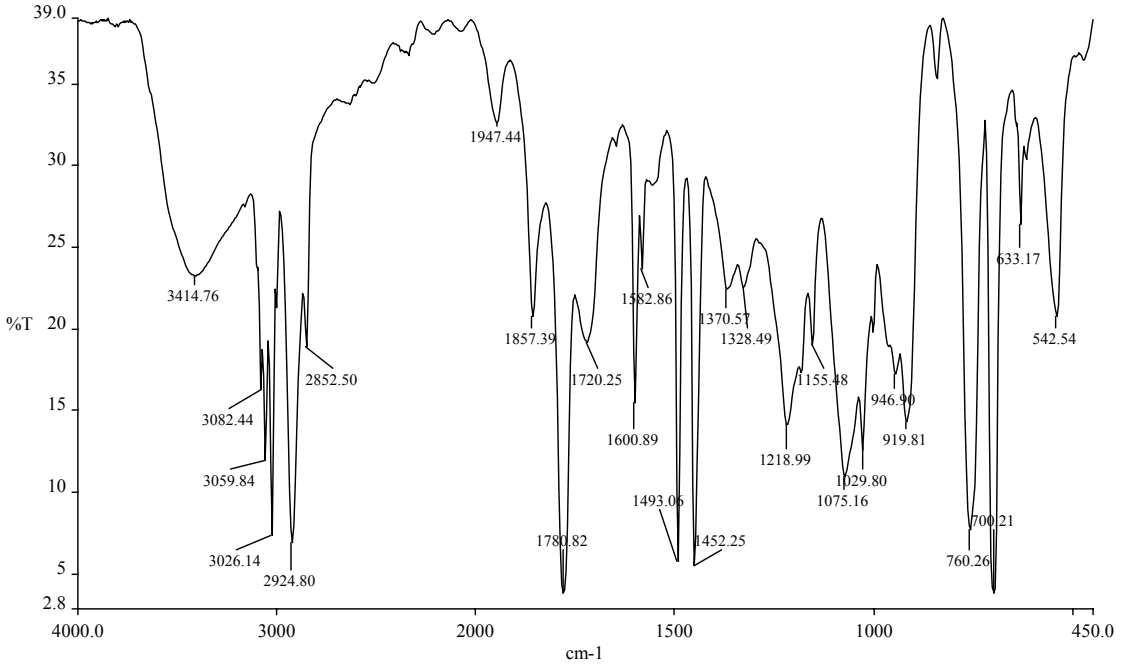


Figure 13: FTIR spectrum of 6-O-trityl D-glucose linked to poly(styrene maleic anhydride)

Figure 13 shows the FTIR spectrum of 6-O-trityl D-glucose linked to poly(styrene maleic anhydride). The FTIR spectrum shows reduction in the intensity of the anhydride carbonyl at 1781 and 1857 cm^{-1} and appearance of a new peak at 1720 cm^{-1} due to the ester and the carboxylic acid carbonyls. The peak at 1220 cm^{-1} slightly broadened due to additional C-O stretching frequencies of carboxylic and ester groups.

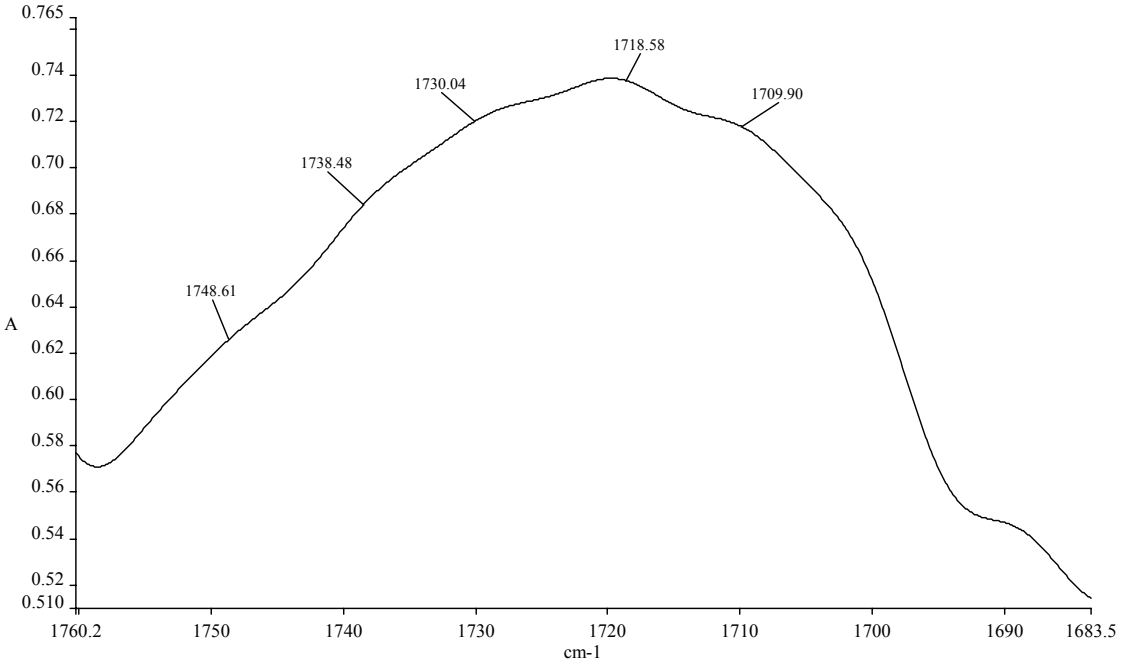


Figure 14: deconvoluted carbonyl of 6-O-trityl D-glucose linked to poly(styrene maleic anhydride)

The deconvoluted FTIR spectrum of 6-O- trityl D-glucose linked to poly(styrene maleic anhydride) (Figure 14) shows splitting of the carbonyl band into 5 peaks at 1710, 1718, 1730, 1738 and 1748 cm^{-1}.

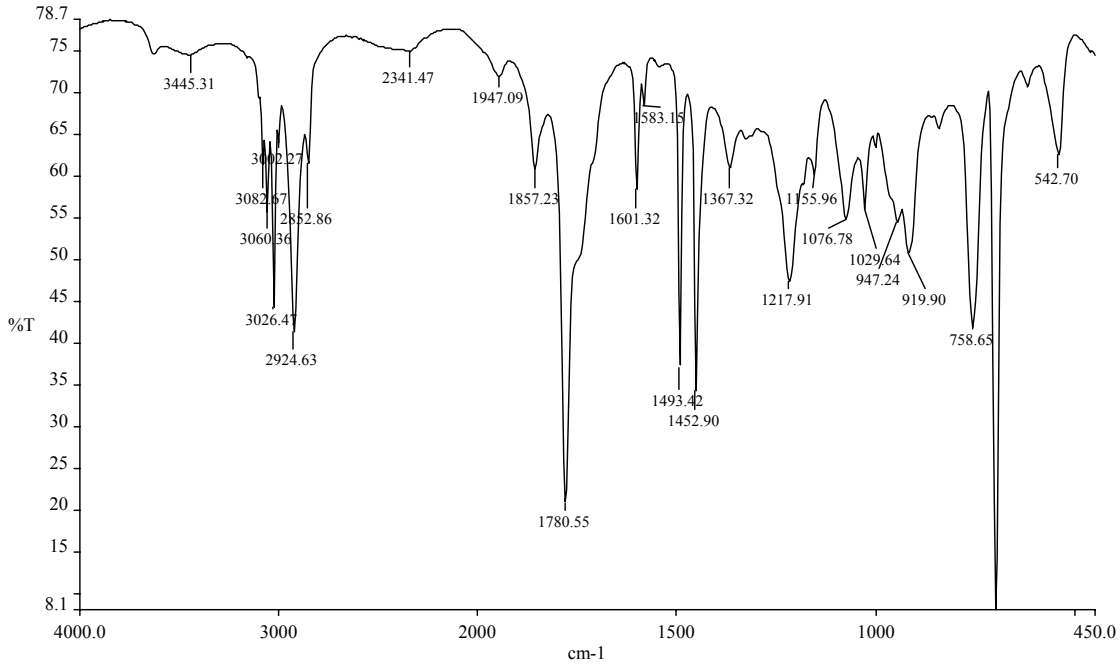


Figure 15: FTIR spectrum of 1,2,3,4- tetra acetyl D-glucose linked to poly(styrene maleic anhydride)

The FTIR spectrum of 1,2,3,4-tetra acetyl D- glucose linked poly(styrene maleic anhydride) shows formation of a small carbonyl peak at ~1730 $cm^{-1}$and at 1710 cm^{-1} (Figure 15) . There are few changes in the spectrum as compared to poly(styrene maleic anhydride). The carbonyl peak was again deconvoluted as seen in Figure 16. Due to large number of ester carbonyls of the of the acetyl groups of the sugar derivative, the deconvolution was not very effective in separating the ester carbonyls formed in the reaction.

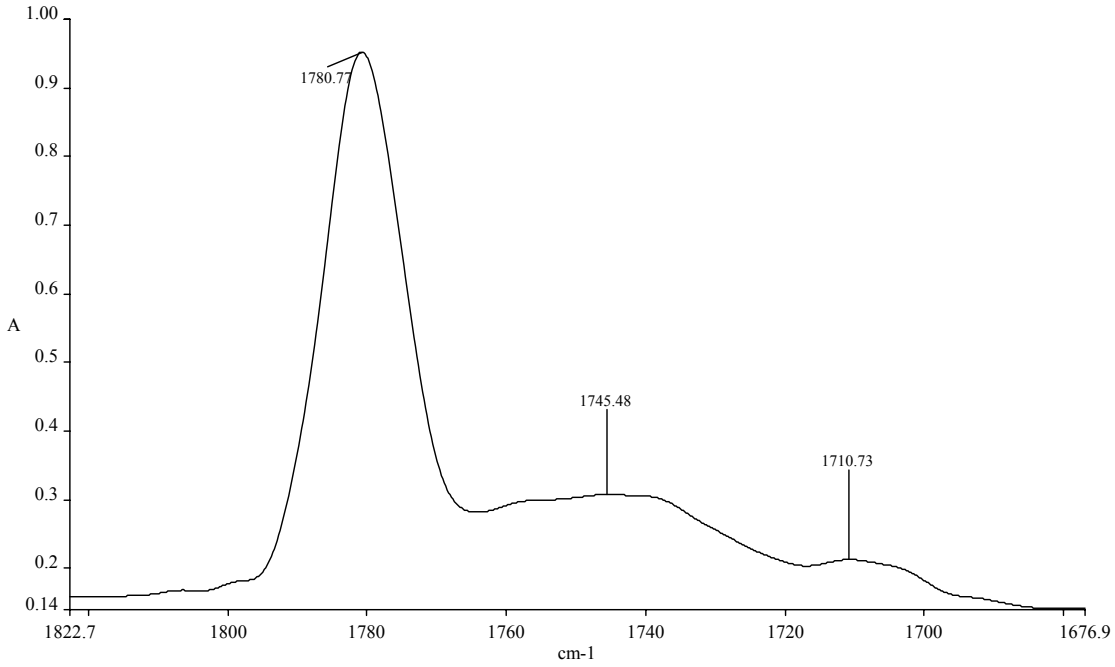


Figure 16: deconvoluted carbonyl of 1,2,3,4- tetraacetyl D-glucose

Determination of the reactivity of the different hydroxyl groups with the anhydride moiety in case of glucose using FTIR spectroscopy:

Since the amount of sugar incorporated into the polymer was in minute quantities, it was difficult to find out as to which of the hydroxyls was involved in the esterification reaction. For this, different derivatives of the sugar using protection-deprotection chemistry of the hydroxyl group, were synthesized and reacted with poly(styrene maleic anhydride). The ratio of their intensities was used for determining the reactivities of the different hydroxyl groups. The peaks chosen for the quantification were 1781 and 1493 cm^{-1}. The 1493 cm^{-1} peak does not vary with the reaction, whereas the peak at 1781 decreases with the progress of the reaction. The ratios are tabulated in table 2.

Table 2: Ratio of peak intensities of 1780 and 1493 cm^{-1}

Name of the derivative linked to PSMAH	No. of free hydroxyls	Nature of the free hydroxyl group	Corrected heights 1780cm^{-1}	1493 cm^{-1}	Ratio $\frac{1780}{1493}$ cm^{-1} cm^{-1}
PSMAH	-	-	1.52	0.55	2.76
D-glucose	5	1: anomeric, 1: primary 3: secondary	0.39	0.568	0.68
Methyl glucoside	4	1: primary 3: secondary	0.59	0.68	0.86
1,2-5,6 Diisopropyli -dene D-glucose	1	1: secondary	0.563	0.33	1.71
*6-O-trityl D-glucose	4	1: anomeric 3: secondary	0.7	0.66	1.06
1,2,3,4-tetra acetyl-D-glucose	1	1: primary	0.425	0.254	1.67

* The ratio of the peak intensities for the derivative 6- O-trityl –D-glucose cannot be accurately calculated, due to the contribution of the intensity at 1493 cm^{-1} by the trityl group.

From the above table it can be inferred that the primary hydroxyl is the most reactive, the anomeric carbon based hydroxyl is next, followed by the secondary hydroxyls. This inference can be drawn from the following observations:

- Both 1,2,3,4-tetra acetyl-D-glucose and diacetone-D-glucose have one free hydroxyl group, but 1,2,3,4- tetra acetyl D-glucose has a free primary hydroxyl group and 1,2-5,6 isopropylidene-D-glucose has a free secondary hydroxyl group. From the data of 1,2,3,4 tetra acetyl glucose and 1,2-5,6 diisopropylidene glucose, the primary is more reactive than the secondary hydroxyl.
- Comparing methyl glucoside and 6-O-trityl D-glucose, the primary hydroxyl is more reactive than the anomeric carbon based hydroxyl
- From the D-glucose reaction data we see that the reaction is fastest since both primary and anomeric hydroxyls are present.

There are several other factors which can play a role in the relative reactivity, such as the steric hindrance and solubility of the derivative in the solvent. Further, the 1,2-5,6 diisopropylidene D-glucose derivative exists in the furanose form, whereas all the other derivatives studied are in the pyranose form. Therefore, a larger variety of carbohydrate derivatives and model compounds have to be studied in order to arrive at more quantitative results.

Table 3: Peak positions of the different ester and acid carbonyls obtained upon reaction of poly(styrene maleic anhydride)

Derivative	**Peak positions after deconvolution (cm^{-1})**					
	1	2	3	4	5	6
D-glucose	1709	1716	1724	1731	1739	1747
Methyl glucoside	1710	-	1725	1732	1739	1747
Diacetone D-glucose	1710	-	-	-	1736	-
6-O-Trityl D-glucose	1710	1718	-	1730	1739	1748
1,2,3,4-Tetraacetyl D-glucose	1710	-	-	-	-	1745 (broad)
Hydrolyzed PSMAH	1710	-	-	-	-	1747

Table 3 shows the peak positions of the different carbonyls after the deconvolution of the overlapping carboxylic acid and ester peaks. From the table it is observed that the hydrolyzed poly(styrene maleic anhydride) shows two peaks, one at 1710 $cm^{-1}$and the other at 1747 cm^{-1} . hence these peaks can be assigned to the carboxylic acid group. Only the derivatives having a free anomeric hydroxyl viz. glucose and 6-O-trityl D-glucose show a peak at 1716- 1718 cm^{-1}. Hence this peak can be assigned to the ester formed by the reaction of the anomeric hydroxyl. Only the derivatives viz. glucose and methyl glucoside containing a primary hydroxyl showed a peak at 1725 cm^{-1}, and so this peak can be assigned to the ester formed by the reaction of a primary hydroxyl group.

5.4. References:

1. Wang M., Zhu X., Wang S. and Zhang L., *Polymer*, 40, 1999, 7387- 7396
2. Zhbankov R.G., translated by Densham A.B., "*Infrared spectra of Cellulose & its Derivatives*", Consultants Bureau, New York, 1966
3. Methods in Carbohydrate Chemistry, vol. 6, pg. 411
4. Galgali P., Varma A.J., Puntambekar U.S., Gokhale D.V., *Chem. Commun.,* . 2002, 2884-2885
5. *Encyclopedia of Polymer Science & Engineering*, Mark H.F. and Bikales N.M., vol.8, John Wiley & Sons, New York, 1987, page 152.
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10. Silverstein R.M., & Webster F.X.,*Spectrometric Identification of Organic Compounds*, sixth edn., John- Wiley & Sons, New York, 1998.
11. Kobayashi K. & Sumitomo H., *Macromolecules*, 1980, 13, 234- 239

5.5. Appendix 5:

Synthesis of sugar derivatives:

1,2-5,6 Diisopropylidene D- glucose:

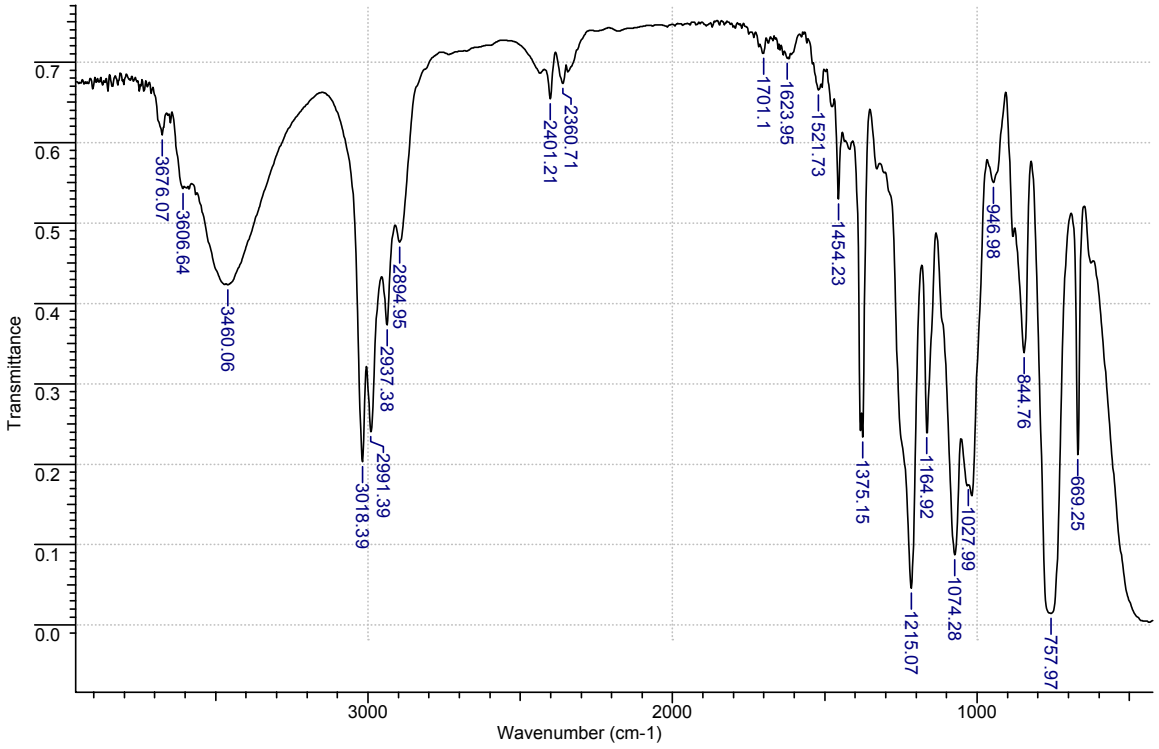
This derivative was synthesized by two different methods

Method 1:

Ref. (Otto Th. Schmidt, Methods in Carbohydrate Chemistry, vol.2,Whistler R.L., Wolfrom M.L., Academic Press, New York, 1963, pg. 320)

D- glucose was dried under vacuum at ~50°C overnight. Acetone was first distilled, refluxed over $KmnO_4$ for 7 hours, distilled, reluxed over K_2CO_3 for 4 hours, redistilled to obtain dry acetone. $ZnCl_2$ was heated at 400- 425°C in a furnace just before use.

To an efficiently stirred suspension of D- glucose (50g) in anhydrous acetone (340 mL) was added anhydrous zinc chloride (40g) followed by 2.5g of orthophosphoric acid (85%). This mixture was stirred at room temperature for 30 hours. The undissolved D-glucose (26.5g) was collected and washed with a little acetone. The filtrate and the washings were cooled and made slightly alkaline with sodium hydroxide (29g in 29 mL water). The insoluble inorganic material was removed by filtration and washed with acetone. The almost colorless filtrate and washings were concentrated under reduced pressure, the residue was diluted with 50mL of water, extracted with chloroform (3× 50mL). The combined chloroform extracts were washed with a little water and concentrated to obtain a white crystalline residue of crude diacetone D- glucose (22g)



FTIR spectrum of recrystallized Diacetone D- glucose

Appearance of a peak at 1375 cm^{-1}, characteristic of a gem dimethyl group, confirms the formation of the product.

Method 2:

Ref. (Methods in Carbohydrate Chemistry, vol., 19, pg. 319,)

D- glucose was dried under vacuum at ~50°C overnight. Acetone was first distilled, refluxed over $KmnO_4$ for 7 hours, distilled, reluxed over K_2CO_3 for 4 hours, redistilled to obtain dry acetone. Sodium carbonate was dried in the oven overnight and activated charcoal was dried for 4 hours in the oven.

D-glucose (5.05g) was suspended in dry acetone (150 mL). Concentrated sulfuric acid was added to the above suspension under stirring. The reaction mixture was stirred for 24 hours at room temperature under in presence of nitrogen atmosphere. The color of the solution turned yellow initially. The color darkened progressively. The sugar remaining undissolved after 24 hours (350mg) was filtered off. Anhydrous sodium carbonate (40g) was added to the filtrate to neutralize the acid. After filtering

off the sodium sulfate, the filtrate was refluxed in presence of sodium carbonate (15g) and activated charcoal (0.72g) for 1 hour. It was then filtered. The solvent was removed under reduced pressure at 55- 60°C. The residue was extracted with a little amount of ether and precipitated in pet- ether to obtain 1.46g of the crude product (crop 1). The supernatant was cooled to get product (crop 2) (m.p. 95°C).

Recrystallization from chloroform- petether (1:2) gave a pure product (m.p. 106°C, lit. m.p. 105- 109°C)

Diacetone D-glucose

2 Mar 2004

Acquisition Time (sec)	2.0480	*Comment*	GPP-A-1/CDCL3		*Date*	07/06/91 11:39:59	
Frequency (MHz)	200.13	*Nucleus*	1H	*Number of Transients* 512	*Original Points Count*	8192	*Points Count* 8192
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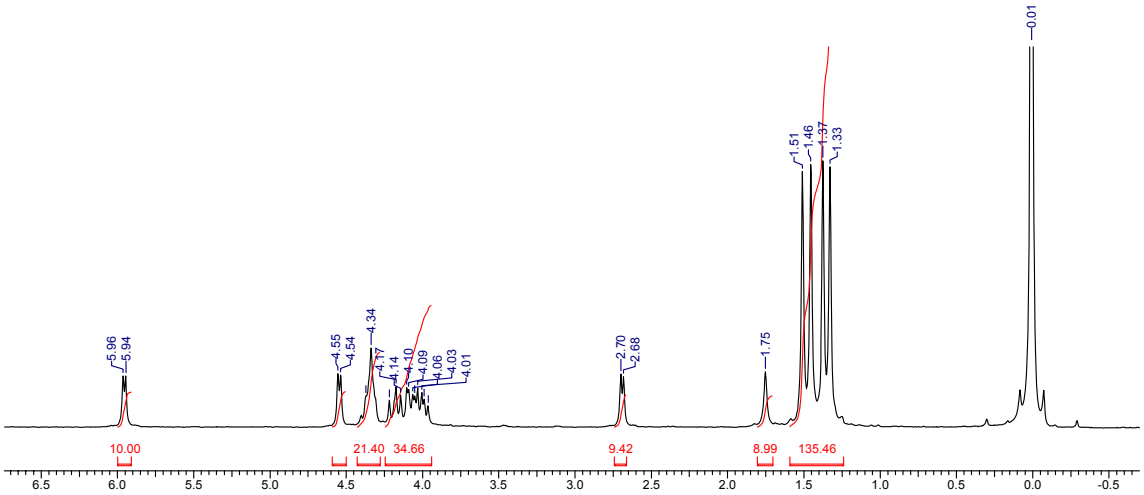

No.	(ppm)	Height	No.	(ppm)	Height
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3	1.37	0.182	16	4.10	0.027
4	1.46	0.180	17	4.14	0.022
5	1.51	0.175	18	4.17	0.028
6	1.75	0.038	19	4.22	0.018
7	2.68	0.035	20	4.34	0.054
8	2.70	0.036	21	4.37	0.021
9	3.96	0.015	22	4.54	0.036
10	3.99	0.019	23	4.55	0.036
11	4.01	0.024	24	5.94	0.035
12	4.03	0.027	25	5.96	0.035
13	4.05	0.021			

NMR spectrum of 1,2 :5,6-diisopropylidene- D- glucose

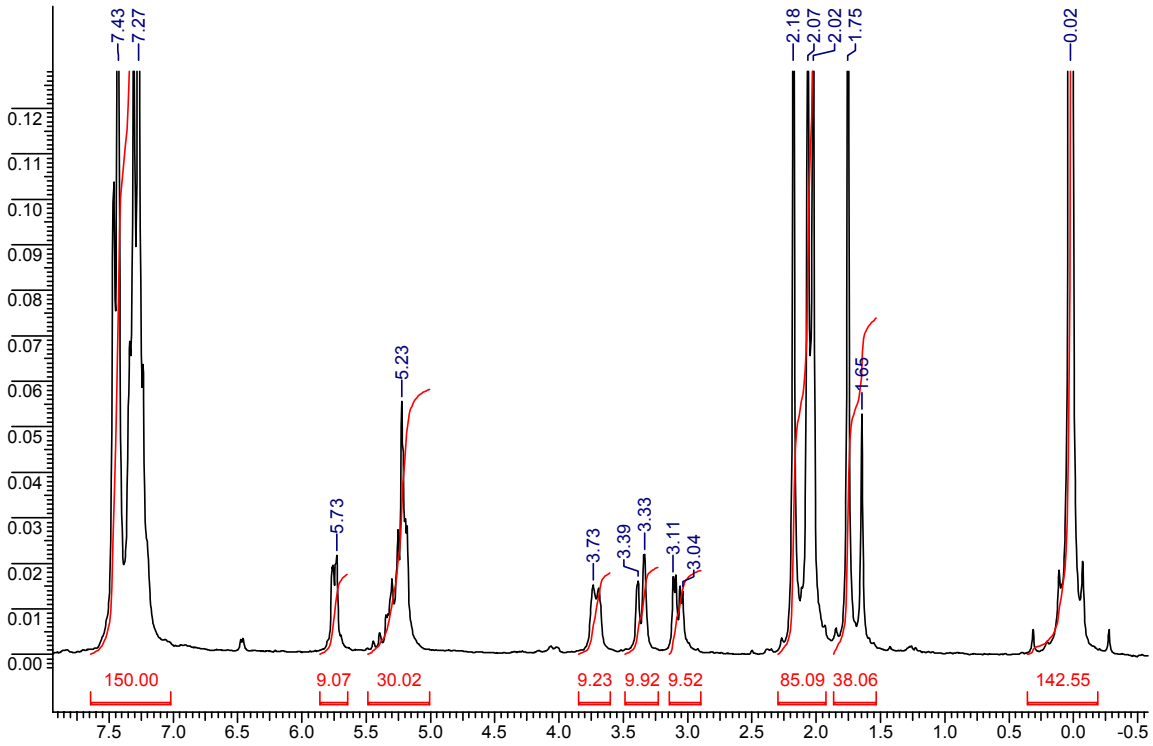
The NMR spectrum shows two doublets at 1.35 and 1.48 δ due to the isopropylidene group. The integration of the isopropylidene protons to the sugar protons is 0.6, whereas the theoretical value is 0.58.

1,2,3,4- Tetra acetyl D-glucose: this derivative was synthesized in two steps

Step 1: Tritylation and acetylation of D- glucose

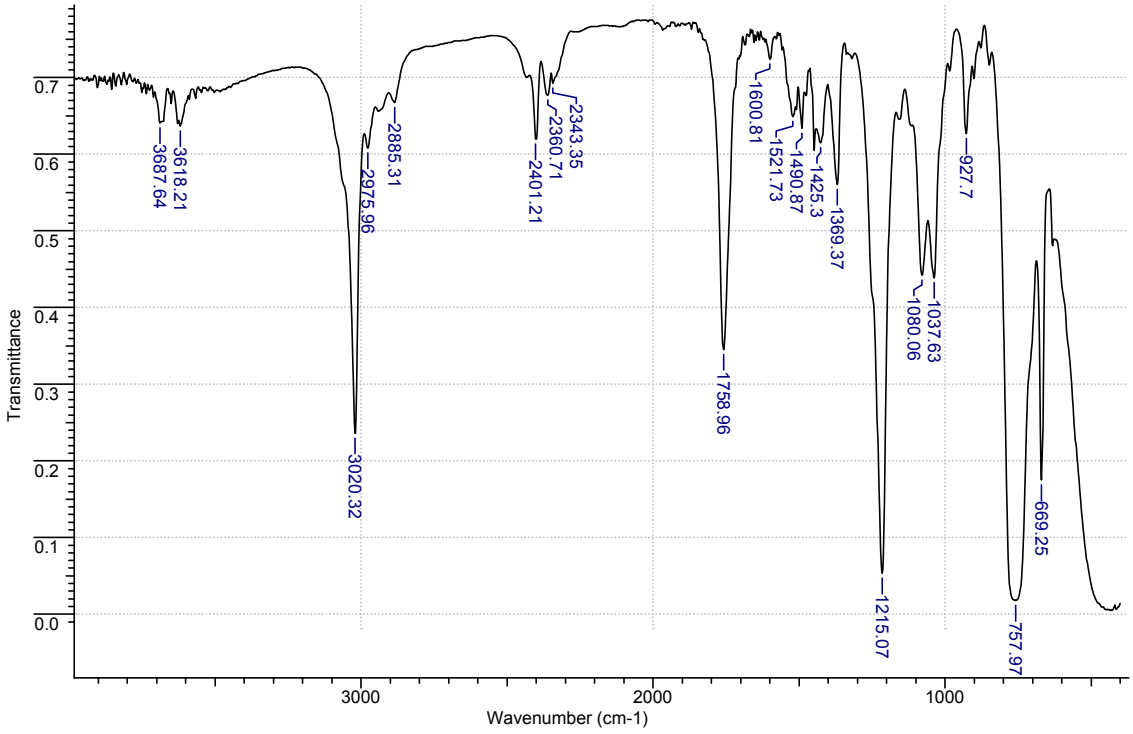
Ref. (Methods in Carbohydrate Chemistry, Whistler R.L., & Bemiller J.N., vol. 6,1972, pg. 411)

Anhydrous D- glucose (9g) and trityl chloride (15g) were stirred in 50 mL of dry pyridine at 40°C for 16 hours. The TLC (chloroform- methanol 5:1) showed a major spot of the product and a very minor spot of the reactant. At this stage the reaction mixture was cooled to approximately 25°C and acetic anhydride (60 mL) was added and the solution was stirred for 2 hours. TLC (benzene- ethyl acetate 6:1) showed completion of the reaction. The reaction mixture was poured into 2 litres of vigorously stirred ice- water. Stirring was continued for 4 hours after which the reaction mixture was extracted with 3x 200 mL chloroform. The combined chloroform extracts were washed three times with 250 mL water, dried over anhydrous sodium sulfate, chloroform distilled off under vacuum at 44°C. Toluene was added and distilled off to remove pyridine. This procedure was repeated once more. A faint colored sticky mass was obtained. Ethanol (100mL) was added to this substance to obtain a white crystalline compound. The product was filtered, washed with ether, filtered to obtain 14.0 g of the crude product (m.p. 157.4°C). This product was again crystallized from 95% ethanol to obtain 13.4g of the product.



NMR of tritylated and acetylated product of D-glucose

The peak at 7.0- 7.5 δ is attributed to the trityl group. The peks between 1.6- 2.2 δ are due to the acetyl group and the peaks between 3.0- 5.8 δ are due to the sugar protons. The observed integration values are consistent with that of the thereotically calculated values (observed 0.79; calculated is 0.79)



FTIR spectrum of tritylated and acetylated product of D-glucose

The FTIR spectrum shows complete disappearance of the sugar hydroxyl stretching peak, thereby, indicating completion of acetylation and tritylation, presence of peak at 1759 cm^{-1} indicating acetylation, presence of peaks at 1600, 758 and 699 cm^{-1} indicating tritylation. This confirms the conversion of D-glucose to 1,2,3,4- tetra acetyl 6-O-trityl D- glucose.

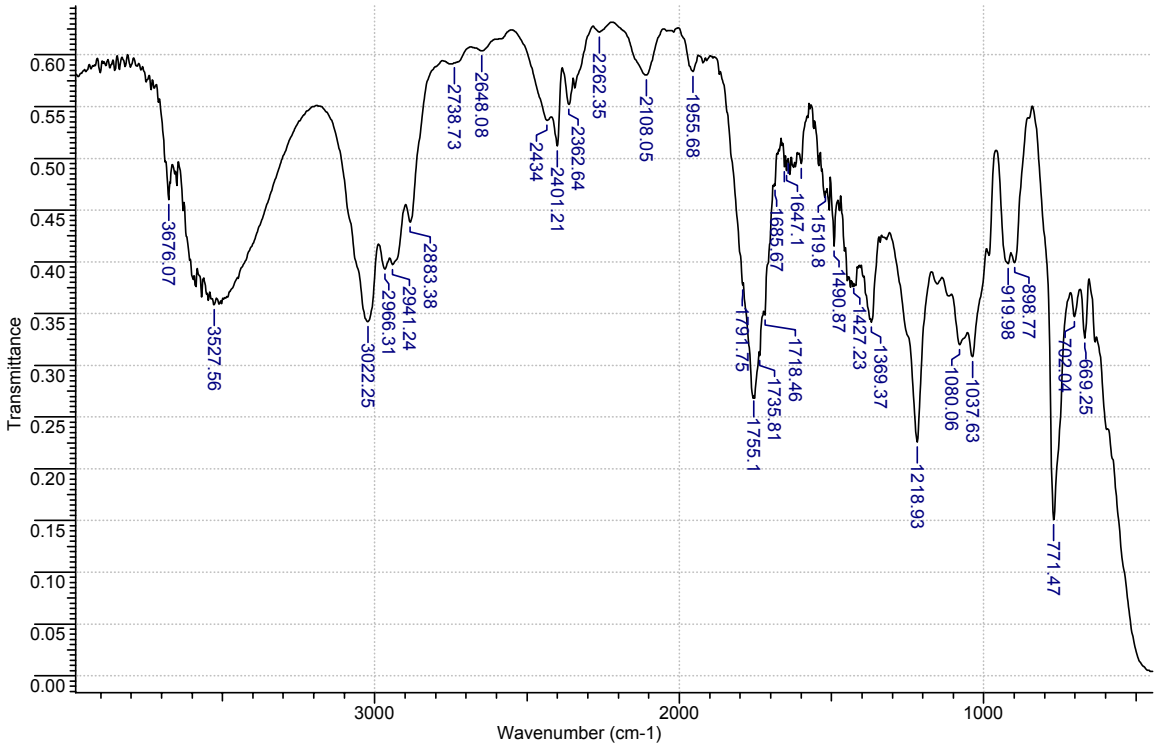
Step 2:

Detritylation of 1,2,3,4- tetra acetyl 6-O-trityl D- glucose:

(Ref. Methods in Carbohydrate Chemistry, Whistler R.L., & Bemiller J.N., vol. 6,1972, pg. 412)

1,2,3,4- tetra acetyl 6-O-trityl D- glucose (3g) was dissolved in 17mL of glacial acetic acid by warming. It was then cooled to 10-12°C. 1.5 mL saturated solution of HBr in glacial acetic acid was added to the above solution. The reaction mixture was shaken for 1 minute. The triphenyl methyl bromide formed was removed immediately by

suction filtration using celite 545 (6g) and the filtrate was at once poured into 100 mL of ice- water mixture. The tetraacetate was removed from the mixture by extraction with ~ 4x 25 mL chloroform. The combined chloroform extracts were washed with 40mL portions of cold water to remove acetic acid and then dried over anhydrous sodium sulfate. The filtrate was concentrated under reduced pressure at 40 °C. the residue was then crystallized from 4:1 v/v ether- chloroform (0.687 + g)

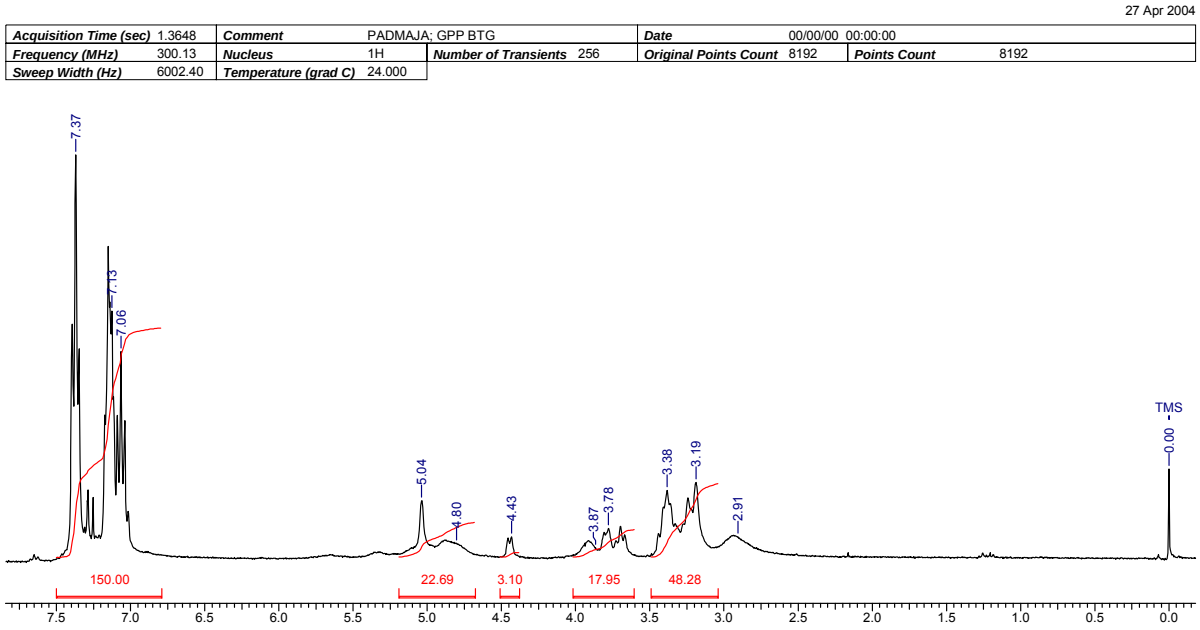


FTIR spectrum of 1,2,3,4-Tetra acetyl D- glucose

Disappearance of the peaks 757, 699 and 1600 cm^{-1}, and appearance of the hydroxyl peak at 3527 indicate the conversion of 1,2,3,4- tetra acetyl 6-O-trityl D- glucose to 1,2,3,4-Tetra acetyl D- glucose.

6-O-trityl D- glucose: D- glucose was dried under vacuum at for 5 hours. Pyridine was dried over calcium hydride and distilled before use.

Anhydrous D- glucose (9g) and trityl chloride (15g) were stirred in 50 mL of dry pyridine at 30- 32°C for 17 hours. The TLC (chloroform- methanol 5:1) showed a major spot of the product and a very minor spot of the reactant. The reaction mixture was poured into 500mL of ice- water. The product was extracted with dichloromethane 4-5 times. The combined extracts were washed with ice- cold water several times to get rid of most of the pyridine. Then the dichloromethane layer was dried over anhydrous sodium sulfate. Dichloromethane was removed under reduced pressure. Ethanol was added to the residue to obtain two crops of a white solid product.



No.	(ppm)	Height
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2	2.91	0.051
3	3.19	0.186
4	3.38	0.166
5	3.78	0.072
6	3.87	0.023
7	4.43	0.050
8	4.80	0.034
9	5.04	0.140
10	7.06	0.511
11	7.13	0.611
12	7.37	1.000

No.	Annotation	(ppm)
1	TMS	0.00

NMR spectrum of 6-O-trityl D-glucose

The NMR shows multiplet at 7.0- 7.5 δ formed due to tritylation of D-glucose. The theoretically calculated integration value of the aliphatic protons to aromatic protons was 0.46, whereas the observed integration value was 0.6.

Procedure for the blank and the hydrolysis reaction products:

Blank reaction of PSMAH in DMF solvent system with 4-DMAP as the catalyst:

Poly(styrene maleic anhydride) (4g) was dissolved in 40mL of dry DMF. To this solution was added 100mg of 4-DMAP. The reaction mixture was stirred at 52°C for 18 hours in presence of dry nitrogen.
Work-up: the reaction mixture was precipitated in brine, filtered, washed with water several times till free of chloride and dried.

Hydrolysis reaction of PSMAH using DMF as the solvent and 4-DMAP as the catalyst

Poly(styrene maleic anhydride) (10g) was dissolved in 150mL of DMF. 4-DMAP (500mg) and 10mL of distilled water was added to the above solution under stirring. The reaction mixture was stirred at 70°C for 17 hours and then the temperature was raised to 90 °C and stirred at this temperature for 2 hours. After 2 hours at 90°C, 10mL of distilled water was added to the reaction mixture. The reaction mixture was further stirred at this temperature for additional 3 hours.
Work-up: the reaction mixtures were precipitated in brine, filtered and washed with water several times till free of chloride and dried.

CHAPTER 6

Thermal Analysis of sugar- linked poly(styrene maleic anhydride)

Abstract:

The thermal properties of poly(styrene maleic anhydride) and sugar- linked poly(styrene maleic anhydride were studied by thermogravimetry in nitrogen atmosphere. Linking of carbohydrate moieties to poly(styrene maleic anhydride), rendered the polymer thermally less stable. After bacterial and fungal degradation, the thermal curves showed a similar trend. An FTIR analysis of the products of thermally treated samples showed that the sugar groups of the sugar- linked poly(styrene maleic anhydride) start to decompose at 150 ℃ and are fully lost by 250℃, which is the range for processing temperature of polystyrene base polymers. Hence the processing of these sugar- linked polymers appears to be difficult at higher temperatures. However, the processing may improve with the aid of suitable lubricants. Therfore, new derivatives will have to be designed to be more stable under processing conditions, or alternate processing methods will have to be designed, such as lower temperatures with high torque and low residence time. New additive packages to improve processability should also be investigated.

6.1. Introduction:

In our recent studies on biodegradable polymers, we synthesized biodegradable polymers by reacting functionalized synthetic polymer with carbohydrate moieties so as to obtain synthetic polymers with pendant sugar units. The percentages of sugars incorporated in the polymers were minute, in the range of 0.1- 4.5 weight percent. These polymers were very interesting since even incorporation of such minute quantities of sugar brought about enhanced rates of biodegradation as compared to unmodified poly(styrene maleic anhydride) (Galgali, Varma, Puntambekar, Gokhale, 2002). Therefore, it would be of great interest to know the differences in the thermal properties of polystyrenes, which incorporate sugar units in low quantities. Earlier, thermal studies have been reported on polymers with larger amounts of sugars, for example, significant quantities of sucrose acrylate graft copolymerized and

incorporated on the surface of poly(vinyl chloride) showed decrease in the thermal stability as compared to poly(vinyl chloride) (Rios & Bertorello, 1997). Poly(vinyl chloride) had an initial decomposition temperature of 248°C, whereas poly(vinyl chloride) grafted with sucrose acrylate had an initial decomposition temperature of 114°C. Copoly(ester amide)s containing L-arabinose units showed single or two-stage degradation pattern depending upon the percentage of the carbohydrate in the polymer, the onset of degradation being 200°C (Pinilla, Martinez, Mata, Galbis, 2002). With higher percentages of carbohydrates in the polymer, i.e. above 20 %, the TGA showed mainly a decomposition pattern with a minor third decomposition stage. The thermal degradation of a copolymer formed by reacting methacryloyl 1,2:3,4-di-O-isopropylidene-α-D-galactopyranose with ethyl acrylate showed a two-stage degradation pattern with the polymer being stable upto 160°C (Carneiro, Fernandes, Figueiredo, Fortes & Freitas, 2001). However, the thermal stability of the unprotected sugar units on the copolymer was not reported. The isopropylidene groups may have been the cause of the somewhat lower thermal stability. Syntheses and studies on such high sugar content polymer systems were carried out generally to obtain biocompatible polymers. However, by the incorporation of such large quantities of sugar, the intrinsic physical and chemical properties of the polymers are drastically altered. In our case, the objective was to attempt to retain the intrinsic physical and chemical properties of the polymer by incorporating minute quantities of sugars, while simultaneously drastically improving its biodegradability.

6.2. Experimental:

The starting polymer viz. poly(styrene maleic anhydride) (PSMAH) with 14 weight percent of maleic anhydride was obtained from Aldrich Co. The synthesis of the glucose, sucrose and lactose- linked poly(styrene maleic anhydride) polymers used in the present study and their bacterial and fungal biodegradation are reported elsewhere (Galgali, Varma, Puntambekar & Gokhale,2002; Galgali, Puntambekar, Gokhale & Varma, 2004) TG and DTG were carried out using a Seiko Instruments TG/ DTA 32 instrument equipped with a SSC 5100 Disk Station and SP-530 Plotter. The studies

were carried out in nitrogen gas at a nitrogen flow rate of 100ml/ min and heating rate of 10°C, in the temperature range of 25- 600°C. A Perkin Elmer FTIR Spectrum 1 spectrometer was used to obtain FTIR spectra. All the spectra were recorded in KBr in diffuse reflectance mode at a resolution of 2 $cm^{-1}$and the number of scans were 50.

6.3. Results and Discussions:

6.3.1. Thermogravimetry

It is reported that the alternating copolymer viz. poly(styrene- alt- maleic anhydride) shows a two-stage degradation pattern, the first stage involving decarboxylation. The decarboxylation occurs at lower temperatures between two adjacent anhydride moieties (Haussler, Weinhold, Albrecht & Zschoche, 1996; Jarm & Bogdanic, 1990). In case of the random copolymer successive maleic anhydride groups are far apart from each other to effect such complex formation, hence the degradation pattern showed only a single stage degradation. This shows that our poly(styrene maleic anhydride) copolymer contains the anhydride groups situated randomly along the polymer chain (Figure 1a).

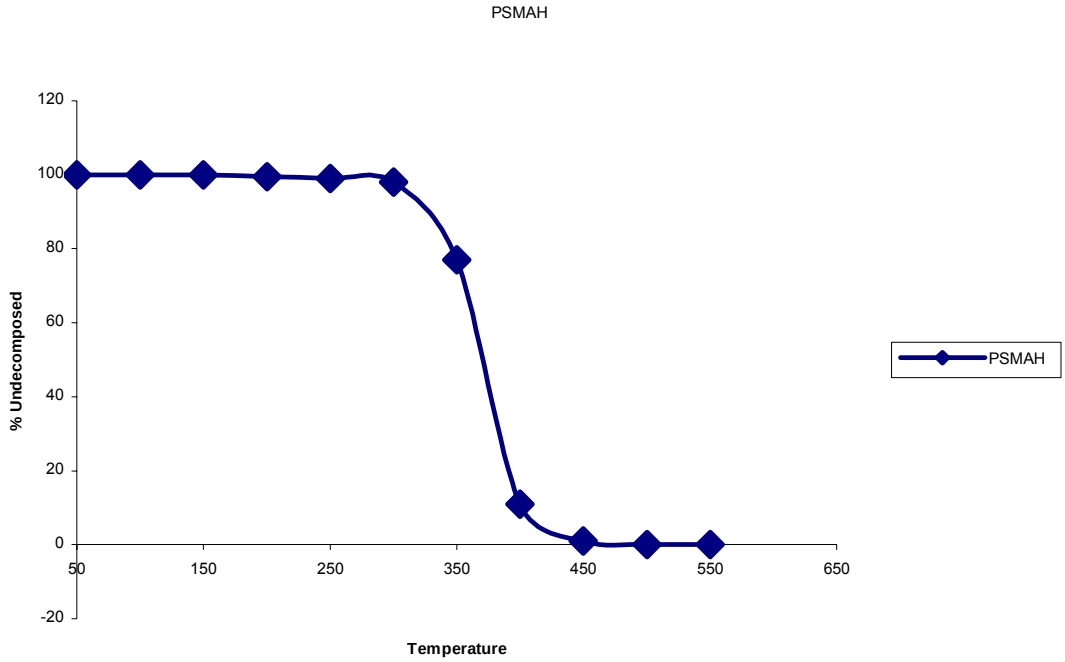

Figure 1a: Thermogravimetric curve of unmodified poly(styrene maleic anhydride) random copolymer with 14% MAH

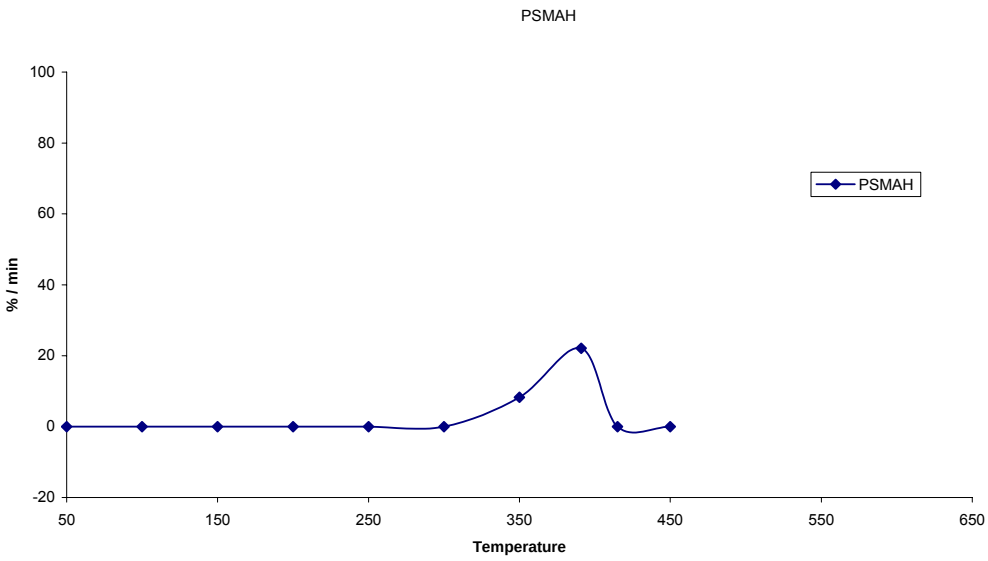

Figure 1b: DTG curve of unmodified poly(styrene maleic anhydride)

The polymer is stable upto 300°C (2% degradation), after which it degrades rapidly and completely leaving no behind residue (Figure 1a). The final degradation temperature is 450°C. The rate of degradation was maximum at 390°C as indicated by the peak maximum in its DTG curve (Figure 1b). The onset of degradation in case of poly(styrene maleic anhydride) is dependent on the content of maleic anhydride in the copolymer. More the content of maleic anhydride in the copolymer, lesser is its thermal stability (Baruah & Laskar, 1996). The onset of degradation for poly(styrene-alt-maleic anhydride) is reported to be 200°C (Urushizaki, Matsui, Sakamoto & Aida, 1975). The evolution of carbon dioxide starts at 260°C and its maximum release from the degrading polymer is at 385°C.

In contrast to the single stage decomposition of PSMAH, the sugar-linked polymers viz. glucose-/ sucrose-/lactose- (sugar content 0.1 to 4.5) linked poly (styrene maleic anhydride) show a two-stage decomposition pattern.

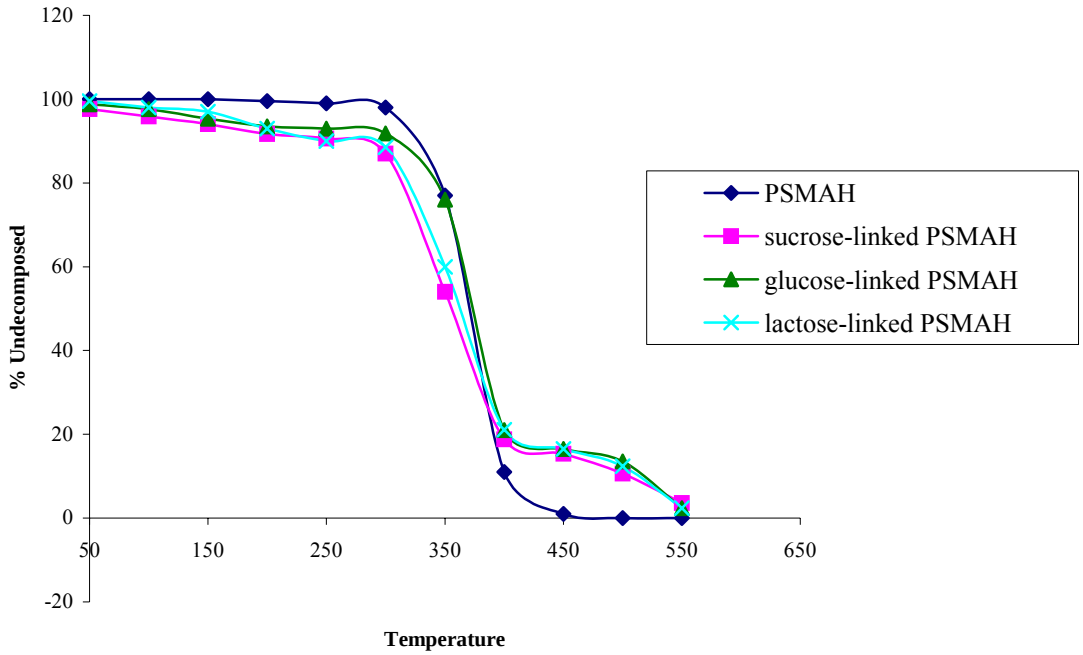

Figure 2: Overlapping TGA curves of sugar-linked poly(styrene maleic anhydride)s

While the poly(styrene maleic anhydride) has an onset of degradation of 300°C, the sugar-linked polystyrenes show initial instability even at 150°C, and at 300°C there is a weight loss of over 10%. Following this, the degradation curve merges with the PSMAH till a temperature of 400°C, after which sugar-linked polymers are more stable than the PSMAH. However the final degradation temperature is the same (550°C) for both PSMAH as well as the sugar- linked PSMAH. No residue is left behind in all cases.

The TGA pattern of the blank reaction is similar to that of poly(styrene maleic anhydride) at lower temperatures. The product of the blank reaction showed a two-stage degradation pattern, with the onset of the first stage of degradation at 300°C. The TG curves of poly(styrene maleic anhydride) and the product of the blank reaction were similar upto 350°C, beyond which the blank reaction product degraded slowly, which involved a small second stage.

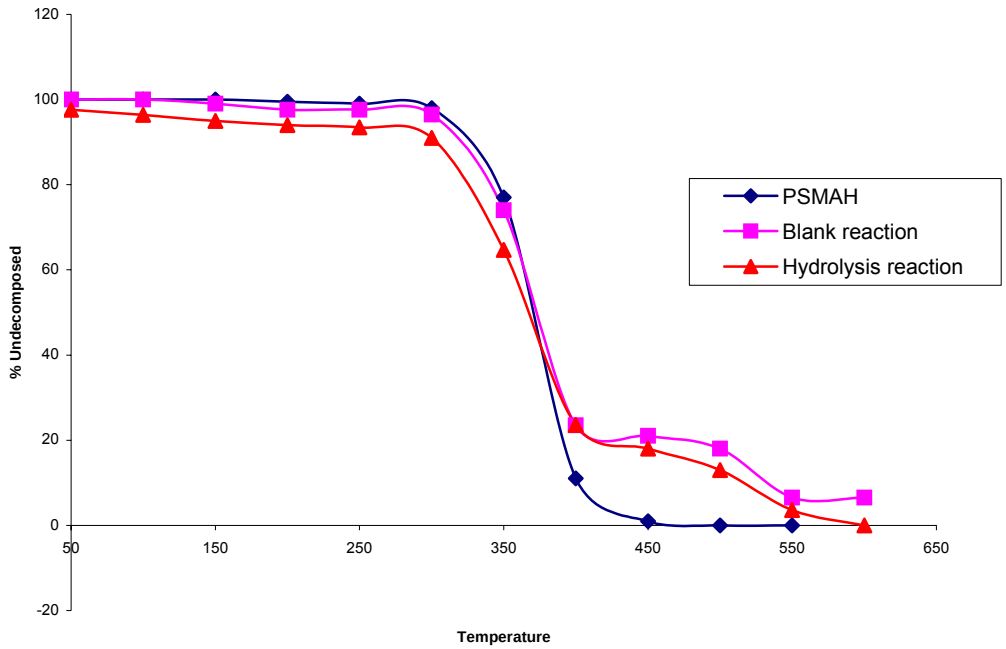

Figure 3: Overlapping TGA curves of poly(styrene maleic anhydride), its hydrolysed product and the blank reaction product.

Figure 3 shows the comparative thermal stability curves of PSMAH and its hydrolyzed derivatives. The similarity in the TGA curve of the anhydride hydrolyzed PSMAH (Figure 3) and the sugar- linked PSMAH (Figure 2) shows that after the decomposition of the sugars in the latter polymers, the anhydride groups are ring opened and give similar degradation products as the anhydride hydrolyzed PSMAH. Thus the percentages of the sugars incorporated into the polymer do influence their degradation pattern. This rationale is seen more clearly from a study of TGA curves of glucose- linked poly(styrene maleic anhydride) polymers with different grafting percentages (0.15%, 1.4% and 2.6%) (Figure 4). It is observed that more the percentage of sugar incorporated into the polymer, more is the rate of degradation in the first stage. Since the overall grafting percentages of the sugars are very less, the differences are less prominent in Figure 4.

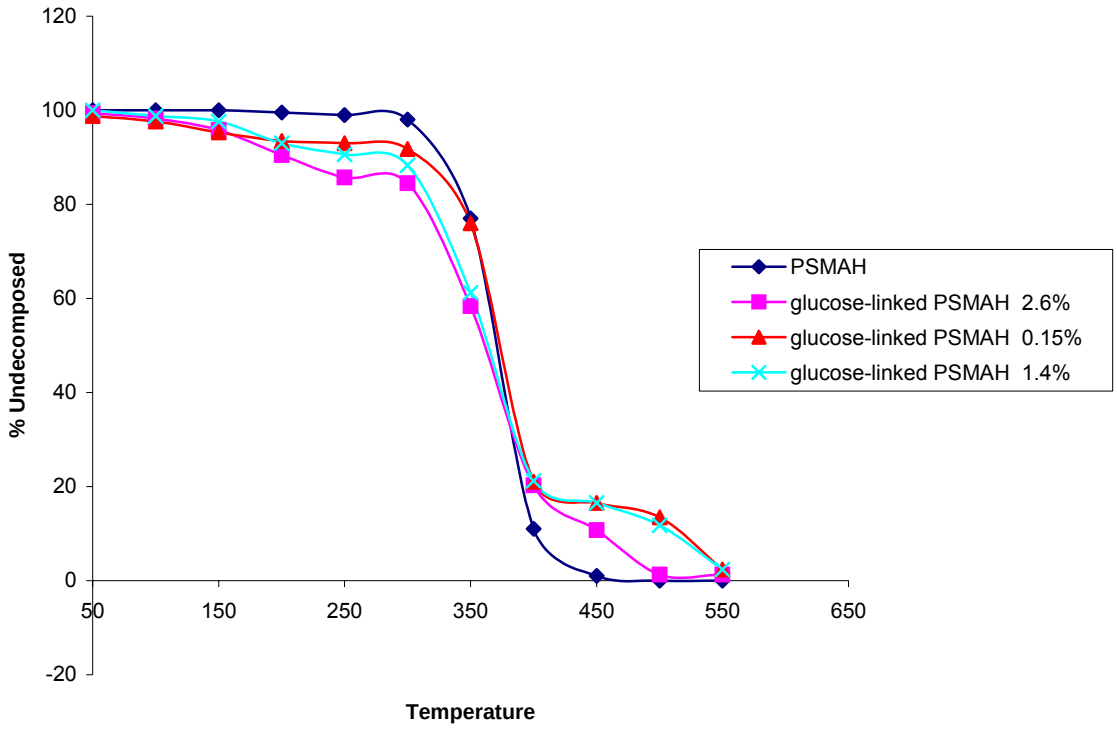

Figure 4: Overlapping TGA curves of glucose-linked poly(styrene maleic anhydride)s with different grafting percentages.

The sugar-linked poly(styrene maleic anhydride)s degraded by micro-organisms (both bacteria viz. Serratia marscecens, Pseudomonas sp. and Bacillus sp. and fungi viz. A.niger, Pullularia.pullulans, Trichoderma sp. and P.ochro-chloron) (Galgali, Varma, Puntambekar & Gokhale, 2002; Galgali, Puntambekar, Gokhale & Varma, 2004) show little difference in their thermal degradation pattern in the first stages. Generally, the residue contents in cases of all biodegraded polymers are significantly higher than the non-biodegraded sugar- linked PSMAH (Figures 5,6,7,8 and table 1). Apparently, the final products of thermal degradation of the PSMAH, sugar- linked PSMAH, and the biodegraded sugar- linked PSMAH are all different. Further studies to identify the structures of the products of thermal degradation of such polymers will be carried out. In conclusion, we have successfully proved a methodology of preparing a series of polymers based on sugar- incorporated PSMAH which do not significantly alter the thermal properties of the base polymer in the temperature range needed to process such polymers for molding applications (200- 300°C), while at the same time achieving the desired goal of biodegradability and making it a "green" polymer. More work is being done to further improve the thermal characteristics of such polymer systems.

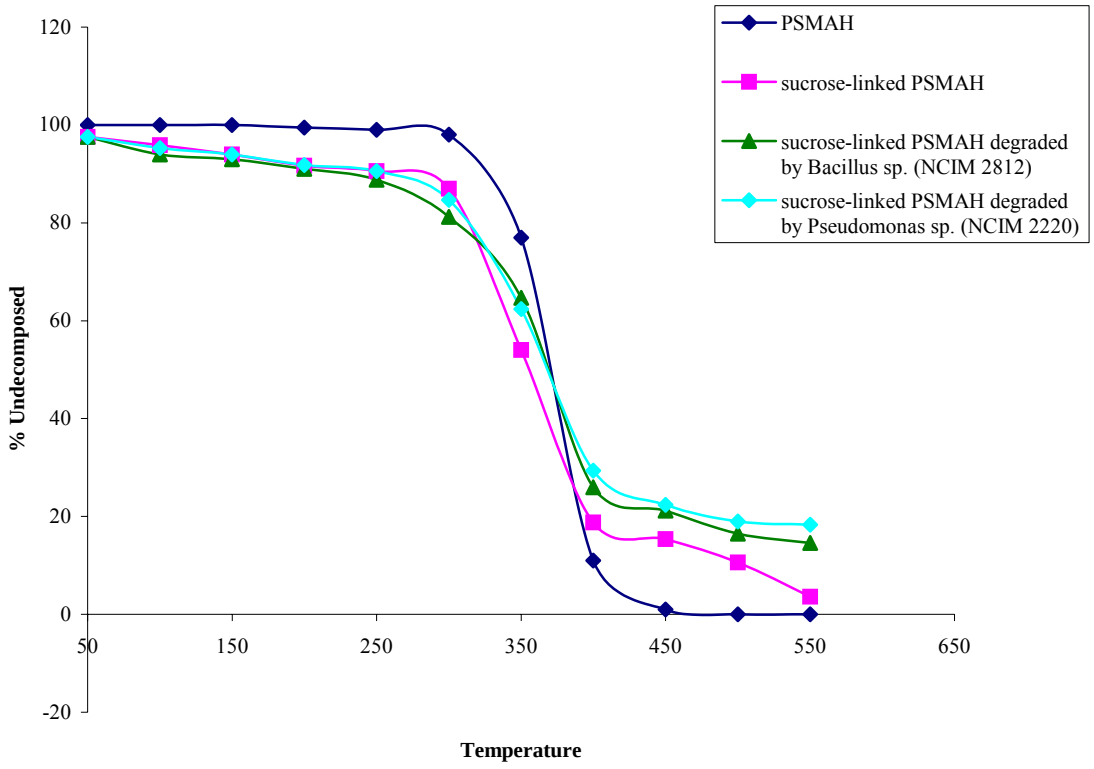

Figure 5: Overlapping TGA curves of sucrose-linked poly(styrene maleic anhydride)s and its bacterial degraded products.

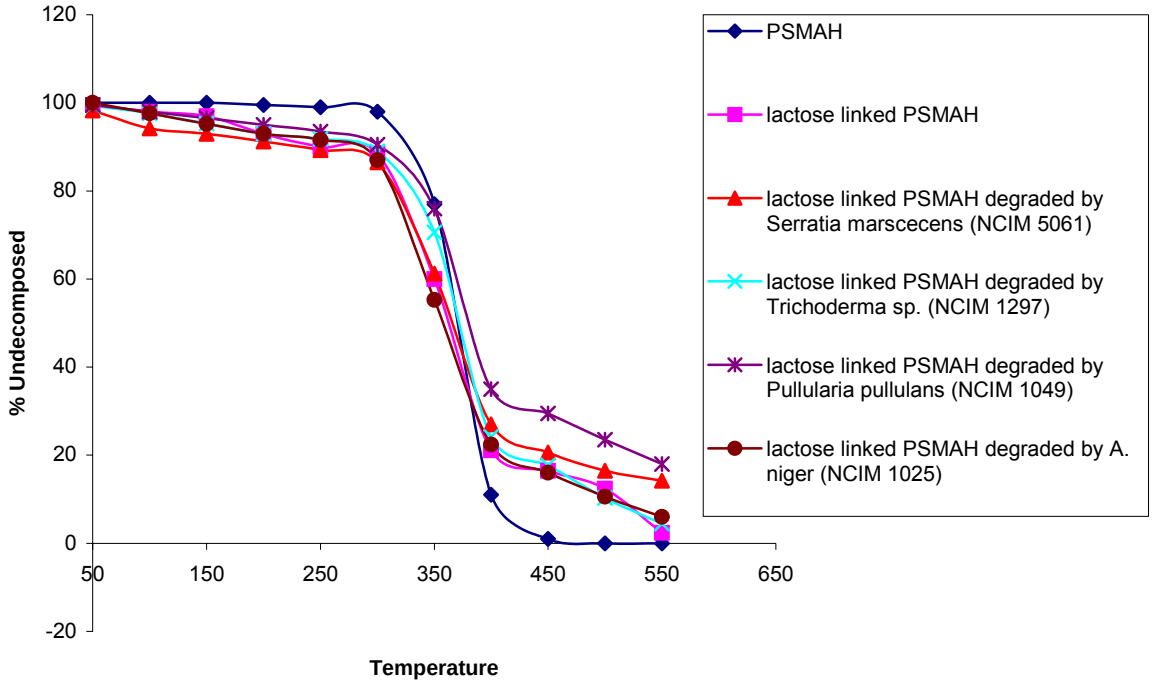

Figure 6: Overlapping TGA curves of lactose-linked poly(styrene maleic anhydride)s and its bacterial and fungal degraded products.

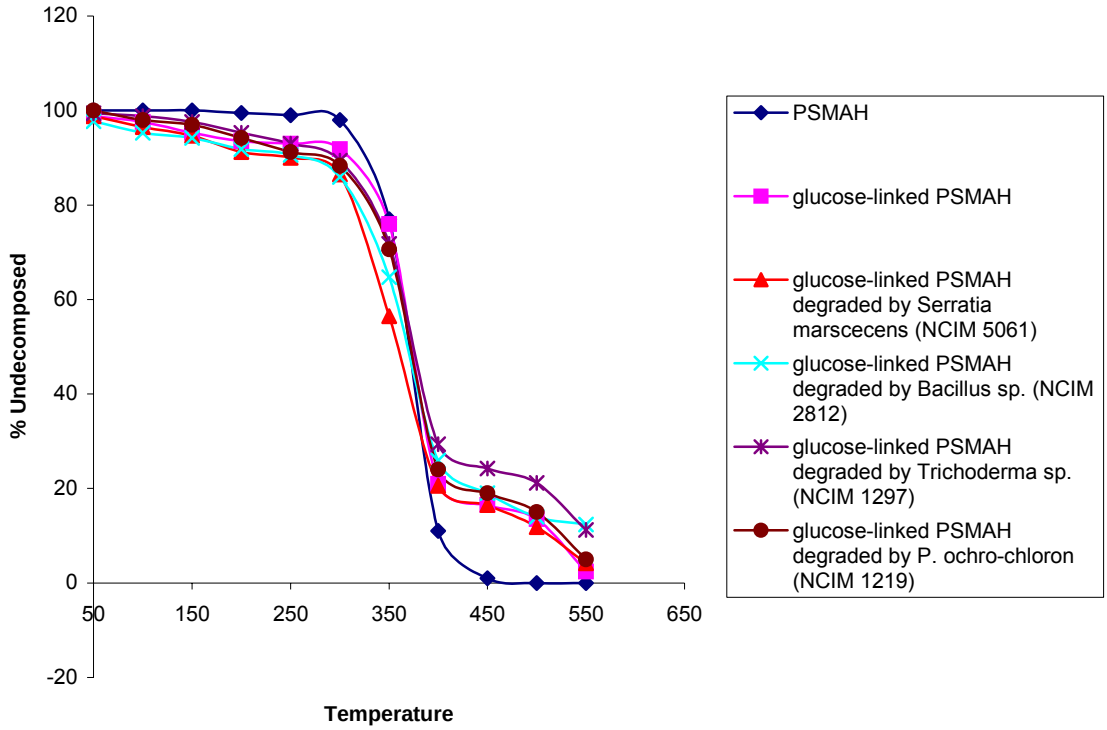

Figure 7: Overlapping TGA curves of glucose-linked poly(styrene maleic anhydride)s and its bacterial and fungal degraded products.

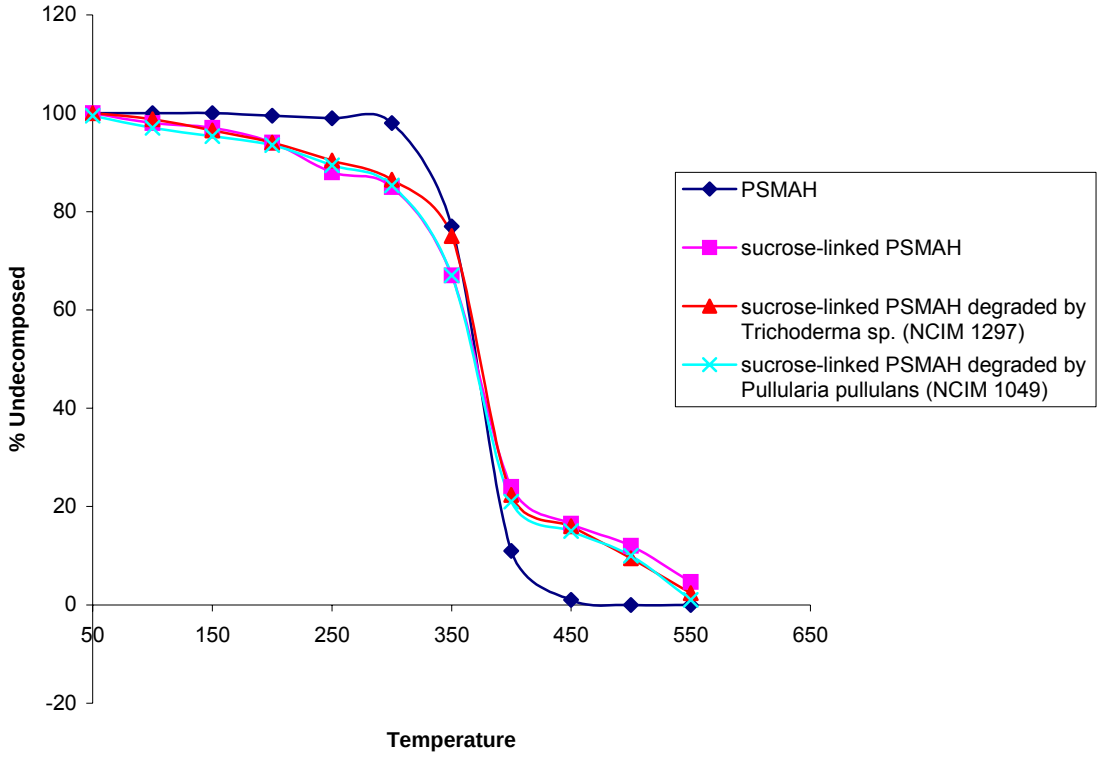

Figure 8: Overlapping TGA curves of sucrose-linked poly(styrene maleic anhydride)s and its fungal degraded products

All the biodegraded poly(styrene maleic anhydride)s show a single-stage degradation pattern. All the biodegraded polymers start rapidly degrading from 250°C to about 400°C. The residue contents in all the biodegraded polymers are reasonably high as compared to their undegraded counterparts.

6.3.2. FTIR characterization of the thermally treated products:

The changes occurring in the polymers at various temperatures were interpreted and correlated with the help of the FTIR spectra of the polymers treated thermally at temperatures of 150°C, 250°C and 350°C for 30 minutes in a furnace. The FTIR spectra of PSMAH and its thermally treated products (Figure 9) show that there are

no changes in the spectra upto 250°C, whereas the only change observed at 350°C is the formation of a very weak band at 1695 cm^{-1}, probably arising due to formation of new carbonyl groups during the degradation of the polymer. The polymer, on the whole appears to be reasonably stable upto 350°C. The FTIR spectra of the product of the blank reaction of PSMAH and its thermally treated products show changes similar to that of PSMAH (Figure10).

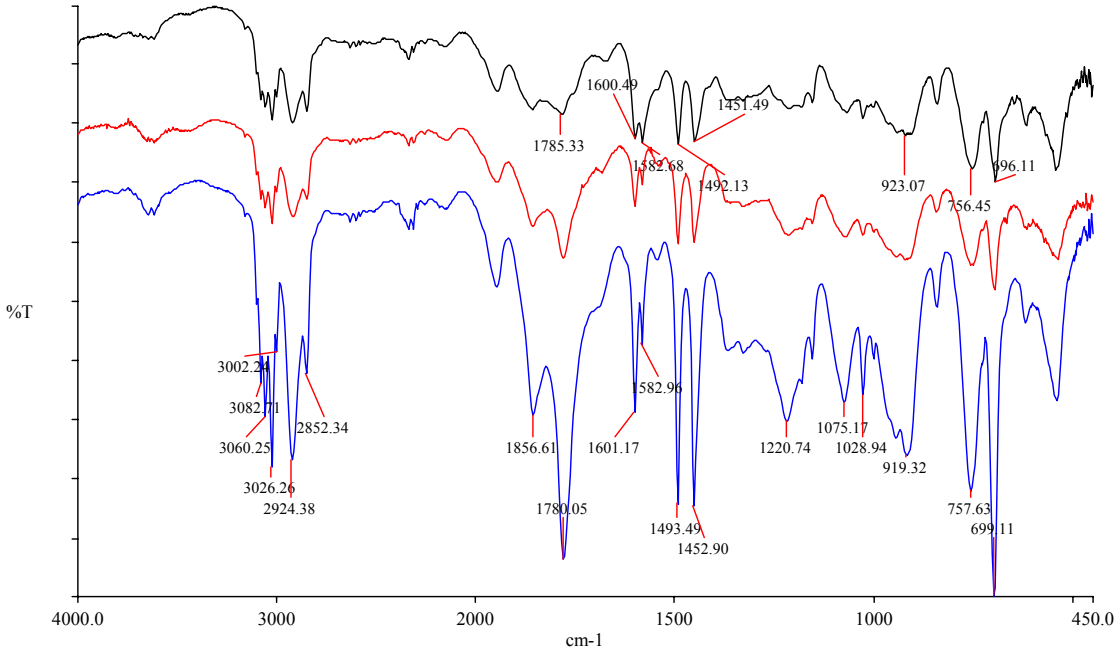

Figure 9: FTIR spectra of PSMAH (14 weight % MAH) and its thermally treated products (top to bottom: PSMAH at RT, 250°C and 350°C)

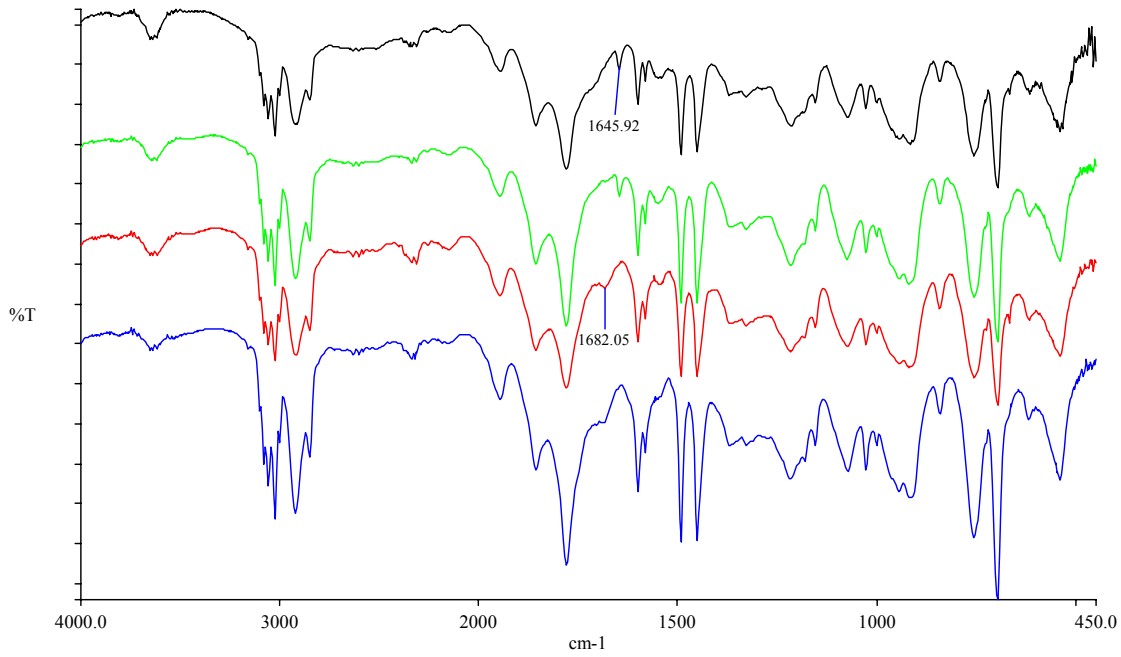

Figure 10: FTIR spectra of blank reaction product at various temperatures (top to bottom: RT, 150°C, 250°C and 350°C)

The FTIR spectra of the glucose linked PSMAH and its thermally treated products indicate several changes during the heating process (Figure 11). At 150°C there is a little decrease in the OH stretching frequency at 3400 cm^{-1} and in the intensity of the acid and ester carbonyl peak, with a shifting of this peak from 1729 to 1736 cm^{-1} and a simultaneous increase in the intensity of the anhydride carbonyl at 1780 and 1857 cm^{-1}. This is also accompanied by changes in the region of 900- 1250 cm^{-1}, with little increase in the intensity of the peaks at 920, 1074, 1029 and 1217 cm^{-1}. At 250°C, the spectrum shows complete disappearance of the acid and ester carbonyl peak with a sharp increase in the anhydride carbonyl peak at 1780 and 1857 cm^{-1}. The spectrum of the glucose- linked PSMAH at 250°C is similar to the spectrum of unreacted PSMAH at room temperature (Figure 12).

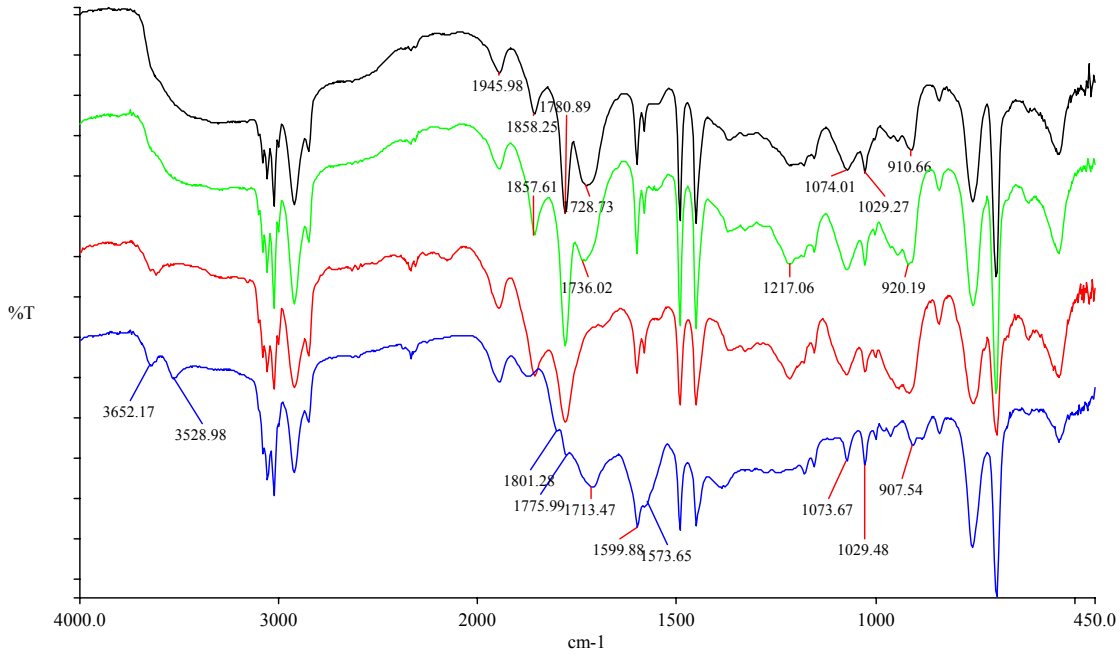

Figure 11: FTIR spectra of glucose-linked PSMAH and its thermally treated products (top to bottom: RT, 150°C, 250°C and 350°C)

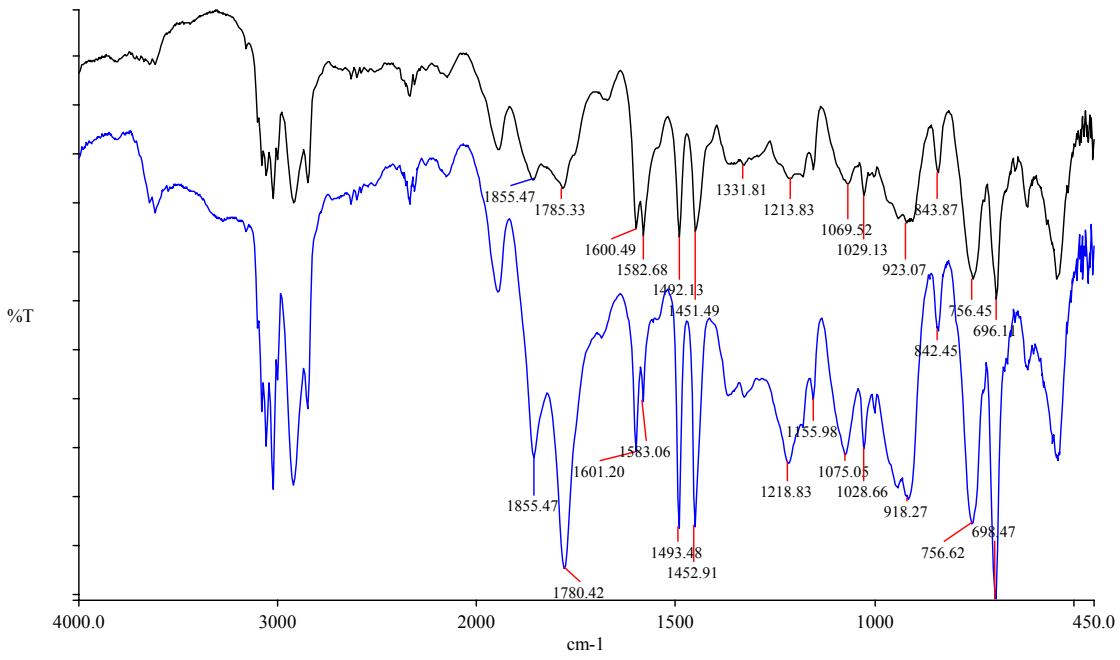

Figure 12: Overlapping FTIR spectra of (PSMAH at RT, top) and (glucose- linked PSMAH at 250 °C, bottom)

Hence the process occurring at 250°C could be just the cleavage of the ester bond of the sugar moiety accompanied by ring closure (anhydride formation), which could be either intramolecular or intermolecular (ref).

The FTIR spectrum of the hydrolysis product of PSMAH at 150°C shows reduction in the intensity of the diacid carbonyl peak with the peak shifting to a higher frequency from 1713 to 1718 cm^{-1} with the formation of an anhydride carbonyl (Figure 13). Peaks at 1215, 1076 and 1029 cm^{-1} also appear in the spectrum. At 250°C, the peak at 1717 cm^{-1} vanishes, with retention of a small peak at 1704 cm^{-1}. There are no changes in the IR spectrum of the sample at 350°C as compared to the spectrum at 250°C.

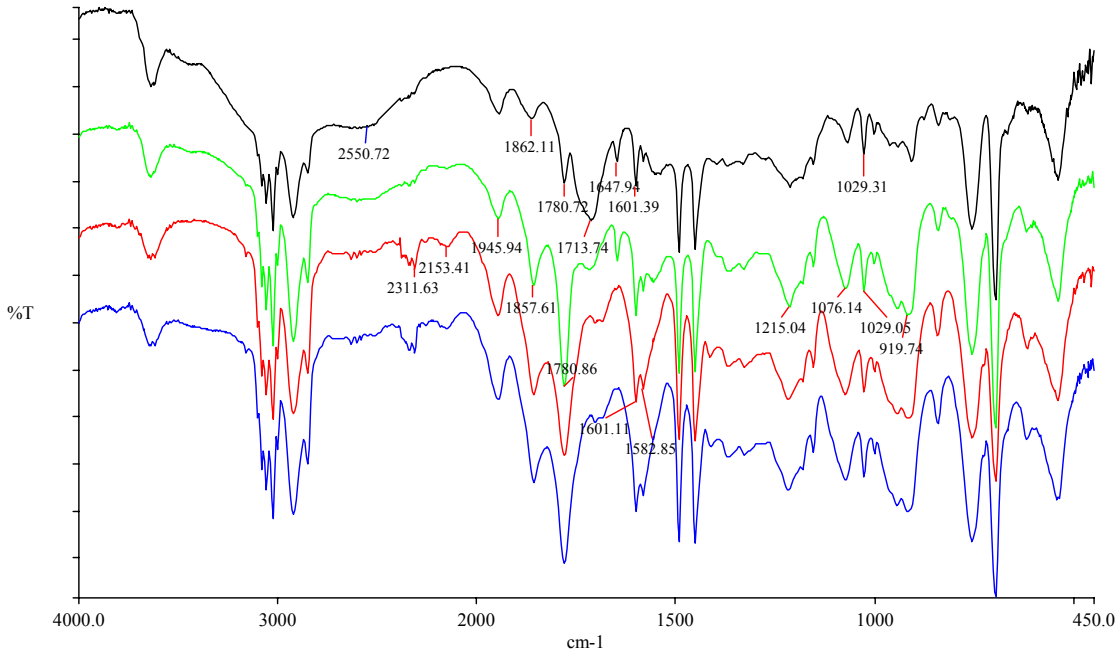

Figure 13: FTIR spectra of hydrolyzed product of PSMAH at various temperatures (top to bottom: RT, 150°C, 250°C and 350°C)

Table 2: Thermogravimetric analysis data of poly(styrene maleic anhydride) and its sugar grafted derivatives.

Polymer	Percent Decomposed at Temperatures (°C) 100	200	250	300	350	400	500
PSMAH	0.0	0.5	1.0	2.0	33.0	89.0	100.0
Glucose-linked PSMAH	1.8	9.5	14.3	15.5	41.6	79.7	98.7
Sucrose-linked PSMAH	2.4	3.6	10.8	14.4	27	78.8	89.4
Lactose-linked PSMAH	1.2	7.0	8.2	10.0	31.7	79.4	94.1
Xylose-linked PSMAH	1.2	5.9	7.6	9.4	29.4	78.2	87.0
Galactose-linked PSMAH	1.7	7.0	8.8	10.0	34.0	78.8	88.8
Glucose-linked PSMAH acetylated	0.6	10.6	14.7	17.6	29.4	90.5	98.2
Glucose-linked PSMAH lauroylated	0.0	8.2	11.7	21.2	33.0	83.5	97.0
Lactose-linked PSMAH acetylated	0.6	14.0	18.8	22.2	25.0	84.0	94.0
Lactose-linked PSMAH hexonylated	0.6	10.6	14.0	15.3	33.0	93.0	100.0
Lactose-linked PSMAH lauroylated	0.6	3.5	6.0	14.1	37.6	91.7	98.8

PSMAH: poly(styrene maleic anhydride)

6.4. Conclusion:

From the above discussion, it is observed that the chemical linking of sugars onto the polymer, viz. poly(styrene maleic anhydride) makes the polymer less stable. There appear to be no appreciable changes in the thermal degradation profile of the sugar-linked polymers and their biodegraded products, after one month treatment with bacterial and two months with fungal cultures. The FTIR spectra of the thermally treated sugar- linked poly(styrene maleic anhydride) polymers indicate that the sugar molecules start to fall off from the polymer at 150 ℃ and are fully lost by 250℃. This indicates that normal processing conditions cannot be employed for the processing of these polymers. The processing conditions have to be optimized by reducing the processing time and by adding lubricants. Also, newer derivatives of sugar linked polymers can be designed which may have superior thermal properties.

6.5. References:

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CHAPTER 7

Biomineralization of the sugar-linked poly(styrene maleic anhydride)

7.1. Introduction:

The term biodegradation involves bioassimilation and biomineralization. Bioassimilation involves ingestion of polymer fragments by microorganisms. Biomineralization is a process in which the organic material or the polymer is completely broken down and converted to carbon dioxide, methane, water, salts, nitrogen and biomass, so that all the elements from the polymer re- enter the natural geochemical and microbial cycles. There are several standards to assess the biodegradation of polymers like the ISO 472:1988, ASTM (D20.96 proposal) and DIN 103.2. However these methods fail to classify them in terms of the rates of biodegradation of the polymer and also do not consider their ultimate fate (Ref: Amass W., Amass A. and Tighe B., Polymer International, 47, 89144 (1998)). The test methods included in the OECD guidelines, which are considered to be appropriate tests, include the modified Strum test, the modified MITI test and the closed bottle test (OECD 301B, C, D). All the tests are carried out under aerobic conditions in aqueous medium wherein, the polymer provides the sole source of carbon for the microorganisms. Any polymer, which degrades to 60 % of the total carbon content, is considered to be readily degradable.

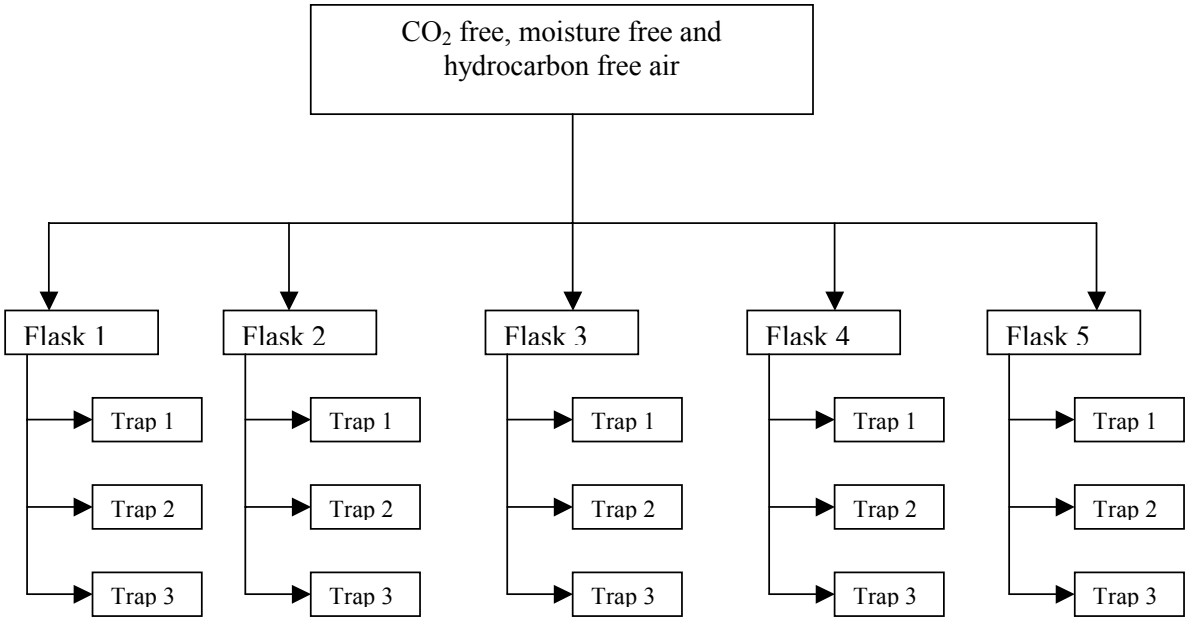
The extraordinary abundance of microorganisms available consists of both autotrophic and heterotrophic bacteria, which share a symbiotic relationship. The autotrophs are capable of utilizing carbon dioxide from the atmosphere directly, whereas the heterotrophs depend on the autotrophs for their carbon source. Hence the carbon dioxide evolved from the degradation of the polymer thus enters the geochemical cycle.

Principle of the Organisation for Economic Co-operation and Development (OECD) method for determination of biodegradation by biomineralization is based on the measuring the amount of carbon dioxide evolved from the sample under consideration, in which the sample is the sole source of carbon for the micro-organisms.

7.2. Experimental:

7.2.1. Experimental set- up:

Carbon dioxide, hydrocarbon and moisture- free air was distributed into five one litre capacity reactor flasks. The reactor flasks were constantly stirred by magnetic stirring bars. All the flasks contained 250 mL of the minimal medium and the inoculum. Flask-1 contained only the minimal medium without any carbon source. In addition to this, Flask-2 contained unmodified poly(styrene maleic anhydride) (PSMAH), Flask-3 contained glucose- linked PSMAH, Flask-4 contained sucrose- linked PSMAH and Flask-5 contained lactose- linked PSMAH. The evolved carbon dioxide was trapped in barium hydroxide traps (0.0125M solution) (three traps arranged consecutively for each flask). The first trap of each set/ flask was removed for titration and the second trap was shifted in place of the first trap, the third was shifted to the place of the second one and a new trap was placed in place of the third trap. 25 mL of barium hydroxide solution withdrawn from the middle portion of each trap was titrated with 0.05M HCl solution using phenolphthalein indicator. Standardization of ~0.05M HCl was carried out using ~0.05M NaOH solution. NaOH solution (~0.05 M) was standardized using potassium hydrogen phthalate solution.



Flask 1: Minimal medium + inoculum

Flask 2: Minimal medium + inoculum + PSMAH

Flask 3: Minimal medium + inoculum + glucose- linked PSMAH

Flask 4: Minimal medium + inoculum + sucrose- linked PSMAH

Flask 5: Minimal medium + inoculum + lactose- linked PSMAH

All traps contain ~ 0.0125M Barium hydroxide solution

Figure 1: Schematic representation of the set-up for determination of evolved carbon dioxide

The photograph of the set-up is shown below:

Figure 2: Photograph showing the set-up for the determination of percent biodegradation by the OECD method.

7.2.2. Composition of minimal medium for 1 litre solution:

Potassium dihydrogen phosphate (KH_2PO_4) :85 mg
Dipotassium hydrogen phosphate (K_2HPO_4) : 217 mg
Disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$) :334 mg
Ammonium chloride (NH_4Cl) : 5 mg
Calcium chloride ($CaCl_2.2H_2O$) : 36.4 mg
Magnesium sulfate ($MgSO_4.7H_2O$) : 22.5 mg
Ferric chloride ($FeCl_3.6H_2O$) : 0.25 mg
pH: 7.4 ± 0.2

7.2.3. Solutions for the titration are as follows:

5M Sodium hydroxide solution: sodium hydroxide (200g) dissolved in distilled water to make 1litre solution
0.05M Barium hydroxide solution: barium hydroxide (1.71g) dissolved in distilled water to make a 500mL solution
0.05M Hydrochloric acid solution: hydrochloric acid (4.5 mL) diluted upto 1litre with distilled water
0.05M Sodium hydroxide solution: sodium hydroxide (1g) dissolved in distilled water to make 500mL solution
0.0125M Barium hydroxide solution: barium hydroxide (3.94g) dissolved in distilled water to make 1litre solution and then filtered to remove insoluble particles
Potassium hydrogen phthalate solution: accurately weighed potassium hydrogen phthalate (0.2 g) dissolved in 50 mL of water for standardization of ~ 0.05M sodium hydroxide solution.

7.2.4. Preparation of the inoculum:

Pure culture viz. Serratia marscecens was used in place of the activated sludge used in the OECD method. The culture was transferred from the slant to 10mL of nutrient broth on day one. On day two, the 10mL-inoculated broth was transferred to 100mL of nutrient broth. This 100mL broth was centrifuged on day three at ~4000rpm for 15 minutes. The cells were washed with saline, centrifuged again, suspended in 10mL saline and used as an inoculum. 1.5 mL of the bacterial suspension was was added to each flask.

7.3. Results and Discussions:

The set- up was standardized using different concentrations of glucose (100 mg /L – 1400 mg /L in terms of total organic carbon). The optimum concentration was found to be 200 mg/ L of carbon based on the amount of mg of CO_2 evolved and percent biodegradation of glucose standard. The results were reproducible and the tests were extended to the test polymers. The starting polymer, viz. poly(styrene maleic anhydride) contained 85% carbon, whereas the sugar- linked poly(styrene maleic anhydride) polymers contained 80% carbon, according to elemental analysis. The amounts of the polymers were chosen such that the concentration of the total organic carbon was 200 mg/ L in the minimal medium.

Formulas used for the calculation of mg of CO_2 evolved & % Biodegradation

$$\text{mg of } CO_2 \text{ evolved} = \frac{\text{Normality of HCl}}{2} \times \text{mL of HCl titrated} \times \text{mol. wt. of } CO_2$$

$$\% \text{ Biodegradation} = \frac{\text{mg of } CO_2 \text{ evolved}}{\text{amount of carbon (g)} \times 3.67} \times 100$$

The amount of carbon was determined from the percentage of carbon in the polymer and the weight of the polymer taken.

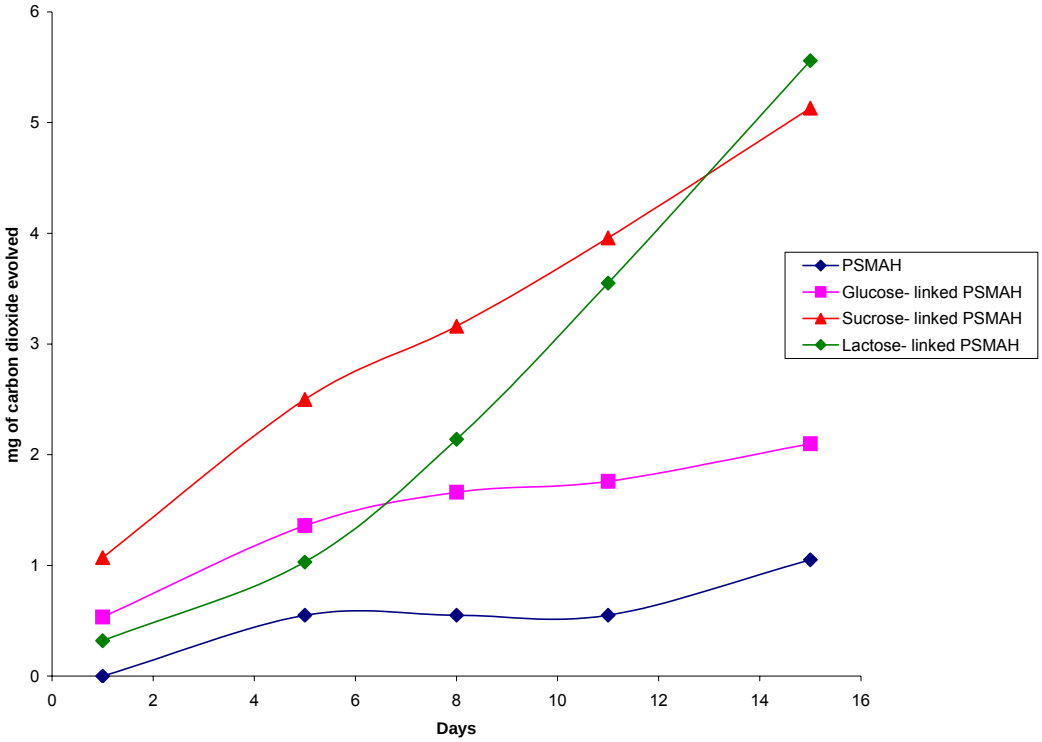


Figure 3: Graph showing the amount of carbondioxide evolved from the polymers.

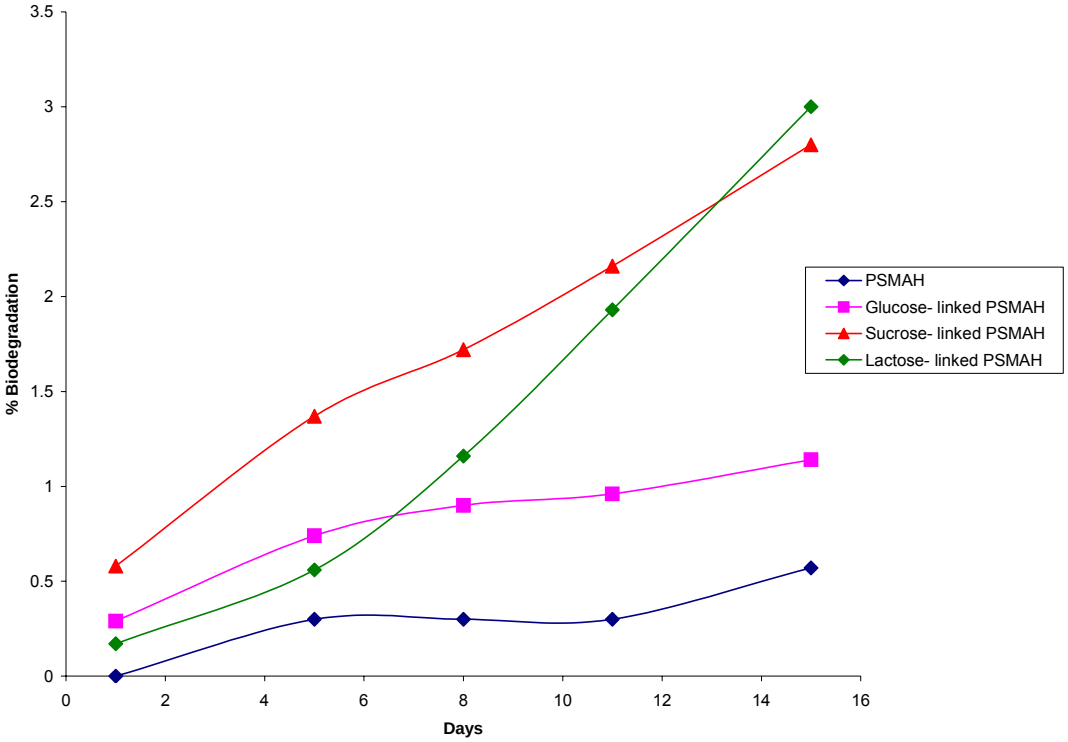


Figure 4: Graph showing the percent biodegradation of the polymers.

CHAPTER 8

Biodegradation of acylated sugar-linked poly(styrene maleic anhydride)

Abstract:

The sugar- linked poly(styrene maleic anhydride) copolymers were acylated using the corresponding acyl halides (viz. acetyl, hexanoyl or lauroyl chloride) in anhydrous pyridine. The acylated polymers were characterized by FTIR and thermal analysis. The thermal stability of the polymers studied by thermogravimetry was in the following order: lauroylated-> hexanoylated-> acetylated- sugar-linked poly(styrene maleic anhydride). The effect of acylation on the biodegradation by both bacterial and fungal cultures were studied in shaker flasks by weight loss data as well as turbidimetry in case of bacterial degradation and weight loss data in case of fungal degradation. The acylated derivatives were generally found to be more biodegradable as compared to poly(styrene maleic anhydride) but less biodegradable as compared to their sugar- linked counterparts, in case of both bacterial and fungal degradation. The protein content in the broth was determined by the Folin-Lowry method to support the turbidimetry data. SEM's of the biodegraded polymers confirmed the observed degradations. In the case of fungal degradation, photographs are presented showing fungal growth in the standard solution, unmodified polymer and the acetylated derivative of sucrose-linked PSMAH.

8.1. Introduction:

The chemical linking of carbohydrates to synthetic polymers was useful in enhancing the biodegradation of these synthetic polymers, the details of which have been described in the previous chapters. Carbohydrates do not have melting points and they undergo charring at the temperatures under which processing is carried out. The general processing temperature for polystyrene and polystyrene base polymers is in the range of 200- 250°C. The charring is due to the large number of hydroxyl groups present in the molecule. They readily undergo dehydration and oxidation reactions at higher temperatures. Hence, one way to facilitate the processing is to block the hydroxyl groups. The easiest and the most convenient method is by acylating the hydroxyl groups. In this chapter, we report the acylation of the sugar- linked

poly(styrene maleic anhydride) polymers, the FTIR characterization of these polymers and the effect of acylation on their biodegradability and thermal stability. The thermal analysis of the sugar- linked poly(styrene maleic anhydride) polymers has been reported in chapter 6. The FTIR of the sugar- linked poly(styrene maleic anhydride) polymers has been described in chapter 5.

8.2. Experimental:

Procedure for Acylation of sugar- linked poly(styrene maleic anhydride) polymers:

The sugar- linked poly(styrene maleic anhydride) polymers were acylated using acyl chloride viz. acetyl chloride, hexanoyl chloride or lauroyl chloride in pyridine solvent at room temperature. After the initial work-up, the polymers were purified by dissolution in organic solvent and reprecipitation in a non-solvent and finally by distillation using soxlet apparatus. They were characterized by FTIR spectroscopy and thermal analysis. They were also subjected to biodegradation using both bacterial cultures and fungal cultures.

8.3. Results and Discussion:

8.3.1. FTIR spectroscopy of the acylated derivatives of sugar-linked poly(styrene maleic anhydride)

The FTIR spectra of the acylated derivatives were all recorded in the diffused reflectance mode on a Perkin Elmer FTIR Spectrophotometer. 2mg of each vacuum oven dried polymer was mulled with 100mg of anhydrous KBr and the spectra recorded at a resolution of 2 cm^{-1} and the number of scans were 50.

In general all the acylated samples showed a reduction in the OH stretching vibration at 3300-3600 cm^{-1}. The carbonyl region shows broadening and merger of the ester carbonyl with the anhydride carbonyl, due to increase in the number of ester groups. The differences are clearly seen in the overlapping spectra of the sugar-linked poly(styrene maleic anhydride) and its acylated products (Figure 4).

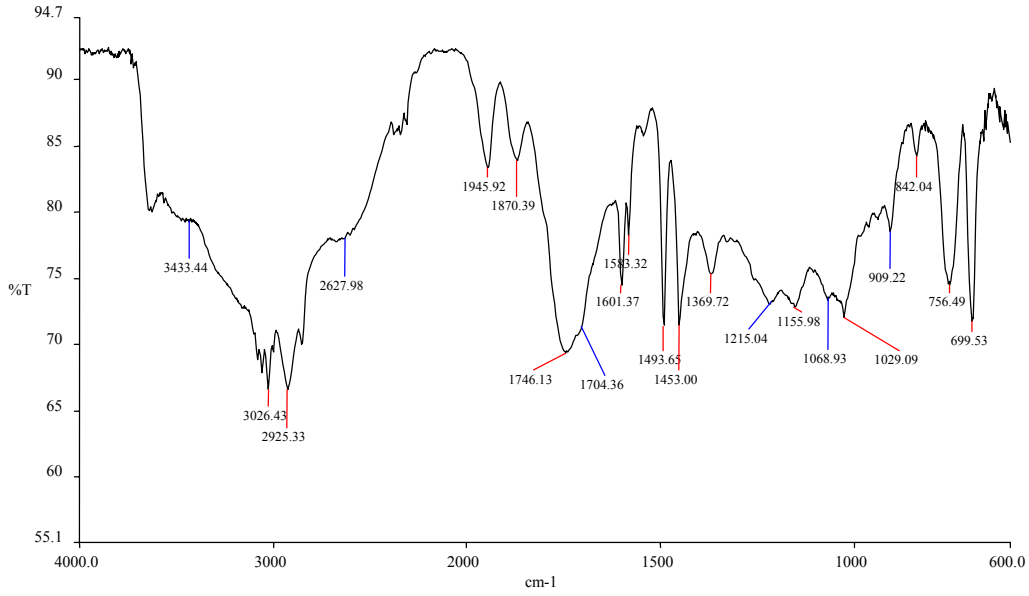


Figure 1: FTIR spectrum of acetylated derivative of glucose- linked poly(styrene maleic anhydride)

Figure 1 shows the FTIR spectrum of the acetylated derivative of glucose- linked poly(styrene maleic anhydride). The spectrum shows reduction in the intensity of the hydroxyl peak at 3300- 3600 $cm^{-1}$and a considerable increase in the intensity and broadening of the carbonyl band centered at 1746 cm^{-1}. The broadening is due to the increase in the number of ester groups formed due to the acylation and due to the contribution from the merging anhydride carbonyl band. Similar trend is observed in case of the lauroylated derivative (Figure 3). These changes can be better viewed in the overlapping spectra of glucose- linked poly(styrene maleic anhydride) and its acetylated and lauroylated derivative (Figure 4).

Due to the broad and asymmetric nature of the carbonyl band, the carbonyl band was deconvoluted (Figure 2)

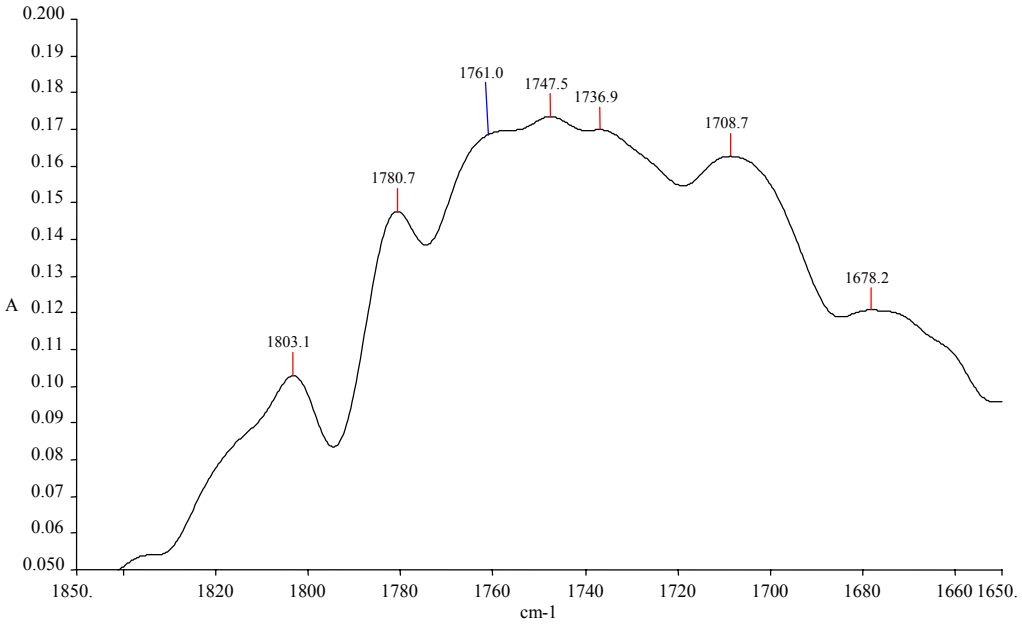


Figure 2: Deconvoluted carbonyl band of acetylated derivative of glucose-linked poly(styrene maleic anhydride).

On deconvolution the anhydride carbonyl band at 1780 cm^{-1}which was embedded in the broad band formed due to the merger of number of ester carbonyls separates out. This is also accompanied by separation of the broad ester carbonyls into various small peaks at 1761, 1747.5, 1737 and 1709 cm^{-1}.

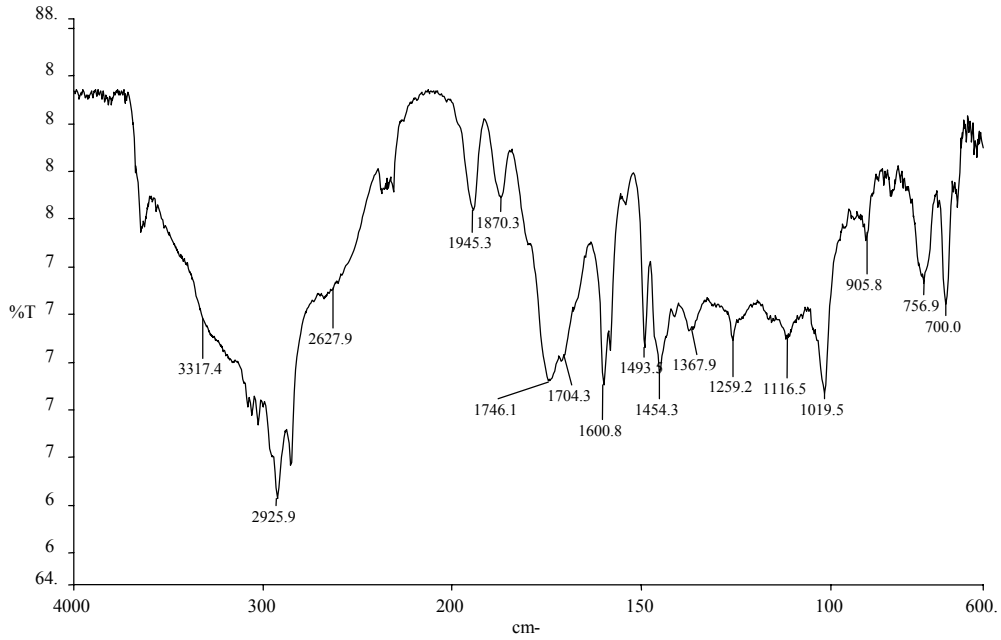


Figure 3: FTIR spectrum of lauroylated derivative of glucose- linked poly(styrene maleic anhydride)

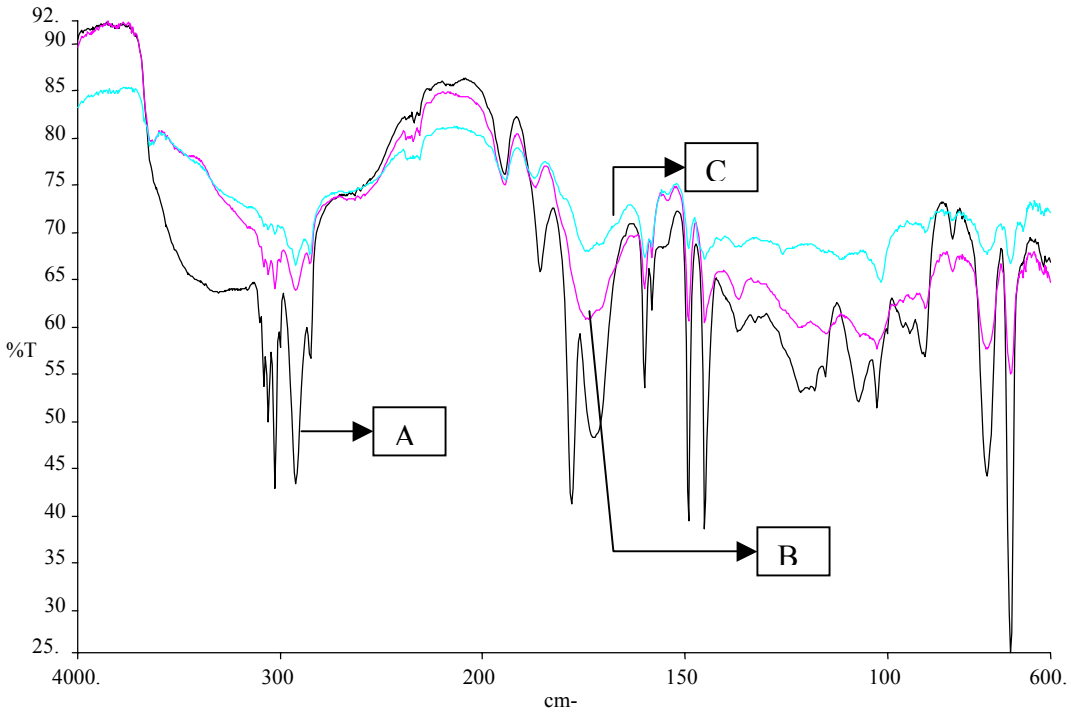


Figure 4: Overlapping FTIR spectra of glucose- linked poly(styrene maleic anhydride) and its acylated derivatives. A= glucose- linked PSMAH; B= acetylated derivative of glucose- linked PSMAH; C= lauroylated derivative of glucose- linked PSMAH.

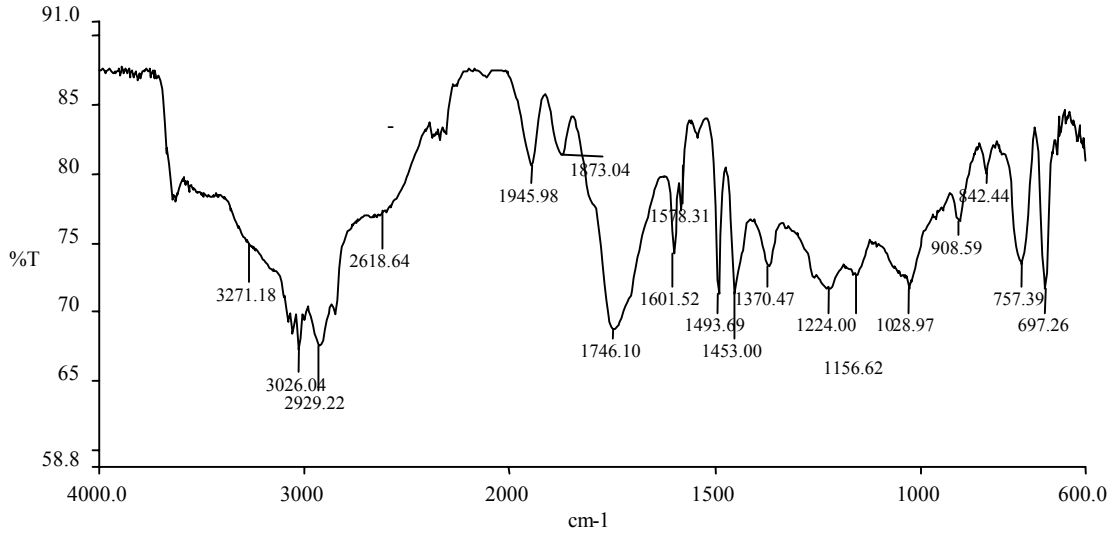


Figure 5: FTIR spectrum of acetylated derivative of sucrose- linked poly(styrene maleic anhydride)

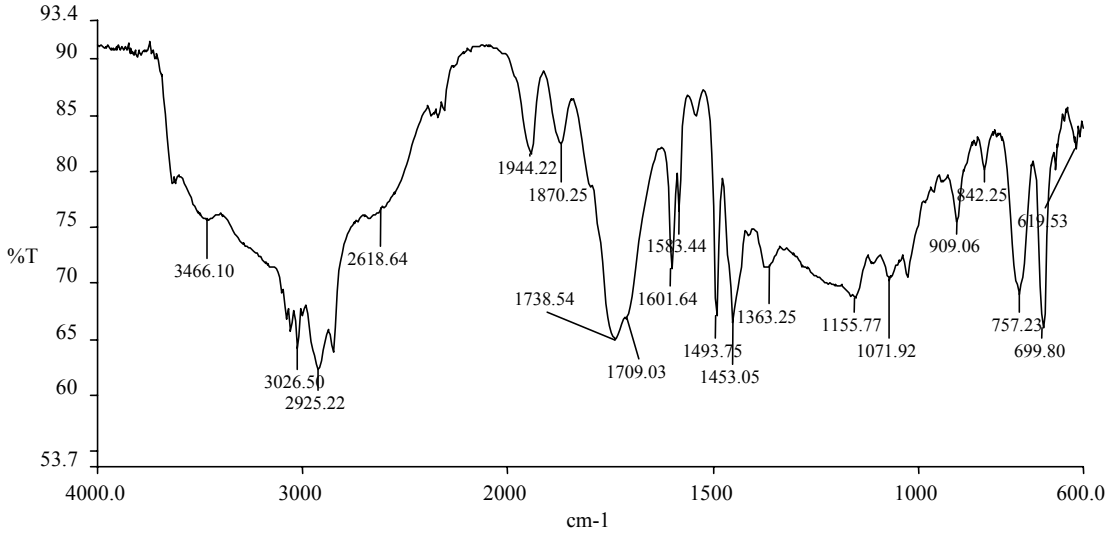


Figure 6: FTIR spectrum of lauroylated derivative of sucrose- linked poly(styrene maleic anhydride)

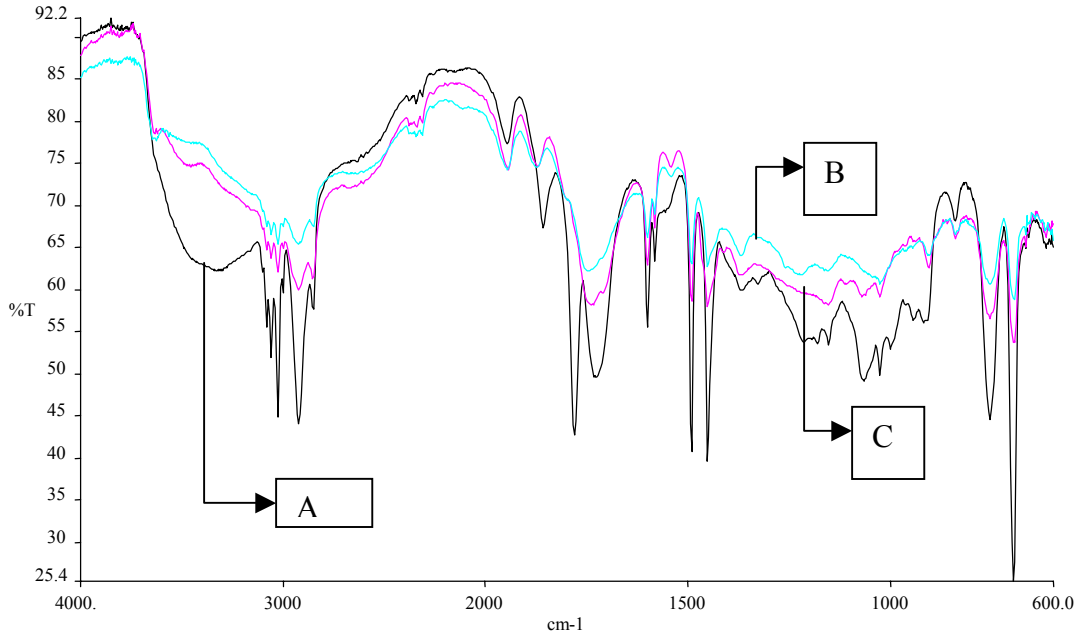


Figure 7: Overlapping FTIR spectra of sucrose-linked poly(styrene maleic anhydride) and its acylated products A= sucrose- linked PSMAH; B= acetylated derivative of sucrose- linked PSMAH; C= lauroylated derivative of sucrose- linked PSMAH.

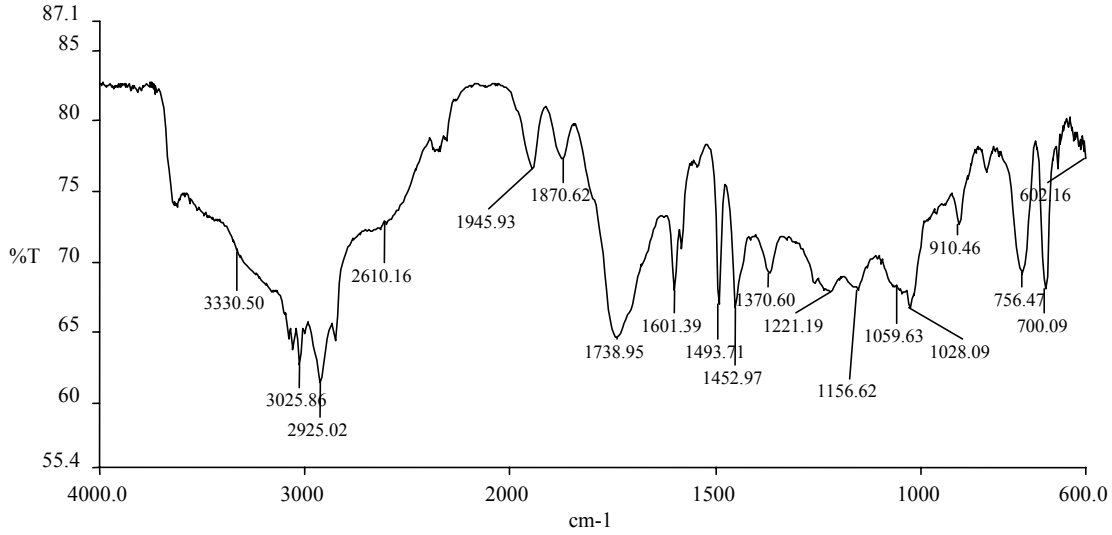


Figure 8: FTIR spectrum of acetylated derivative of lactose- linked poly(styrene maleic anhydride)

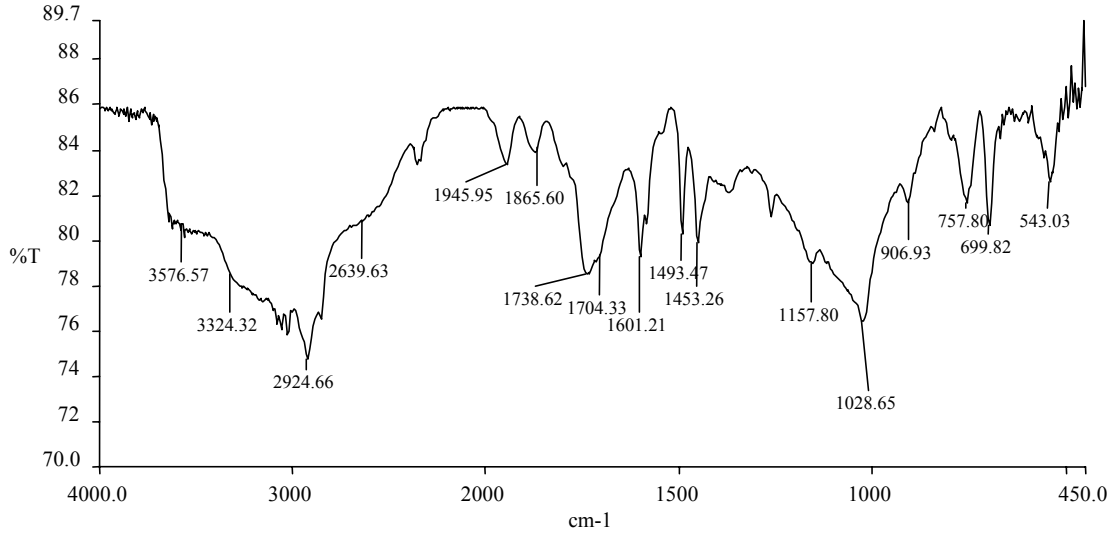


Figure 9: FTIR spectrum of hexanoylated derivative of lactose- linked poly(styrene maleic anhydride)

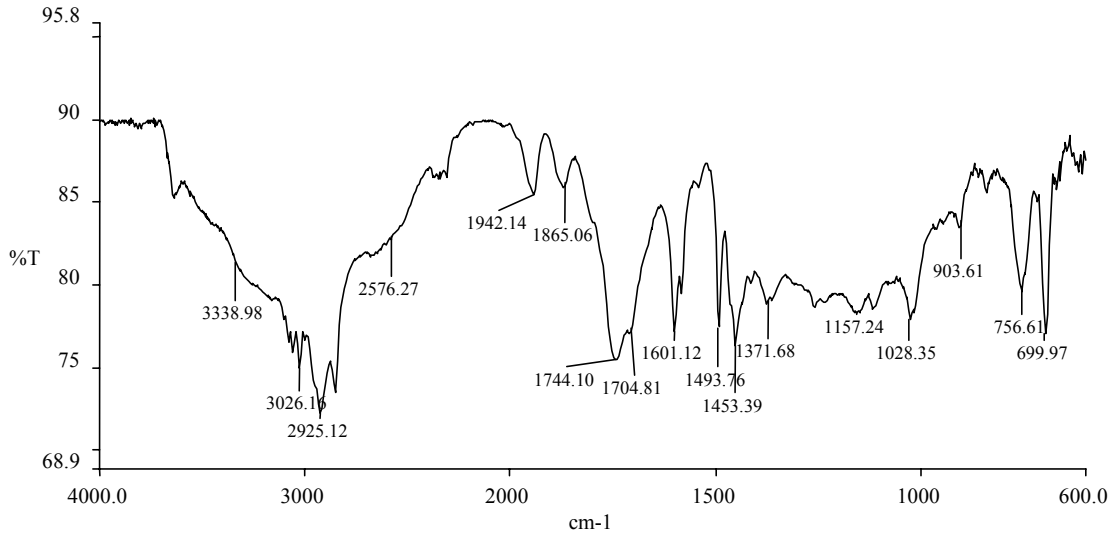


Figure 10: FTIR spectrum of lauroylated derivative of lactose- linked poly(styrene maleic anhydride)

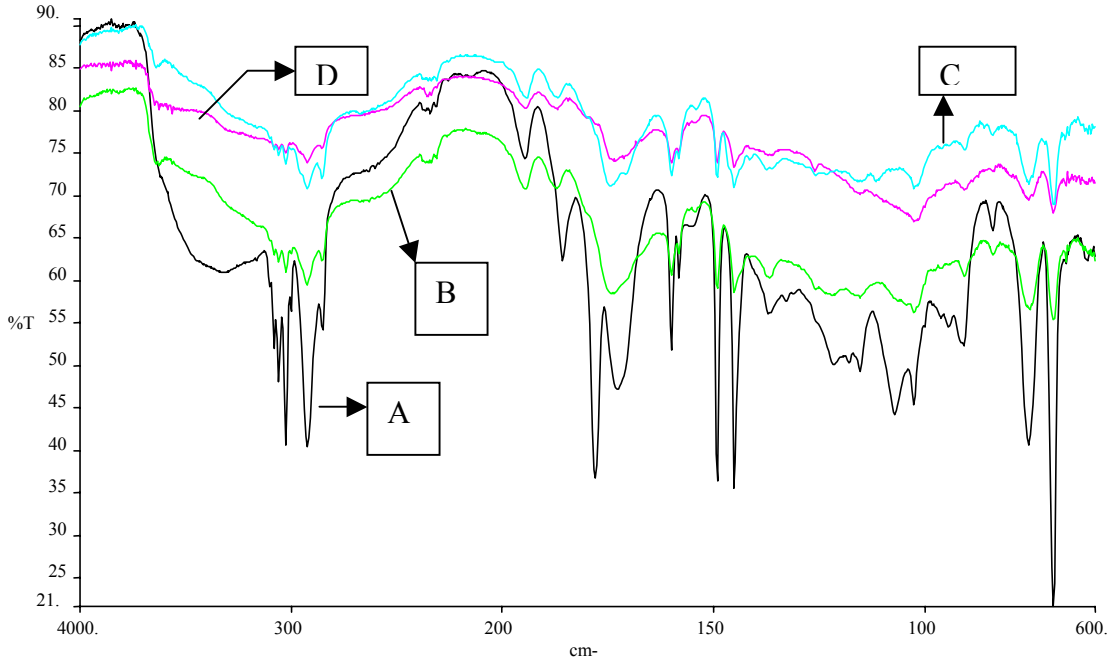


Figure 11: Overlapping FTIR spectra of lactose- linked poly(styrene maleic anhydride) and its acylated derivatives. A= lactose- linked PSMAH; B= acetylated derivative of lactose- linked PSMAH; C= lauroylated derivative of lactose- linked PSMAH; D= hexanoylated derivative of lactose- linked PSMAH

8.3.2. Thermal studies of acylated derivatives of sugar- linked poly(styrene maleic anhydride) polymers:

The sugar-linked poly(styrene maleic anhydride)s after acylation generally showed increased amounts of weight losses uto 300°C as compared to their sugar-linked counterparts. Amongst the acylated derivatives, the thermal stability of the polymers was in the following order: lauroylated-> hexanoylated-> acetylated- sugar-linked poly(styrene maleic anhydride).

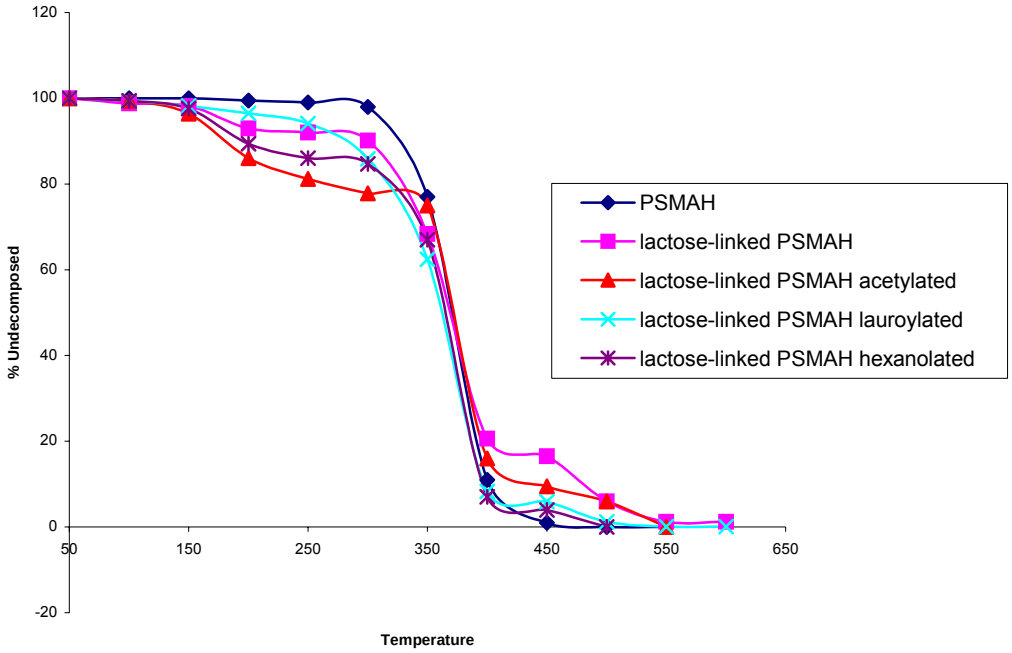


Figure 12: Overlapping TGA curves of lactose-linked poly(styrene maleic anhydride) and its acylated products.

The same trend is observed in case of acylated products of glucose-linked poly(styrene maleic anhydride).

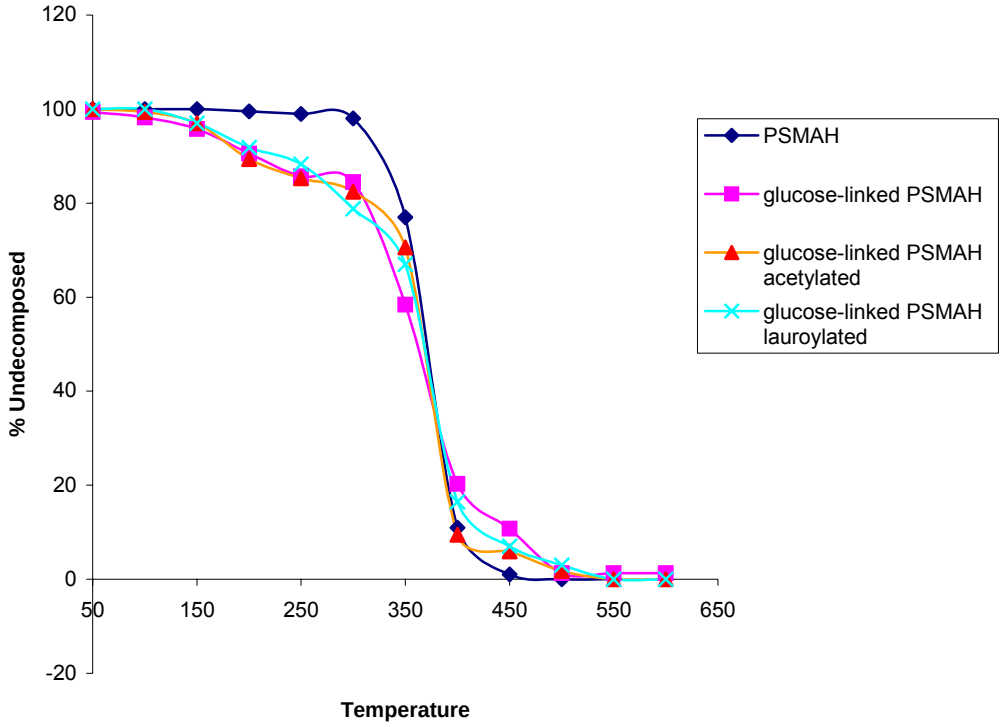


Figure 13: Overlapping TGA curves of glucose-linked poly(styrene maleic anhydride) and its acylated products

The weight losses occurring at various temperatures for the acylated derivatives of the glucose- linked and lactose- linked derivatives, from the thermogravimetry are presented in the table on page 192.

The changes occurring in the polymers at various temperatures were interpreted and correlated with the help of the FTIR spectra of the polymers treated thermally at temperatures of 150°C, 250°C and 350°C for 30 minutes in a furnace. The FTIR spectra of poly(styrene maleic anhydride) and sugar- linked poly(styrene maleic anhydride) are discussed in chapter 6.

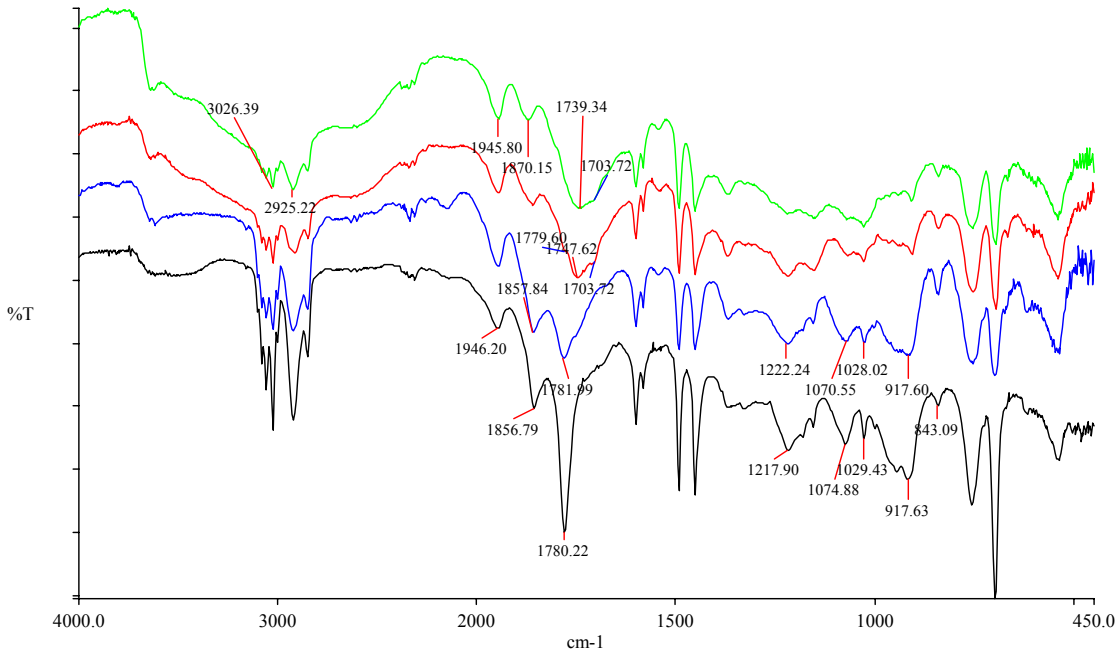


Figure 14: Overlapping FTIR spectra of acetylated derivative of PSMAH (14 wt % MAH) linked by glucose, after thermal treatment (Green: room temp. 25 °C, Red: 150 °C; Blue: 250 °C; and Black: 350 °C

There are no changes in the polymer at 150°C, except that there is a narrowing of the peak between 1700- 1800 cm^{-1}. At 250°C, there is a considerable reduction of the ester and carboxylic acid peak, followed by increase in the intensity of the anhydride carbonyl peaks at 1780 and 1856 cm^{-1}. This is indicative of the fact, that the acylated sugars start detaching from the polymer at this temperature. The FTIR spectrum at 350°C shows complete diappearance of the acid and ester peak, with a further increase in the intensity of the anhydride carbonyls. The spectrum of the acetylated derivative of the glucose- linked PSMAH at 350°C resembles to that of PSMAH at room temperature.

8.4. Biodegradation of acylated derivatives of sugar-linked poly(styrene maleic anhydride) by Serratia marscecens and Pseudomonas sp.

8.4.1. Biodegradation by Serratia marscecens:

The sugar-linked poly(styrene maleic anhydride) and their acylated derivatives were subjected to biodegradation by Serratia marscecens. All the modified poly(styrene maleic anhydride) polymers were more degradable as compared to unmodified poly(styrene maleic anhydride)

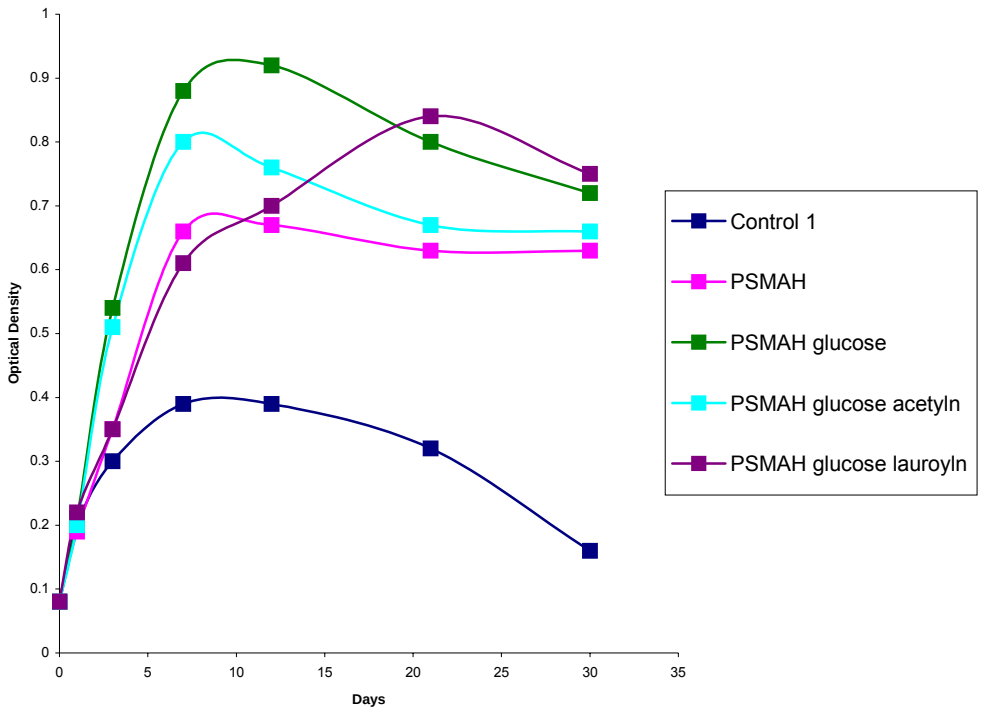


Figure 15: Growth pattern of Serratia marcecens. for glucose- linked poly(styrene maleic anhydride) and its acylated products

From the figure it is observed that the lauroylated derivative was more degradable as compared to the acetylated counterpart. The glucose-linked poly(styrene maleic

anhydride) showed a OD maximum after a week, whereas the lauroylated derivative showed a maximum after three weeks.

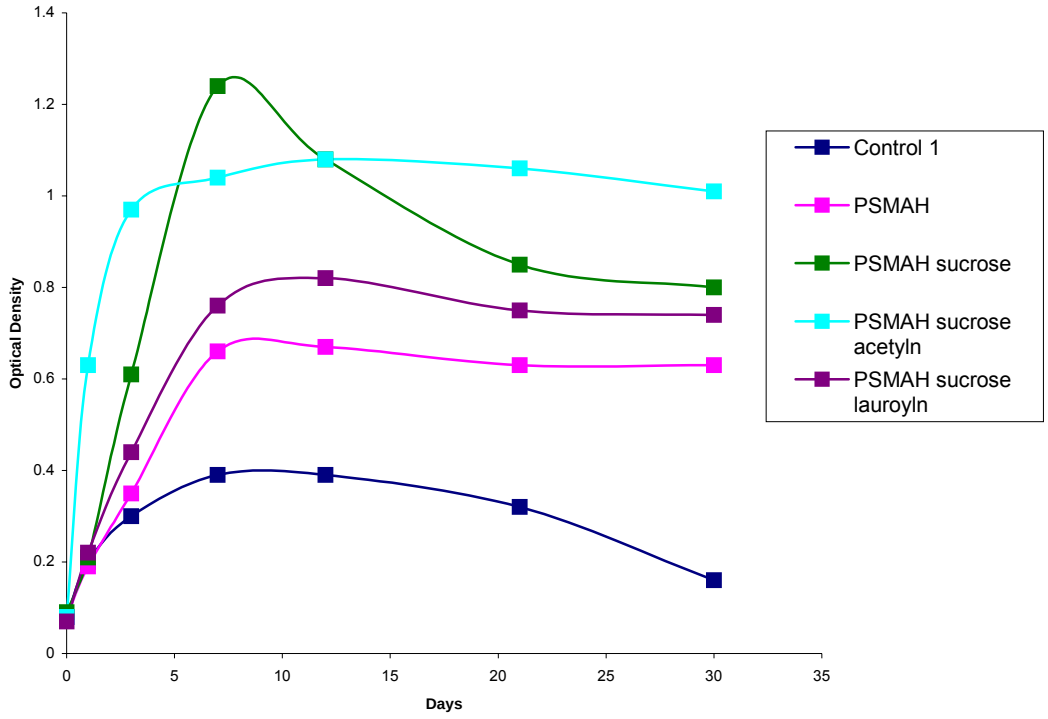


Figure 16: Growth pattern of Serratia marscecens on sucrose-linked poly(styrene maleic anhydride) and its acylated derivatives

From the figure, it is observed that the sucrose-linked poly(styrene maleic anhydride) showed maximum rate of biodegradation, followed by its acetylated and lauroylated derivative. The OD was maximum for all the polymers after approximately 5 days.

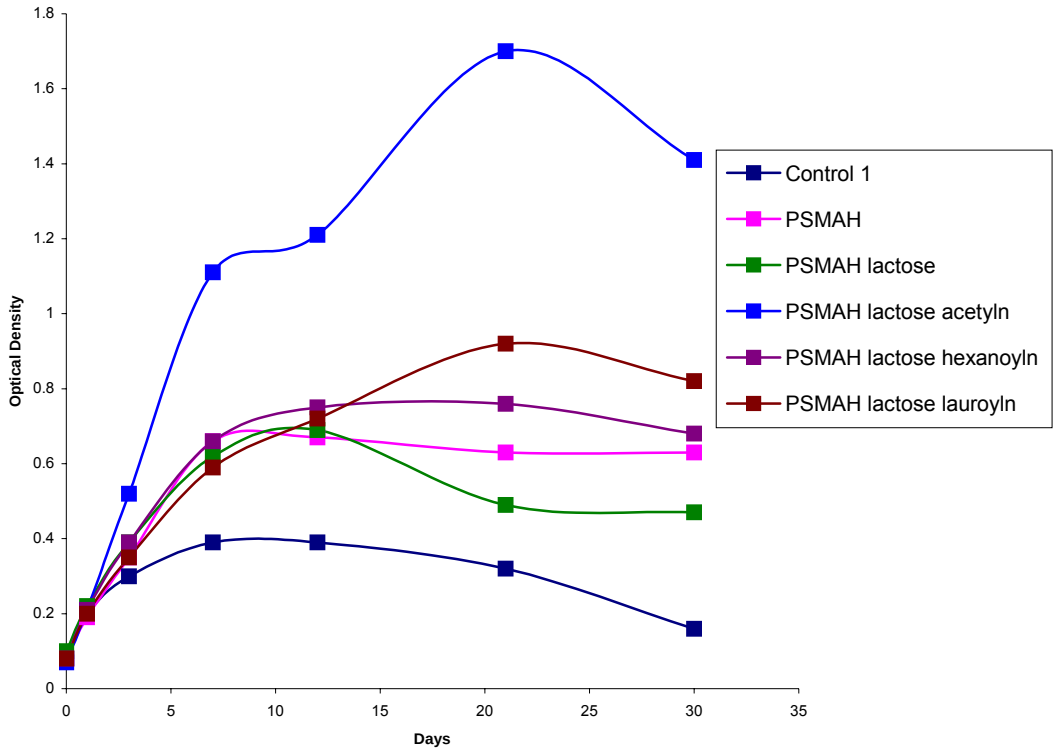


Figure 17: Growth pattern of Serratia marscecens on lactose-linked poly(styrene maleic anhydride and its acylated products

From the figure, it is observed that the lactose-linked poly(styrene maleic anhydride) did not show appreciable difference in the biodegradation rate as compared to poly(styrene maleic anhydride). The hexanoylated and lauroylated derivatives were more biodegradable as compared to poly(styrene maleic anhydride). The acetylated derivative showed significant increases in the OD.

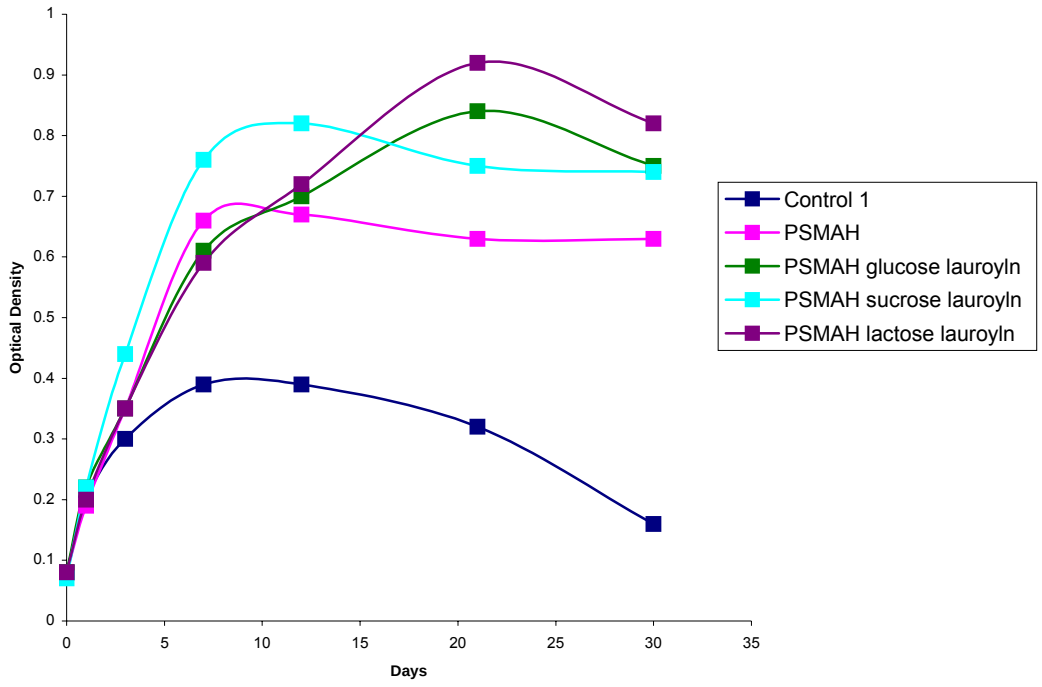


Figure 18: Growth pattern of Serratia marscecens on lauroylated derivatives of sugar-linked poly(styrene maleic anhydride)

Figure 18 shows the growth pattern of Serratia marscecens on lauroylated derivatives of sugar-linked poly(styrene maleic anhydride). All the lauroylated derivatives were more biodegradable as compared to poly(styrene maleic anhydride). Amongst them, the lauroylated derivative of lactose-linked poly(styrene maleic anhydride) showed maximum OD followed by its glucose and sucrose counterparts. The lauroylated derivative of sucrose-linked poly(styrene maleic anhydride) showed OD maximum after one week, whereas the glucose and lactose-linked lauroylated derivatives showed a OD maximum after three weeks.

8.4.2. Biodegradation by Pseudomonas sp.:

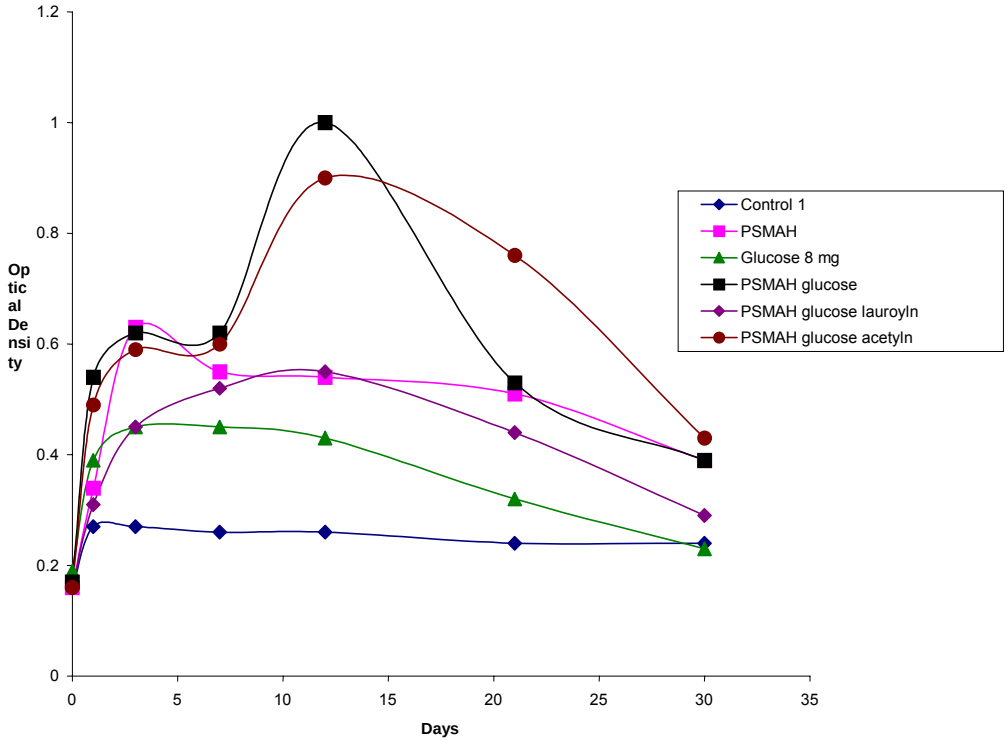


Figure 19: Growth pattern of Pseudomonas sp. on glucose- linked poly(styrene maleic anhydride) and its acylated produducts

Figure shows the growth pattern of Pseudomonas sp. on glucose- linked poly(styrene maleic anhydride) and its acylated produducts. The glucose- linked polymer is more degradable than its acylated derivatives. However, the acetylated derivative is almost as degradable as the glucose- linked polymers. The lauroylated derivative degrades poorly.

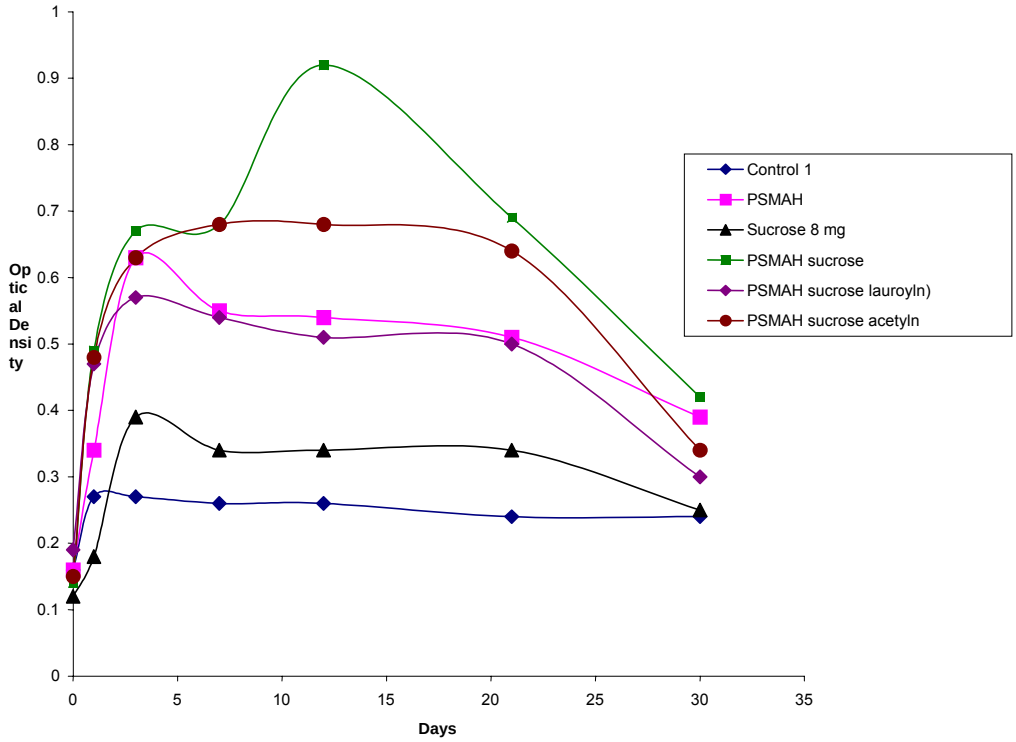


Figure 20: Growth pattern of Pseudomonas sp. on sucrose- linked poly(styrene maleic anhydride) and its acylated produducts

From figure , it is observed that the sucrose- linked poly(styrene maleic anhydride) is the most degradable one followed by its acetylated derivative. Both these polymers are more degradable as compared to poly(styrene maleic anhydride), however, the lauroylated derivative is almost as degradable as compared to poly(styrene maleic anhydride).

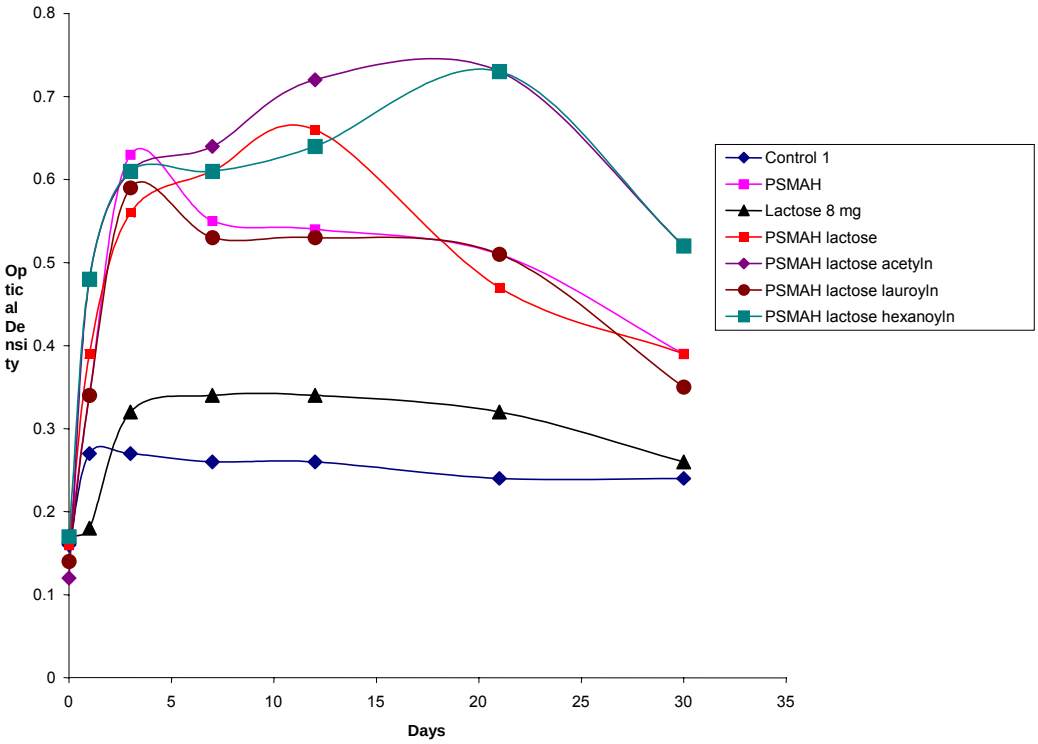


Figure 21: Growth pattern of Pseudomonas sp. on lactose- linked poly(styrene maleic anhydride) and its acylated produducts

From the figure it is observed that the acetylated and hexanoylated derivatives of the lactose- linked poly(styrene maleic anhydride) are the most degradable ones. The lactose-linked poly(styrene maleic anhydride) is less degradable as compared to its acetylated and hexanoylated derivatives. The lauroylated derivative does not show any enhanced rate of degradation as compared to poly(styrene maleic anhydride).

8.5. Characterization of the biodegradaed products:

8.5.1. Weight loss data:

Table 1: Weight loss of different acylated derivatives of sugar linked poly(styrene - maleic anhydride) (PSMAH) copolymers in presence of Serratia marscecens

Sample	**Initial weight (mg)**	**Final weight (mg)**	**% weight loss**
Acetylated derivate of sucrose- linked PSMAH degraded by Serratia sp.	339	317	6.5
Lauroylated derivate of sucrose- linked PSMAH degraded by Serratia sp.	264	249.5	5.5
Acetylated derivate of lactose- linked PSMAH degraded by Serratia sp.	242	203.9	15.7
Hexanoylated derivate of lactose- linked PSMAH degraded by Serratia sp.	236	220.9	6.4

Table 2: Weight loss of different acylated derivatives of sugar linked polystyrene - maleic anhydride (PSMAH) copolymers in presence of Pseudomonas sp.

Sample	**Initial weight (mg)**	**Final weight (mg)**	**% weight loss**
Acetylated derivate of glucose- linked PSMAH degraded by Pseudomonas sp.	245	236.6	3.43
Lauroylated derivate of glucose - linked PSMAH degraded by Pseudomonas sp.	280	276.2	1.36
Acetylated derivate of sucrose- linked PSMAH degraded by Pseudomonas sp.	245	233.8	4.6
Lauroylated derivate of sucrose- linked PSMAH degraded by Pseudomonas sp.	275	264.8	3.71
Acetylated derivate of lactose- linked PSMAH degraded by Pseudomonas sp.	262	243.1	7.2
Lauroylated derivate of lactose- linked PSMAH degraded by Pseudomonas sp.	272	268.9	1.14

8.5.2. Scanning electron micrographs of the acylated derivatives of the sugar-linked polymers before and after degradation:

All the samples were in the form of thick films. In some cases the surfaces were smooth and in some cases they were rough. After subjecting the polymers to biodegradtion, the films were thoroughly washed with water and suspended in 70% ethanol overnight and then dried. The cases in which, SEMs were recorded before the treatment, have been specified.

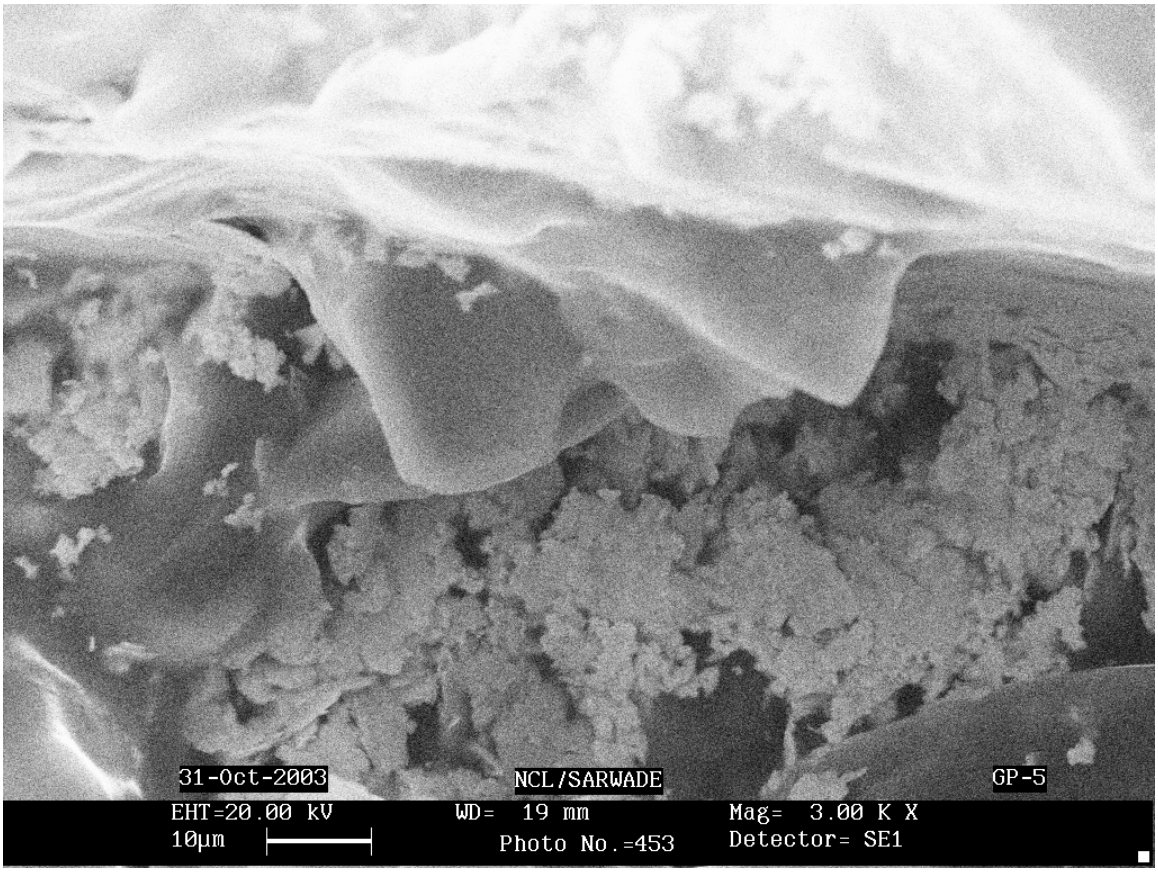


Figure 22: SEM of acetylated derivative of glucose- linked PSMAH before being subjected to biodegradation.

The surface of the polymer is uneven and rough, since it was cast from a gelled solution

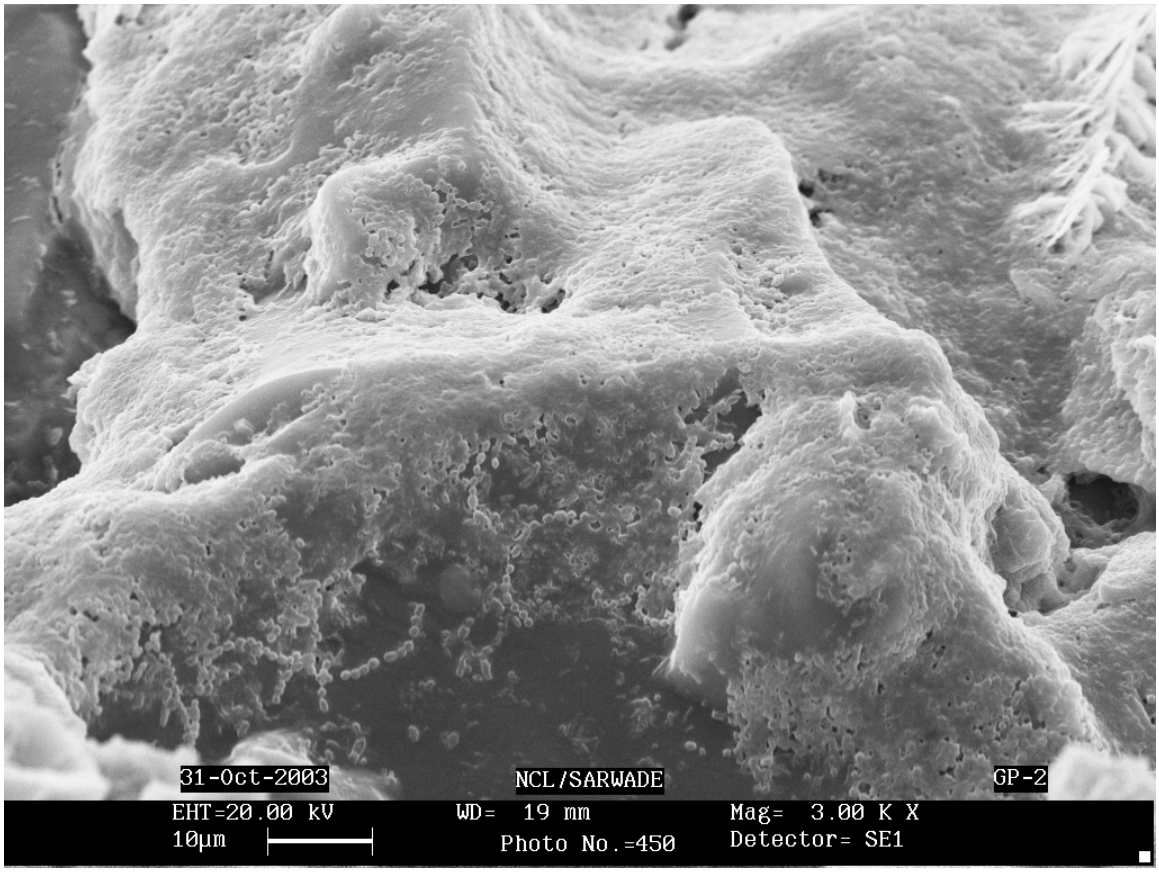


Figure 23: SEM of acetylated derivative of glucose- linked PSMAH after being subjected to biodegradation by Serratia marscecens prior to washing and ethanol treatment.

The surface of the polymer shows dense population of microbes on the surface of the polymer. The bacteria show specific growth patterns.

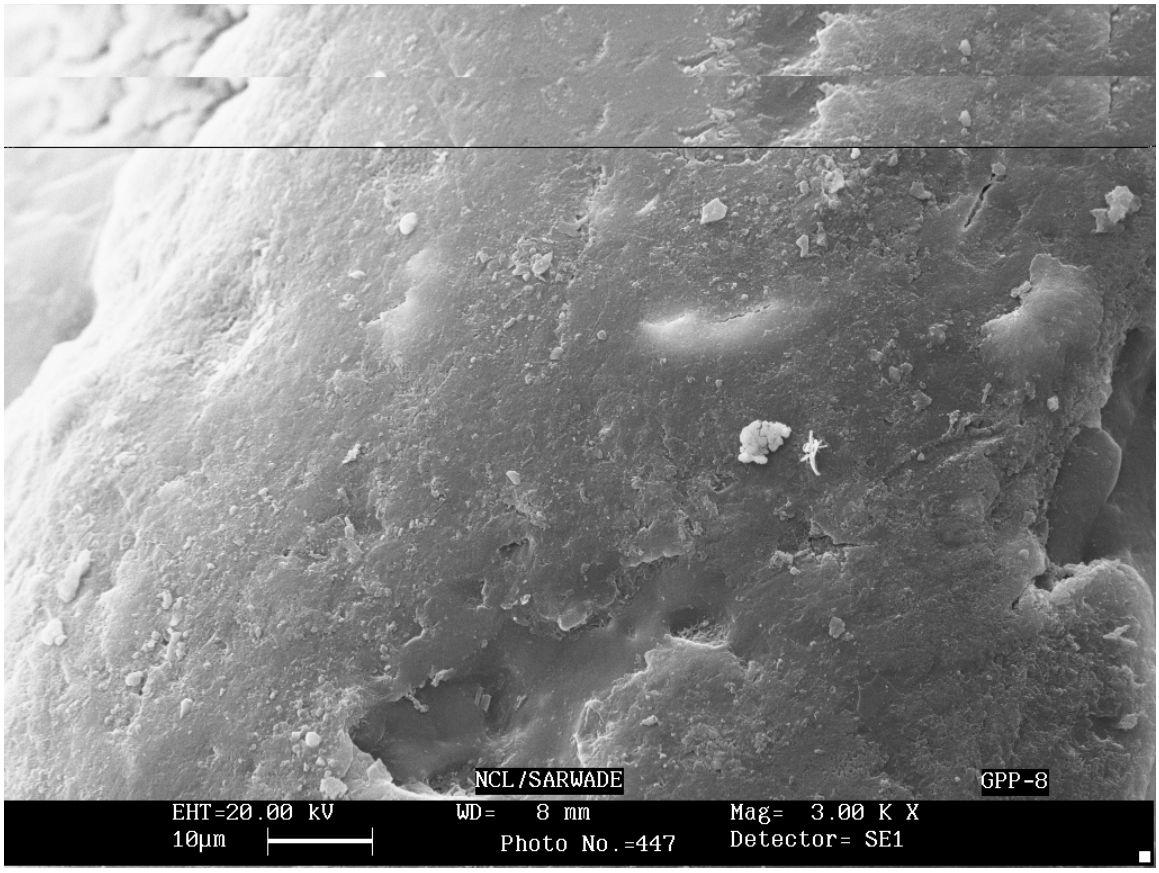


Figure 24: SEM of the acetylated derivative of glucose-linked poly(styrene maleic anhydride) degraded by Serratia marscecens (NCIM 5061) after washing and ethanol treatment.

Some degardation is seen on the surface.

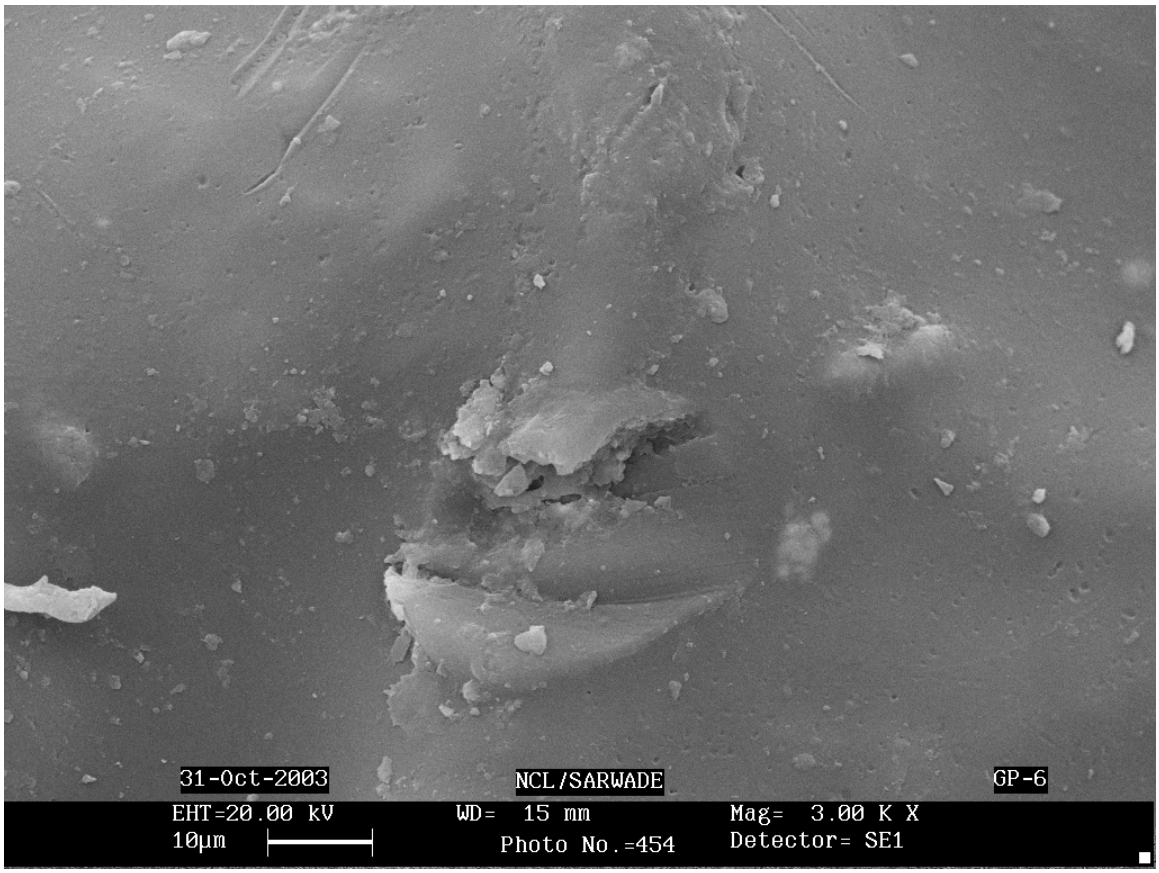


Figure 25: SEM of the lauroylated derivative of glucose-linked poly(styrene maleic anhydride) before subjecting to biodegradation

The surface shows some unevenness due to the casting of the polymer from a gelled solution. The surface of the polymer, on the whole, is smooth.

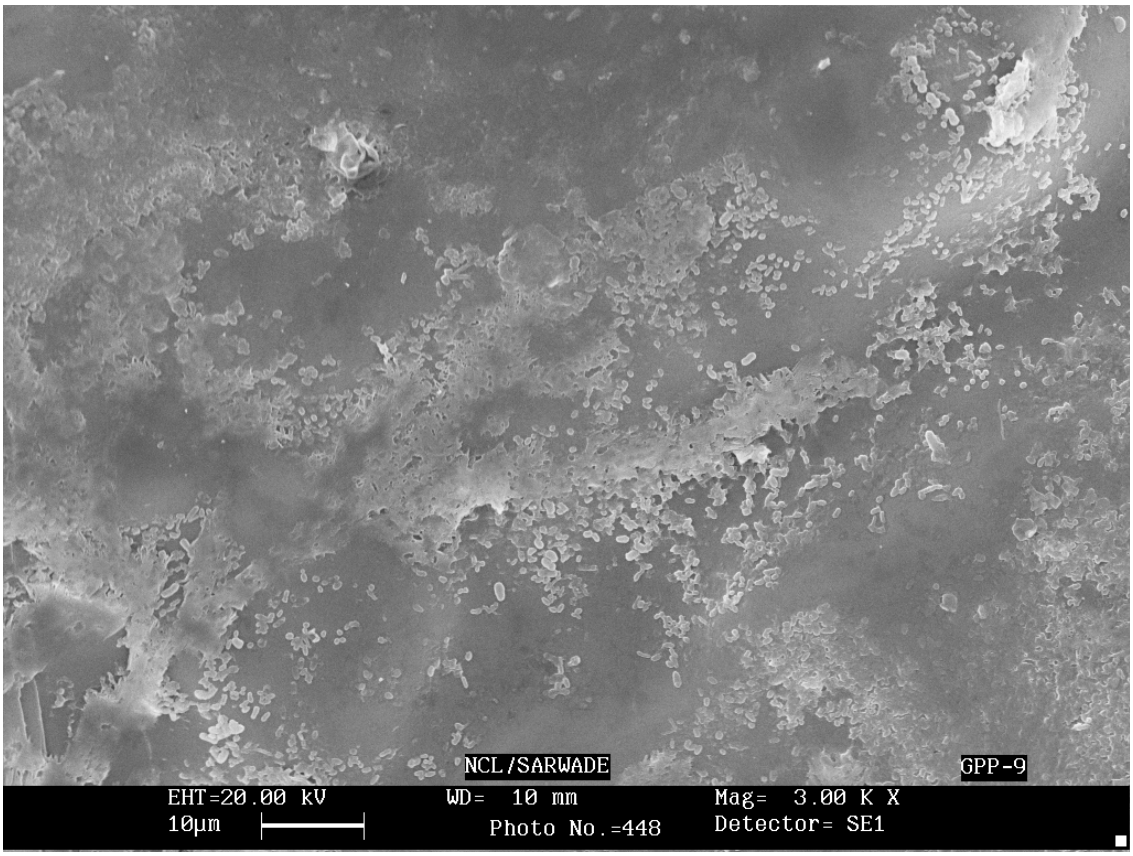


Figure 26: SEM of the lauroylated derivative of glucose-linked poly(styrene maleic anhydride) degraded by Serratia marscecens (NCIM 5061) before washing and treatment with ethanol.

The SEM shows bacteria all along the surface of the polymer

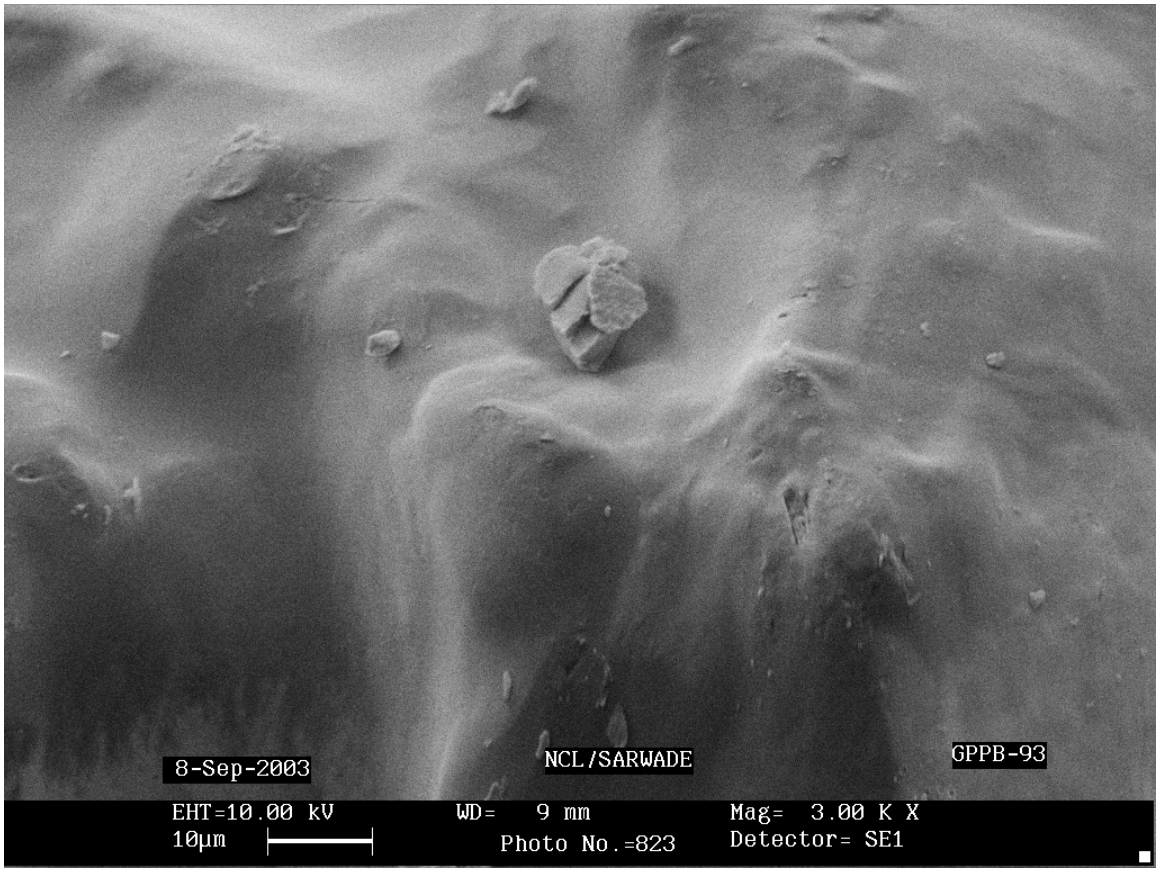


Figure 27: SEM of lauroylated derivative of lactose- linked PSMAH before being subjected to biodegradation

The surface of the polymer appears uneven and smooth. This film did not dissolve completely, and the film was cast from a gelled solution. This could be the reason for the un-evenness of the surface

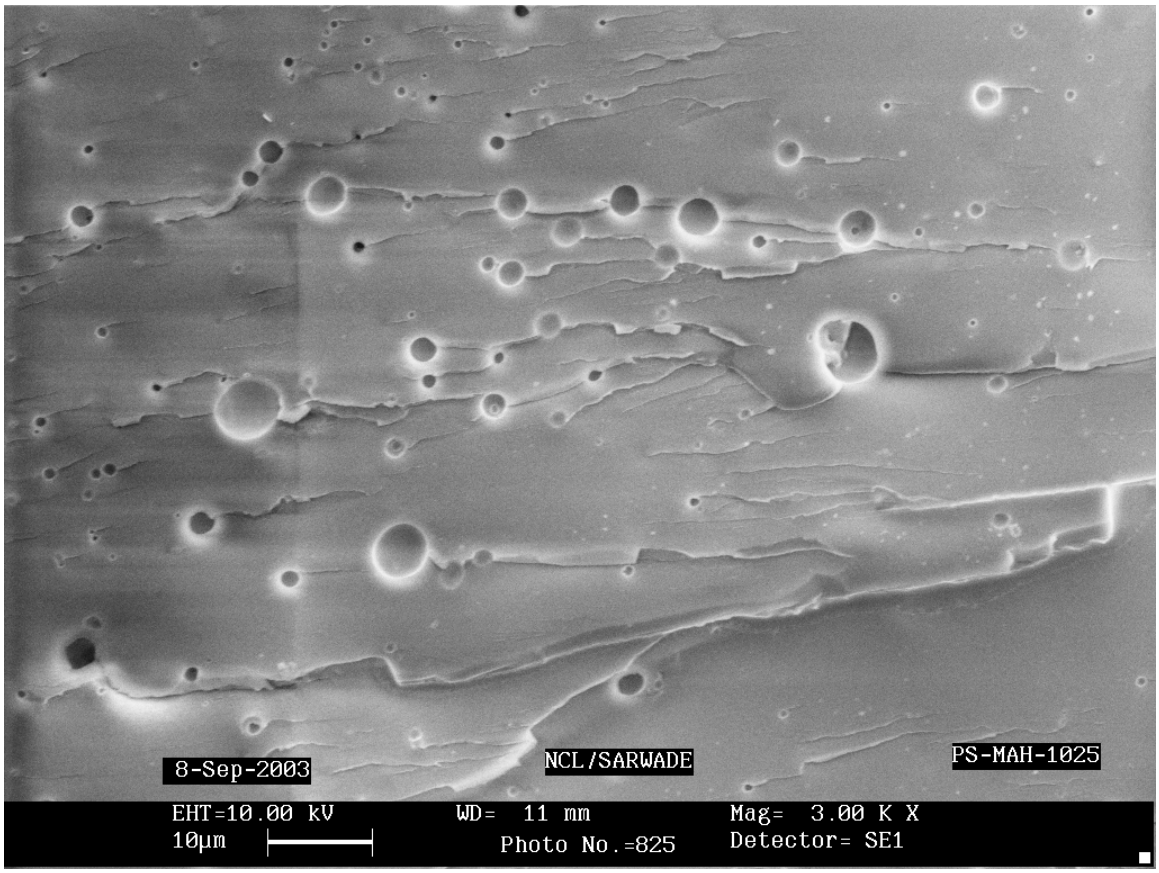


Figure 28: SEM of lauroylated derivative of lactose- linked PSMAH after being subjected to degradation by Serratia marscecens

The surface of the polymer after degradation shows several changes in the morphology of the polymer. The surface of the polymer shows holes and appears eroded.

8.6. Fungal degradation of acylated derivatives of sugar- linked poly(styrene maleic anhydride)s:

8.6.1. Materials :

Poly(styrene-co-maleic anhydride) (PS-MAH) (maleic anhydride content 14% by weight) was obtained from Aldrich, USA. All solvents were obtained from SD Fine Chemicals, pune, India, and were distilled and dried before use. The microorganisms used *Aspergillus niger* NCIM 1025 (ATCC 9642), *Trichoderma* sp. NCIM 1297 (ATCC 9645) and *Pullularia pullulans* NCIM 1049 (ATCC 9348) were obtained from the National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, India.

8.6.2. Test microorganisms:

The selected microorganisms *Aspergillus niger* NCIM 1025 (ATCC 9642), *Trichoderma* sp. NCIM 1297 (ATCC 9645) and *Pullularia pullulans* NCIM 1049 (ATCC 9348) were standard fungal strains (ASTM Standards, G-21, 1980) and *Penicillium ocro-chloron* NCIM 1219 (IS 9000, Part X, 1979) recommended for testing the fungal resistance. All the microorganisms used for the test were obtained from our culture collection, National Collection of Industrial Microorganisms (NCIM). These cultures were routinely maintained on Potato Dextrose Agar (PDA) slopes.

8.6.3. Testing of the samples:

The cultures were grown in minimal medium (ASTM Nutrient Salts Medium) containing (g/l): KH_2PO_4 0.7; K_2HPO_4 0.7; $MgSO_4.7H_2O$ 0.7; NH_4NO_3 1.0; NaCl 0.005; $FeSO_4.7H_2O$ 0.002.; $ZnSO_4.7H_2O$ 0.002.; $MnSO_4.H_2O$ 0.001. The pH of the medium was adjusted to 6.5 ± 0.2 and sterilized at 121° C for 20 minutes. The test

samples (250mg/ 50 ml) were surface sterilized with 70% ethanol for 2 h and added aseptically to the sterilized medium.

The inoculum was prepared by suspending the spores from fully grown cultures on PDA slopes in 10 ml of sterile saline. This suspension (1.0 ml) was added to 50ml of minimal medium (MM) in 250mL Erlenmeyer conical flasks. Flasks were incubated at 30°C for 10 weeks under stationary condition.

Test samples were harvested, washed in sterile distilled water several times to remove mycelia and spores followed by three washings with 70% ethanol and drying at 37°C for 48h.

8.7. Characterization of the acylated derivatives of the sugar- linked poly(styrene maleic anhydride) polymers degraded by fungi

Weight loss data:

Table 3: Weight loss of the sugar- linked polymers and their acylated derivatives degraded by fungal strains

Sample	% weight loss by different strains			
	Aspergillus niger NCIM 1025	*Penicillium ochro-cloron* NCIM 1219	*Pullularia pullulans* NCIM 1049	*Trichoderma* sp. NCIM 1297
Control (without any carbon source)	-	-	-	-
Glucose linked PS-MAH (acetylated)	3.1	2.7	5.6	2.3
Glucose linked PS-MAH (lauroylated)	0.8	0.9	0.6	7.0
Sucrose linked PS-MAH (acetylated)	2.8	2.8	2.8	-
Sucrose linked PS-MAH (lauroylated)	-	1.4	-	7.8
Lactose linked PS-MAH (acetylated)	2.9	4.5	3.6	3.5
Lactose linked PS-MAH (lauroylated)	0.4	0.5	3.4	0.7
Lactose linked PS-MAH (hexanoylated)	3.6	4.4	6.1	4.4

The visual growth of the fungus (Aspergillus niger NCIM 1025) is seen from the photograph below:

Figure 29: Photograph showing growth of Aspergillus niger NCIM 1025 on minimal medium (left), PSMAH (middle) and the acetylated derivative of sucrose- linked PSMAH (right)

8.8. Appendix 5: Protein Estimation by Folin-Lowry method:

(Sugar-linked PSMAH and their acylated products degraded by Serratia marscecens and Pseudomonas sp.)

The purpose of estimating the protein in the broth after separation of the bacterial cells from the broth is to check which of the enzymes (extracellular or intracellular) is involved in the degradation process. The other purpose is to evaluate wether the protein contents are relative to the growth patterns (ODs) of the bacteria degrading the sugar-linked polymers and their acylated products.

Preparation of Reagent A, B, C, and D:

Reagent A: 0.5g copper sulfate + 1g sodium citrate in 100mL water
Reagent B: 10g sodium carbonate + 2g sodium hydroxide in 500mL water
Reagent C: 50mL Reagent B + 1mL Reagent A
Reagent D: 10mL Folin + 10mL water

Approximately 2mL broth from each of the flasks was withdrawn and transferred to Eppendorf tubes / centrifuge tubes and then centrifuged at a speed of ~7000 rpm for 6 mins. The bacterial cells settled down as pellets and 0.5 mL of each of the supernatent liquids was transferred to test tubes. 0.5 mL of distilled water was taken as a blank. In each of the tubes 2.5mL of Reagent C was added. The tubes were allowed to stand at room temperature for 10 mins. Then in each of the tubes 0.25mL of Reagent D was added and the flasks were incubated at room temperature for 30 mins. Due to some precipitations in the tubes, the supernatents were again centrifuged at 7000 rpm for 6 mins. The ODs were recorded at 660nm. Unknown protein concentrations were recorded from the standard plot of Bovine Serum Albumin (BSA).

Standard plot of Bovine Serum Albumin:

A stock solution of BSA (1mg/mL, total volume of 5mL) was prepared. Solutions of different concentration were prepared as follows:

Table 4: Preparation of stock solutions for BSA

Solution	**Amount of stock soln. (1mg/mL) or (1000ug/mL) added (uL)**	**Amount of distd. water added (uL)**	**Concen-tration (ug/mL)**	**Optical Density (OD)**
Blank	0	500	0	-
1	10	490	20	0.053
2	20	480	40	0.104
3	30	470	60	0.114
4	50	450	100	0.211
5	100	400	200	0.340
6	200	300	400	0.618
7	500	0	1000	1.132

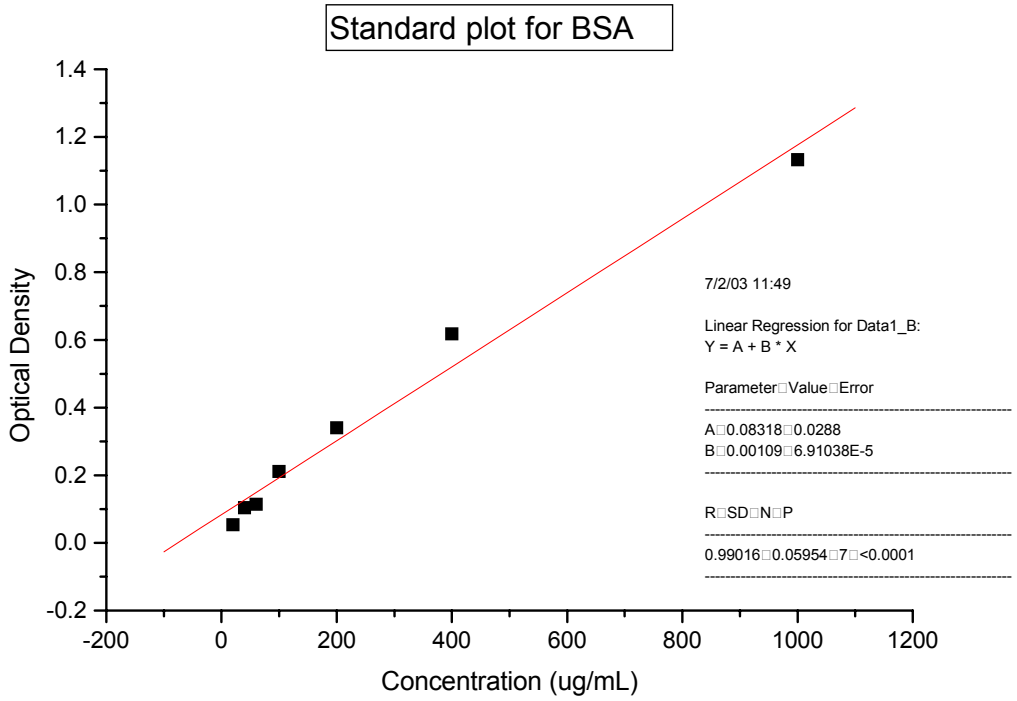


Figure 29: Standard plot for protein estimation of BSA

Table 5: Table for determination of protein concentrations of polymers subjected to biodegradation by Serratia marscecens

Sr.no.	Sample	OD	Concentration (ug/ mL)
1	Minimal medium	0.014	-
2	Control 1: Minimal medium with inoculum	0.219	128
3	Control 2: Glucose 500mg	0.648	517
4	Sucrose-linked PSMAH	0.309	202.5
5	Glucose-linked PSMAH	0.217	128
6	Lactose-linked PSMAH	0.223	133.7
7	Sucrose-linked PSMAH lauroylated	0.259	162.5
8	Glucose-linked PSMAH lauroylated	0.289	192
9	Lactose-linked PSMAH lauroylated	0.221	133.5
10	Sucrose-linked PSMAH acetylated	0.260	165.7

11	Glucose-linked PSMAH acetylated	0.280	186
12	Lactose-linked PSMAH acetylated	0.289	190
13	Lactose-linked PSMAH hexanoylated	0.227	133.7
14	Unmodified PSMAH	0.221	133.5
15	Glucose 8mg	0.177	87
16	Sucrose 8mg	0.160	71.5
17	Lactose 8mg	0.160	71.5
18	Acetic acid	0.250	157
19	Lauric acid 35.5mg	0.699	573
20	Hexanoic acid	0.282	184

The minimal medium showed a low protein concentration of 128ug/mL, whereas lauric acid control and glucose 500mg control showed very high protein concentration of 573 and 517 ug/mL respectively. The glucose 8mg, sucrose 8mg and lactose 8mg controls showed a very low protein content of 70-90ug/mL concentration. The sugar-linked poly(styrene maleic anhydride) polymers and their acylated derivatives showed intermediate contents of protein. Amongst them, the sucrose-linked poly(styrene maleic anhydride) showed the maximum amount of protein, which is consistent with the OD values.

Table 6: Table for determination of protein concentrations of polymers subjected to biodegradation by Pseudomonas sp.

Sr.no.	Sample	OD	Concentration (ug/ mL)
1	Control 1: Minimal medium with inoculum	0.416	303.2
2	Control 2: Glucose 500mg	1.157	980.7
3	Sucrose-linked PSMAH	0.447	333
4	Glucose-linked PSMAH	0.556	430

5	Lactose-linked PSMAH	-	-
6	Sucrose-linked PSMAH lauroylated	0.318	210
7	Glucose-linked PSMAH lauroylated	0.343	241
8	Lactose-linked PSMAH lauroylated	0.383	277
9	Sucrose-linked PSMAH acetylated	0.390	285
10	Glucose-linked PSMAH acetylated	0.410	297
11	Lactose-linked PSMAH acetylated	0.368	264
12	Lactose-linked PSMAH hexanoylated	0.424	313
13	Unmodified PSMAH	0.369	264
14	Glucose 8mg	0.369	264
15	Sucrose 8mg	0.342	239
16	Lactose 8mg	0.369	264

CHAPTER 9

Conclusions and suggestions for future work

Conclusions:

In conclusion, we were successful in developing a new strategy for synthesizing biodegradable polymers by linking minute quantities of carbohydrate molecules onto functionalized synthetic polymer, by polymer analogous reactions. Such polymers belong to a class of semi- synthetic polymers which have a unique blend of properties: biodegradability due to the natural component and processability due to the synthetic component. The work presented in this thesis is based on this new strategy. Poly(styrene maleic anhydride) was chosen as a model functionalized synthetic polymer. The overall conclusions from the present work are summarized below.

- A detailed literature search was done on the different methods employed for the synthesis of synthetic polymers bearing carbohydrates. The applications and the potential applications of these polymers have been listed. The advantages and the disadvantages associated with each method of synthesis have been described in detail. After a thorough study, we found that the synthesis of such polymers by polymer analogous reactions needs to be further explored, particularly for cases where low degrees of carbohydrate incorporation is desired.

- The biodegradation of such polymers with low degrees of carbohydrate incorporation by bacteria was studied by turbidimetry measurements after screening of various bacterial strains. It was found the rates of biodegradation using soil bacteria (viz. Serratia marscecens, Pseudomonas sp. and Bacillus sp.) were enhanced by incorporation of even minute quantities of sugars (typically in the range of 0.1-4.5 weight percentage). Weight loss measurements, GPC, FTIR spectroscopy and SEM confirmed the degradation

of the polymer. For biodegradation using fungal strains (Aspergillus niger, Pullularia pullulans, trichoderma sp. and P. ochro- chloron), only weight loss measurements were used to estimate degradation.

- Aerobic biodegradation would result in conversion of organic carbon into carbon dioxide and water. Hence the evolution of carbon dioxide was studied over a period of 15 days by a standard OECD method. However, we modified this method by using a pure strain of Serratia marscecens instead of activated sludge. The results were consistent with the turbidimetry data.

- It is well known that carbohydrates are not readily processable since they char at processing temperatures, rather than melting. Hence the sugars attached to the polymer were acylated using C_2 to C_{12} carboxylic acids. The effect of such a modification on the biodegradation and the thermal properties of the polymers was studied. The acylated derivatives were found to be more degradable as compared to poly(styrene maleic anhydride) but less degradable as compared to their sugar- linked counterparts, in case of both bacterial and fungal degradation. The sugar-linked poly(styrene maleic anhydride)s after acylation generally showed more weight losses upto 300°C as compared to their sugar-linked counterparts. The FTIR of the sugar- linked poly(styrene maleic anhydride) polymers and their acylated counterparts exposed to temperatures of 150°C, 250°C and 350°C for 30 mins. suggest that the carbohydrate molecule detaches from the polymeric backbone at a temperature of about 250°C, hence the processing times should be as minimum as possible.

- Glucose has five hydroxyl groups, which are all potentially reactive towards esterification. Fourier Deconvolution software of FTIR spectroscopy was used for the elucidation of the reactivities of the different hydroxyl groups of the glucose molecule towards esterifcation with the maleic anhydride groups on the polymer chain of PSMAH.

Suggestions for future work:

- The linking of sugars onto other functionalized polyolefins should be tried out, and the effect of such linking on the biodegradation of the polymer should be evaluated.
- In our study, we acylated the sugar hydroxyls for obtaining polymers possessing both biodegradability and processability. It would be interesting to study the effect of other hydroxy protecting groups like benzyl, phenyl isocyanate, etc. on these properties.
- The kinetics of the reactions should be studied to obtain process data.
- The mechanism of biodegradation needs to be studied, including the enzymes involved in the degradation process at various stages. This is particularly difficult since the medium also contains, in addition to the breakdown products of the polymer, products of the bacterial cell lysis. Methods will have to be developed to identify the products of bacterial and fungal degradation.
- Detailed studies on biominerization has to be carried out with a variety of microorganisms
- Exposure of the sugar- linked poly(styrene maleic anhydride) polymers and their acylated counterparts to processing temperatures for greater time intervals results in detachment of the carbohydrate molecule from the main polymer chain and results into weight losses during processing. Hence processing conditions should be optimized using minimum processing time.
- Efforts are also needed to synthesize different polymers using this strategy to obtain biodegradable polymers, which are cost effective. This is of particular importance, since the main limiting factor of the commercialization of the synthetic biodegradable polymers is its high cost.

List of Publications:

1. Towards biodegradable polyolefins: strategy of anchoring minute quantities of monosaccharides and disaccharides onto functionalized polystyrene, and their effect on facilitating polymer biodegradation
   Padmaja Galgali, Anjani J. Varma, Ulka S. Puntambekar, Digambar V. Gokhale, Chem. Commun., 2002, 2884-2885.

2. Fungal degradation of carbohydrate-linked polystyrenes
   P. Galgali, U. S. Puntambekar, D. V. Gokhale, and A. J. Varma
   Carbohydrate Polymers, 55, 393-399, 2004

3. Synthetic Polymers Functionalized by Carbohydrates : A Review
   A.J. Varma, J. F. Kennedy, and **Padmaja Galgali**,
   Carbohydrate Polymers (2004, Article in press)

4. Thermal Analysis of sugar- linked poly(styrene maleic anhydride)
   (Manuscript)

5. FTIR study: Elucidation of the reactivity of different glucose hydroxyls towards reaction with maleic anhydride
   (Manuscript)

Patents:

1. Biodegradable synthetic polymers and process for preparation thereof
   Anjani J. Varma, **Padmaja Galgali**, Digambar V. Gokhale, Ulka S. Puntambekar (December 2002)