# Synthesis and Characterization of Biodegradable Aliphatic Polyesters

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A series of aliphatic copolyesters were prepared from, 1,4-butane diol and suberic acid, succinic acid/adipic acid through a two step process of melt polycondensation using titanium tetra isopropoxide as catalyst. The synthesized polyesters were characterized by viscosity measurements, nuclear magnetic resonance spectroscopy, gel permeation chromatography, X-ray diffraction analysis and differential scanning calorimetry. The polyesters biodegradation was investigated by enzymatic hydrolysis using the enzyme, in a buffer solution with *Candida cylindracea* lipase and *Mucor miehei* lipase. The enzymatic degradation rates for the two different enzymes were observed and compared. The changes in the structure morphology during the degradation process were studied by scanning electron microscopy. It appears that biodegradation was affected by polyester crystallinity. The lowest crystalline polyester shows highest rate of degradation in both enzymes.

**Key Words: Biodegradable polyesters, Aliphatic polymers, Enzymatic degradation.**

## INTRODUCTION

Polymeric materials have invaded almost every human activity. This success is primarily due to their low cost, reproducibility at high speed and durability related to a high resistance to physical ageing and biological attacks. The rapid growth of plastics production is considered as a serious source of environmental pollution. Biodegradable polymer is an area that has great promise for being used to integrate life science into the chemical engineering curriculum. A current research area of significant environmental, economic and scientific improvements is biodegradable polymers. In recent years biodegradable polymers have attracted considerable attention as green material and biomaterials in pharmaceutical, medical and biomedical engineering applications, including drug delivery system, artificial implants and functional materials in tissue engineering.

Great efforts have been and are still being carried out towards the development of novel polymers that are able to undergo controlled biological degradation[1]. Montaudo *et al.*[2]. Suggested that there is strong evidence, that microbial biodegradation

of synthetic polyesters in nature is also initially caused by hydrolase's secreted from the microorganisms. Tokiwa and Suzuki[3] reported that synthetic polyesters can be attacked by hydrolase's. A number of other investigations on the degradation of synthetic polyesters by hydrolases have been published. There are strong evidences that microbial biodegradation of synthetic polyesters in nature is also initially caused by hydrolases secreted from the microorganisms[4-6].

Biodegradability of a certain polymer in the form of enzymatic hydrolysis is controlled by several factors. The most important one is the nature of the polymer itself, *i.e.*, its chemical structure, the occurrence of specific bonds along its chain, which might be susceptible to hydrolysis. It is also well known that the degree of crystallinity may be a crucial factor, since enzymes mainly attack the amorphous domains of a polymer. Copolymers with low crystallinity show increased hydrolysis rates. Conditions like temperature, pH and concentration of enzyme also play a particular role[7-10].

Recent reports have demonstrated that the dominating factor controlling biodegra-dability of aliphatic polyesters is the mobility of the polymer chains, which is correlated with the difference in the melting temperature of the crystalline action of the polyester and the temperature at which the degradation takes place[11].

Considering the high commercial potential of aliphatic copolyesters and their interesting properties, the present investigation deals with the synthesis and charac-terization of new type of biodegradable polymer with considerable enzymatic degradability was prepared from suberic acid, succinic acid/adipic acid and 1,4-butane diol. The structure of their repeating units and the effect of the copolymer composition on the physical and thermal properties as well as enzymatic degradation are investigated. The enzymes *Candida cylindracea* lipase and *Mucor miehei* lipase were used in the degradation studies.

## EXPERIMENTAL

Succinic acid (Merck, AR grade), adipic acid, succinic acid and suberic acid (Lancaster) were recrystallized from deionized water. 1,4-Butane diol (Lancaster) was dried with CaO overnight and then distilled under reduced pressure. Titanium tetra isoproxide (TTiPO) purchased from Lancaster was used as such. *Candida cylindracea* and *Mucor miehei* lipase were purchased from Sigma Aldrich. All the other materials and solvents used for the analytical methods were of analytical grade.

**Synthesis of polyesters:** Synthesis of polyesters was performed by using three-necked flask containing a mechanical stirrer, condenser and nitrogen inlet tube. The apparatus was charged with 0.1 mol of succinic acid/adipic acid and 0.1 mol suberic acid, 0.2 mol of 1,4-butane diol. The mixture was heated to 210 °C under nitrogen atmosphere and water was removed as the esterification by product. When water ceased to be generated, predetermined amount of titanium tetra isopropoxide catalyst was placed into the reaction flask. Subsequently, the pressure of the reaction

system was gradually decreased and melt polycondensation polymerization was continued at 210 °C under a final reduced pressure lower than 0.5 mm Hg. The second phase of the reaction was carried out under this condition for 20 h.

The viscous slurry was cooled in the nitrogen atmosphere. The crude polyester was dissolved in chloroform, precipitated into a 10-fold amount of vigorously stirred ice-cold methanol and dried under reduced pressure at room temperature. To remove the catalyst residues in the original polyesters, the corresponding original precipitated polyesters were further refluxed at 90 °C for a predetermined period using 8 fold molar excess of dilute HCl and then the reaction solutions were similarly precipitated in ice-cold methanol and dried.

The molecular weight and polydispersity index were calculated by gel permeation chromatography (GPC). Physical properties of the synthesized random copolyesters were characterized by $^1$H NMR, solution viscosity measurement, differential scanning calorimetry (DSC) and Wide Angle X-ray diffraction analysis (WAXD). For all polyesters melting point ($T_m$), glass transition temperature ($T_g$) and enthalpy of fusion ($\Delta H_m$) was recorded. The enzymatic degradation was performed in a buffer solution with *Candida cylindracea* lipase and *Mucor miehei* lipase. The enzymatic degradation rates for the two different enzymes were observed and compared.

**Enzymatic hydrolysis:** Copolyester thin films were obtained by hot pressing method. The thin films of area 10 mm$^2$ × 10 mm$^2$ and about 200 mm thickness were placed in a Petri dish containing 10 mL of phosphate buffer solution (pH 7.00 ± 0.01) with 1 mg/mL lipase from *Candida cylindracea*. After a specific period of incubation, the films were removed from the dish, washed with distilled water and weighed till the constant weight was obtained. This procedure was repeated for every chosen time interval: 9, 24, 48, 72 and 90 h.

The enzymatic hydrolysis was also carried out with another enzyme *Mucor miehei* using the same procedure as followed for *Candida cylindracea*. The degree of biodegradation was estimated from the weight loss percentage (D)

$$D = \frac{(m_0 - m_t)}{m_0} \times 100\,\%$$

(2)

where $m_0$ = weight of original films, $m_t$ = weight of residual films after degradation for different times.

## RESULTS AND DISCUSSION

**Gel permeation chromatography analysis (GPC):** Gel permeation chromatography analysis was used for the determination of the molecular weight distribution and the average molecular weights of the samples. The molecular weight and poly dispersity index of polymers were determined by GPC analysis and are presented in Table-1.

**Thermal studies:** Differential scanning calorimetry thermograms of the synthesized aliphatic polyesters are shown in Fig. 1. The thermal properties like melting

TABLE-1
GPC MEASUREMENTS OF POLYESTERS

| Polymer | $[\eta]^a$ (dL/g) | $M_n$ (g/mol) | $M_w$ (g/mol) | Poly dispersity index ($M_w/M_n$) |
|---------|---------|---------|---------|---------|
| PBSSu | 0.225 | 5621.8 | 12627.2 | 2.25 |
| PBASu | 0.335 | 8548.4 | 20134.8 | 2.35 |

temperature ($T_m$), glass transition temperature ($T_g$), enthalpy of melting ($\Delta H_m$) and decomposition temperature ($T_d$) have been evaluated and are summarized in Table-2.
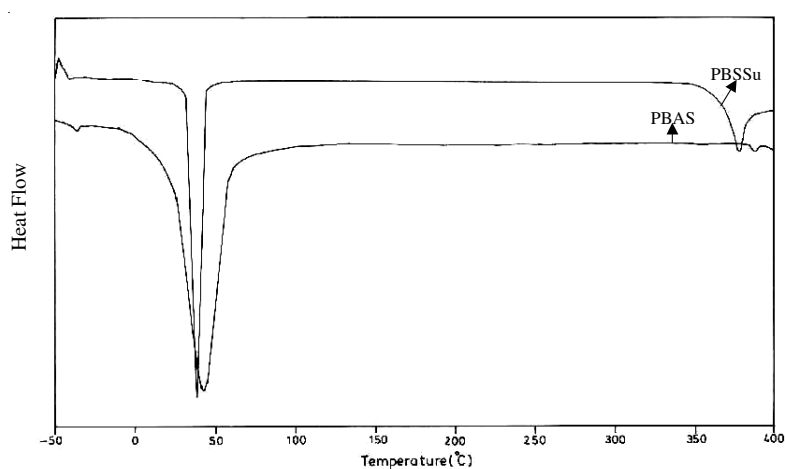


Fig. 1. DSC thermogram of polyesters

TABLE-2
THERMAL DATA OF SUBERIC ACID POLYESTER SERIES

| Polymer | $T_g$ (ºC) | $T_m$ (ºC) | $T_d$ (ºC) | $\Delta H_m$ (J/g) |
|---------|---------|---------|---------|---------|
| PBSSu | -43.2 | 40.0 | 382.8 | 93.61 |
| PBASu | -36.2 | 43.20 | 375.0 | 80.6 |

**^{1}H NMR studies:** ^{1}H NMR spectra of the three polymers are given in Fig. 2. The ^{1}H NMR spectra of these polyesters show signals characteristic of terminal and central methylene proton resonance of the tetramethylene group of 1,4-butane diol at 4.04-4.10 ppm and 1.69 ppm, respectively. The methylene protons of the succinic ester moiety give signals at 2.3 ppm. The terminal and central methylene protons of the sebacic ester residue appear at 1.60 and 1.25 ppm, respectively. The terminal and central methylene protons of suberic ester residue appear at about 1.69 and 1.25 ppm, respectively.

**X-Ray diffraction analysis:** WAXD was used to analyze the crystallinity of the copolyesters. Indicative WAXD patterns of the two polyesters are presented in Fig. 3. The PBSSu sample exhibited high degree of amorphous nature, due to the
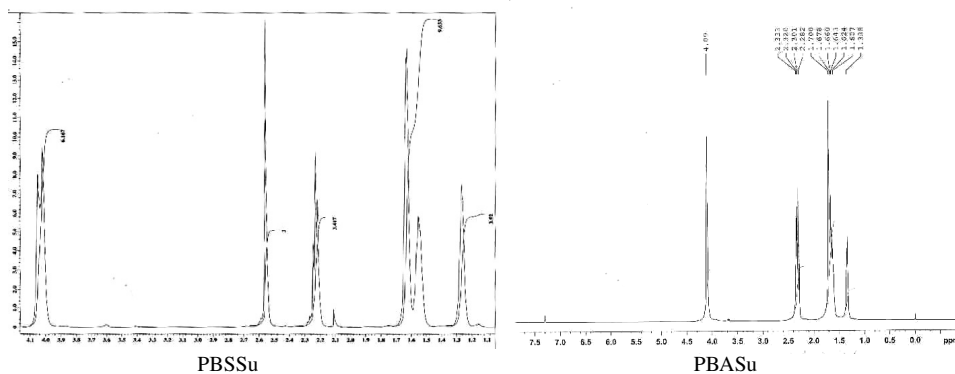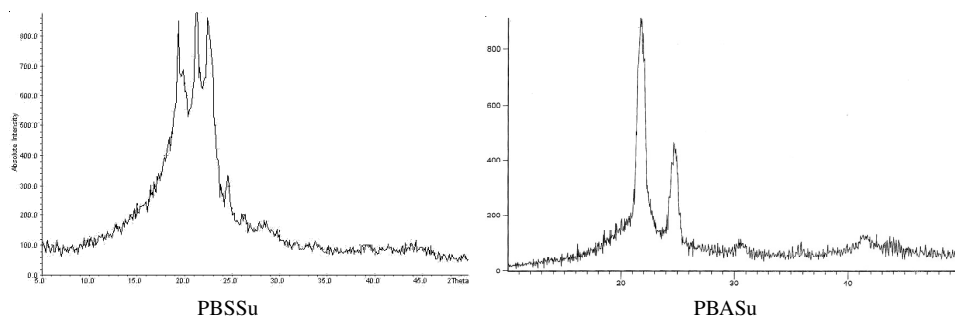
PBSSu

PBASu

Fig. 2. ¹H NMR spectra of copolyesters



PBSSu

PBASu

Fig. 3. WAXD diffrctogram of polyesters

lower crystallinity compared to PBASu samples. The comparison of the diffracto-grams of the polyesters reveals that the crystallinity of polymers increases with the increase in the number of methylene groups in the repeating unit of the polymer chain.

**Biodegradation studies:** Hydrolytic cleavage of the polyester chains takes place at random in the polymers. As degradation advances, smaller oligomeric species are formed leading to increase in weight loss and fragmentation of the polymers. Enzymatic hydrolysis is a heterogeneous process. Enzymes are attached on the surface of an insoluble substrate and hydrolysis takes place *via* surface erosion. In general, the internal parts of polyester thin films are not attacked until extended holes are created onto surface allowing the enzymes to penetrate and attack the main body.

The enzymatic degradation of the two polyesters PBSSu and PBASu were inves-tigated by *Candida cylindracea* and *Mucor miehei* lipase enzymes. The weight loss of the two copolyesters as a function of time is plotted. The enzymatic degradation of co polyesters by *Candida cylindracea* and *Mucor miehei* lipase is exhibited in Figs. 4 and 5, respectively.
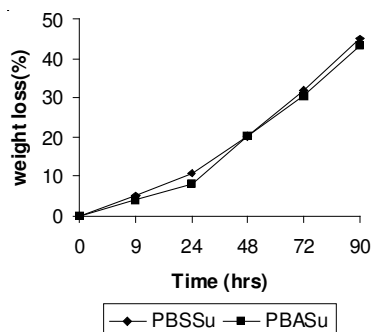
Fig. 4.    Biodegradation analysis of polyesters using *Candida cylindracea* lipase enzyme
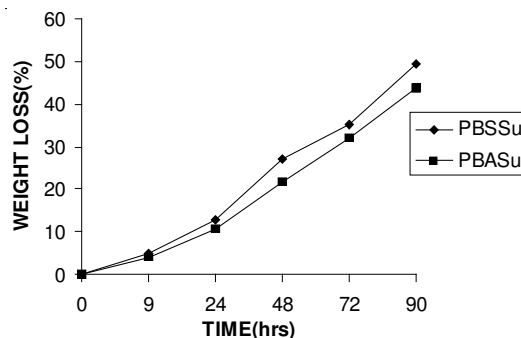


Fig. 5. Biodegradation analysis of polyesters using *Mucor miehei* lipase enzyme

**Scanning electron microscope analysis:** The hydrolytic effect of the two enzymes *Mucor miehei* lipase and *Candida cylindracea* lipase enzymes on copolyesters can be seen in SEM micrographs.

The effect of biodegradation upon the polymer surfaces was examined using scanning electron microscope. The morphology of the aliphatic polyesters was observed using SEM after enzymatic degradation. SEM micrographs of thin films taken before and after enzymatic hydrolysis are presented in Fig. 6. The SEM micrographs of the polyesters before degradation exhibits a homogenous and smooth surface. The image of polyester films after enzymatic hydrolysis shows small holes and cracks which influence the surface erosion of thin films. The enzymatic degradation was performed using *Candida cylindracea* and *Mucor miehei*. Though the erosion
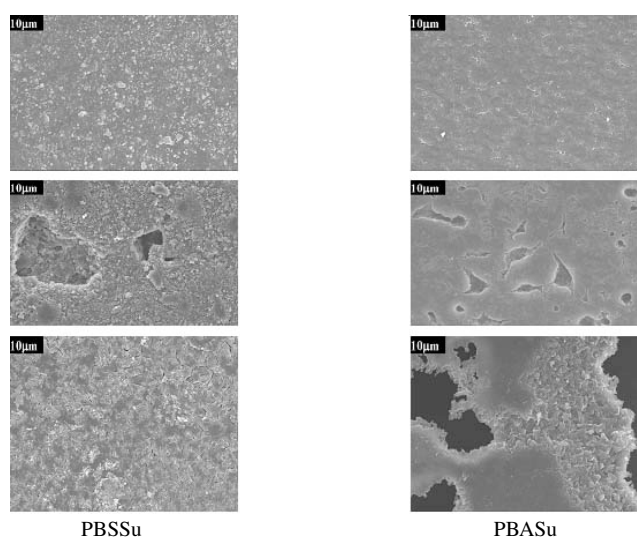


PBSSu                                PBASu

Fig. 6.    SEM micrographs of polyesters (PBASu, PBSSu) during enzymatic hydrolysis. (A) Before degradation (B) after degradation using *Candida cylindracea* lipase enzyme (C) After degradation using *Mucor miehei* lipase enzyme

was observed in both, using *Candida cylindracea* and *Mucor miehei*, it was slightly higher in the later case than the former. The SEM micrographs of PBSSu demonstrated that hydrolysis occurred all over the surface of polyester thin film creating holes, in contrast to PBASu films, where degradation was observed only at specific regions inferring that PBASu have less amorphous regions than PBSSu.

**Conclusion**

A new type of biodegradable polymer with considerable enzymatic degradability was prepared from suberic acid, succinic acid/adipic acid and 1,4-butane diol. Enzymatic degradability was determined on the basis of weight loss in buffer solution with lipase enzyme. Disintegration of the copolyesters occurred during degradation. Disintegration of the copolyesters occurred during degradation. The hydrolytic degradation of polyester films in the presence of *Mucor miehei* lipase is relatively faster than *Candida cylindracea* lipase. Biodegradation showed that amongst the various parameters affecting polymer degradation, crystallinity was the dominant factor. For this reason, the higher biodegradation rates are observed for the copolymers PBSSu, which exhibit lower crystallinity. SEM micrographs reveal that hydrolysis occurred at the specific area of the surface of PBSA and PBSSu specimens, where as the hydrolysis occurred all over the surface of the polyester PBSSu and creating holes. From the thermal analysis data and in comparison with the SEM micrographs, it is established that amorphous areas of polyesters are preferably hydrolyzed. It was concluded that hydrolysis of PBSSu film using both enzymes are higher than that of PBSA as expected due to lower crystallinity and lower melting point of the polymer. Thus, the rate of enzymatic degradation is influenced by the degree of crystallinity of the polymer.

## REFERENCES

1.  D.N. Bikiaris, G.Z. Papageorgiou and D.S. Achilias, *Polym. Degrad. Stab.*, **91**, 31 (2006).
2.  G. Montaudo and P. Rizzarelli, *Polym. Degrad. Stab.*, **70**, 305 (2000).
3.  Y. Tokiwa and T. Suzuki, *Nature*, **270**, 76 (1977).
4.  J.M. Raquez, P. Degee, R. Narayan and S.P. Duboi, *Macromolecules*, **34**, 8419 (2001).
5.  V. Tserki, P. Matzinos, E. Pavlidou, D. Vachliotis and C. Panayiotou, *Polym. Degrad. Stab.*, **91**, 367 (2006).
6.  Y. Ikada and T.H. Suji, *Macromol. Rapid Commun.*, **21**, 17 ( 2000).
7.  D. Bikiaris, J. Aburto, I. Alric, E. Borredon, M. Botev, C. Betchev and C. Panayiotou, *J. Appl. Polym. Sci.*, **71**, 1089 (1999).
8.  J. Aburto, I. Alric, S. Thiebaud, E. Borredon, D. Bikiaris, J. Prinos and C. Panayiotou, *J. Appl. Polym. Sci.*, **74**, 1440 (1999).
9.  G. Seretoudi, D. Bikaris and C. Panijiotot, *Polymer*, **43**, 5405 (2002).
10. C.-J. Tsai, W.-C. Chang, C.-H. Chen, H.-Y. Lu and M. Chen, *Eur. Polym. J.*, **44**, 2339 (2008).
11. V. Jaisankar, R. Nanthini, A. Ravi and M. Karunanidhi, *J. Polym. Mater.*, **26**, 157 (2009).