ENZYMATIC DEGRADATION OF POLY(SOYBEAN OIL-g-METHYL METHACRYLATE)

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ABSTRACT

This study discusses grafting of methyl methacrylate units from the polymeric soybean oil peroxide to produce poly(soybean oil-graft-methyl methacrylate) (PSO-g-PMMA). The degradation of this copolymer in solution was evaluated in the presence of different lipases, viz., *Candida rugosa* (CR), Lipolase 100T (LP), Novozym 435 (N435) and *Porcine pancreas* (PP), at different temperatures. The copolymer degraded by specific chain end scission and the mass fraction of the specific product evolved was determined. The degradation was modeled using continuous distribution kinetics to determine the rate coefficients of enzymatic chain end scission and deactivation of the enzyme. The enzymes, CR, LP and N435 exhibited maximum activity for the degradation of PSO-g-PMMA at 60 °C, while PP was most active at 50 °C. The thermal degradability of the copolymer, assessed by thermo-gravimetry, indicated that the activation energy of degradation of the copolymer was 154 kJ mol^{-1}, which was lesser than that of the PMMA homopolymer.

Keywords: PMMA; soybean oil; lipases; biodegradable polymers; continuous distribution kinetics

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INTRODUCTION

Synthetic polymers (plastics) have widespread applications and the worldwide production is over 100 million tons. However, these materials have serious disposal problems and thus occupy nearly 10% by volume of the buried waste /1/. Many reviews /2-4/ have dealt with the various methods of plastics waste disposal and the importance of synthesizing new biodegradable polymers.

Biodegradation of polymers is defined as a process that is capable of decomposing polymeric materials into carbon dioxide, methane, water, inorganic compounds, or biomass in which the predominant mechanism is the enzymatic action of the microorganisms /4/. The two key steps that occur during the biodegradation of polymers are (i) depolymerization or chain cleavage and (ii) mineralization /5/. The first step is characterized by an increase in surface area of the polymer, resulting in the weakening of the polymer matrix, thereby leading to the unzipping of the monomeric and oligomeric units from the chain terminal. Extracellular enzymes are responsible for this step. In the second step, the oligomeric fragments are converted to biomass, minerals, salts and water with the evolution of gases. Lipases represent a group of carboxylic acid hydrolases, a subclass of esterase enzymes, which are isolated from a wide variety of mammalian and microbial sources /5/. These enzymes primarily catalyze the hydrolysis of triglycerides into diglycerides, monoglycerides, glycerol and fatty acids. Lipases also exhibit substrate and positional specificity and are used in stereoselective catalysis for the degradation of aliphatic polyesters in organic solvents /6/. We have previously studied the lipase catalyzed degradation of poly(bisphenol-A carbonate) /7/, poly(ε-caprolactone) /8/, poly(vinyl acetate) /9/, poly(D,L-lactide) /10/, and their blends /10,11/ in organic solvents.

Most of the commercial plastics like poly(methyl methacrylate) (PMMA), poly(styrene), poly(ethylene), poly(propylene) resist biodegradation by enzymes due to their hydrophobic character, high molecular weight and the absence of hydrolyzable bond in their structure. Therefore, there is a need to modify these plastics by blending or copolymerizing with other natural polymers, so that the enzymes could diffuse into the polymer and break the carbon components, thereby resulting in degradation.

In this work, we study the mechanism and kinetics of degradation of a hybrid copolymer of PMMA using lipases. PMMA is a transparent, glassy and dimensionally stable polymer that is used in intraocular lenses, dental cements and hip implants, amongst a host of other applications. As the PMMA homopolymer is non-biodegradable, attempts have been carried out to graft copolymerize it with 3-hydroxybutyrate /12/, δ-valerolactone /13/, starch /14/, and mercaptochitin /15/. Apart from utilizing natural polymers for the graft copolymerization, recent investigations have also been on the synthesis of polymers from renewable resources like vegetable oils. Among the various vegetable oils, polymeric soybean oil and soybean curd have found potential applications in lubricants and hydraulic fluids /16/ and bone filling /17/, respectively. Soybean oil contains residues of linoleic (51%), oleic (25%), palmitic (11%), α-linolenic (9%) and stearic acids (4%) /18/. Hence, the different levels of unsaturation it offers can be utilized for hydroxylation, epoxidation and perepoxidation to produce polymerizable triglycerides via autoxidation /19/.

Recently, polymeric soybean oil-graft-methyl methacrylate (PSO-g-PMMA) has been synthesized /18/ by peroxidation, epoxidation and/or perepoxidation of soybean oil and subsequent graft copolymerization with MMA. Though the adhesion of fibroblast and macrophage cells on the copolymer has been investigated /20,21/, the enzymatic degradation of the copolymer was not examined. We aim at augmenting the current literature by evaluating the biodegradation of this potential copolymer, which has a non-biodegradable polymer grafted onto a biodegradable polymer backbone.

The objective of the current study is four fold. Firstly, we synthesize and study the enzymatic degradation of PSO-g-PMMA in the presence of four lipases, from different sources, viz., *Candida rugosa* (CR), *Thermomyces lanuginosus* (Lipolase 100T, LP), *Candida antarctica* (Novozym 435, N435) and *Porcine pancreas* (PP). Secondly, we develop kinetic models for the enzymatic degradation based on continuous distribution kinetics and evaluate the rate coefficients for degradation. Thirdly, we compare the effectiveness of each of the enzyme at different temperatures for maximum degradation. Finally, we compare the biodegradation with the conventional pyrolytic degradation and examine the thermal stability of the copolymer with that of the homopolymer.

EXPERIMENTAL

Materials

The enzymes LP and N435 were gifted by Novo Nordisk, Denmark. The enzymes CR and PP were procured from Sigma Aldrich, USA. MMA (Sigma Aldrich, USA) was purified by washing with 5% caustic solution and distilled water, and distilled prior to use. The solvents, tetrahydrofuran (THF), toluene, chloroform and methanol (Merck, India) were of HPLC grade.

Synthesis of polymeric soybean oil peroxide

Polymeric soybean oil was prepared in a photoreactor using 125 W high pressure mercury vapor lamp as the light source. The lamp radiated predominantly at 365 nm and the light intensity and photon flux determined by o-nitrobenzaldehyde actinometry /22/ were 5.8×10^{-6} Einstein L^{-1} s^{-1} and 12.8 W m^{-2}, respectively. The lamp was placed inside a jacketed quartz tube of 3.4 cm i.d., 4 cm o.d. and 21 cm length. Water was circulated in the annulus to quench the heat generated by the UV radiation. 20 mL of soybean oil was taken in a glass container of 6 cm i.d. and 16 cm height, and the quartz tube was immersed into the container so that the oil film in between the glass container and the quartz tube was irradiated. The soybean oil was stirred well for proper mixing. The formation of the polymeric peroxide of soybean oil was monitored by determining the number average molecular weight (M_n) by gel permeation chromatography (GPC). The molecular weight initially increased with time and reached a maximum value of 1780, with a polydispersity (PDI) of 1.3 at the end of 14 h, after which it remained constant.

Synthesis of graft copolymer

PSO-g-PMMA was prepared by bulk polymerizing polymeric soybean oil peroxide with MMA in culture tubes. In a typical synthesis, 50/50 mol% of both the reactants were taken in 10 mL and heated to 80 °C in a water bath with a PID temperature controller. The reactant mixture was purged with nitrogen for 15 min before polymerization. After 12 h of polymerization, the unreacted monomer and polymeric soybean oil were separated from the

polymer by fractional precipitation. Chloroform was used as the solvent and methanol was the non-solvent. The precipitated polymer was dried at 100 °C till constant weight was obtained.

Characterization of the copolymer

Fourier transform infrared (FTIR) spectrum of PSO-g-PMMA was recorded in transmission mode at a resolution of 4 cm^{-1}, in Perkin Elmer Spectrum RX-I spectrometer. The copolymer was cast as a pellet with KBr and analyzed.

The copolymer was also characterized by ^1H NMR spectroscopy. ^1H NMR spectra was recorded in AMX 400 MHz multinuclear FT-NMR spectrometer using $CDCl_3$ as the solvent and tetramethyl silane as the internal reference.

The molecular weight of PSO-g-PMMA was determined using GPC. The GPC consisted of a Waters 510 isocratic pump, a Rheodyne injector (sample loop - 50 µl), three size exclusion columns of varying pore size (HR 5E, HR 3 and HR 0.5, measuring 300 mm x 7.5 mm and maintained at 50 °C), a Waters 2410 differential refractometer and a data acquisition system. THF was used as the eluent at a flow rate of 1 mL min^{-1}. The chromatogram was converted to molecular weight distribution (MWD) using a conventional calibration curve determined using poly(styrene) narrow standards (Polymer Lab, USA).

Enzymatic degradation experiments

Copolymer of concentration 2 g L^{-1} in toluene was used for the degradation studies. 15 ml of the polymer solution was taken in a screw cap culture tube along with 15 mg of the enzyme and placed in a thermostated incubator shaker to maintain the temperature of the reaction mixture within ± 0.1 °C. The reaction mixture was stirred at regular intervals for efficient mass transfer of the reactants and products. Aliquots of 200 µL were withdrawn for the analysis of molecular weight by GPC. The sample was centrifuged to isolate the enzymes. These experiments were conducted to investigate the effect of various enzymes – CR, LP, N435 and PP at four different temperatures (40, 50, 60 and 70 °C). The experiments were also carried out without any enzyme under the same experimental conditions and it was

found that no significant degradation of the copolymer occurred at the end of 15 days. Experiments were also conducted by varying the amount of enzyme and it was determined that increasing the enzyme loading above 15 mg did not have significant effect on the rate of degradation. All experiments were conducted in triplicate and the standard deviation in the rate coefficients was less than 3%.

Pyrolytic degradation

PSO-g-PMMA was subjected to pyrolysis in a Perkin Elmer Pyris Diamond thermogravimetric-differential thermal analyzer (TG/DTA). Approximately 7 mg of the copolymer was pyrolyzed at different heating rates from room temperature to 700 °C in the presence of inert nitrogen at a flow rate of 150 cc min^{-1}.

THEORETICAL MODEL

The present continuous distribution kinetics model for the chain end scission of the copolymer in the presence of enzymes is the same as proposed earlier /8,9/. Let *P(x)* denote a polymer chain of molecular weight *x*, where *x* is a continuous variable. The chain end scission of PSO-g-PMMA can be represented as

$$P(x) \xrightarrow{k_s} Q(x_s) + P(x - x_s) \tag{1}$$

where, $Q(x_s)$ denotes the chain end product of constant molecular weight x_s and k_s represents the chain end scission rate coefficient. The population balance equation (PBE) for the polymer species and the specific products, involved in reaction (1), is /8,9,23/

$$\frac{\partial p(x,t)}{\partial t} = a(t)\int_x^\infty k_s p(x',t)\Omega(x,x'-x_s)\,dx' - k_s a(t) p(x,t) \tag{2}$$

$$\frac{\partial q(t)}{\partial t} = \int_{x_s}^\infty k_s a(t) p(x',t)\Omega(x_s,x')\,dx' \tag{3}$$

where, *a(t)* is the activity of the enzyme, which is assumed to decrease

exponentially with time, based on the equation

$$a(t) = a_0 \exp(-k_d t) \tag{4}$$

where, a_0 is the activity of the enzyme at time, t = 0 and k_d is the enzyme deactivation rate constant.

The stoichiometric kernel, $\Omega(x_s, x')$ in equation (3) determines the distribution of scission products. For chain end scission, the scission products follow a delta function given by, $\Omega(x_s, x') = \delta(x_s, x)$ /23/. Since the polymer undergoes chain end scission, k_s is assumed to be independent of chain length /8,9,23/. The integro-differential PBEs (2) and (3) can be reduced to ordinary differential equations by applying the moment operation given by /23/

$$p^{(j)}(t) = \int_0^\infty x^j p(x,t) dx \tag{5}$$

The equations (2) and (3) get transformed to

$$\frac{dp^{(j)}(t)}{dt} = k_s a(t) \sum_{k=0}^{j} (-x_s)^j p^{(j-k)}(t) - k_s a(t) p^{(j)}(t) \tag{6}$$

$$\frac{dq^{(j)}(t)}{dt} = k_s a(t) p^{(0)}(t) x_s^j \tag{7}$$

By setting j=0 and j=1 in equations (6) and (7), the rate of change of molar and mass concentration of the polymer and chain end products can be obtained as

$$\frac{dp^{(0)}}{dt} = 0 \tag{8}$$

$$\frac{dp^{(1)}}{dt} = -k_s a(t) x_s p^{(0)} \tag{9}$$

$$\frac{dq^{(0)}}{dt} = k_s a(t) p^{(0)} \tag{10}$$

$$\frac{dq^{(1)}}{dt} = k_s a(t) x_s p^{(0)} \tag{11}$$

where $p^{(0)}$, $p^{(1)}$, $q^{(0)}$ and $q^{(1)}$ represent the molar concentration and mass concentration of the polymer and specific product, respectively. Equation (8) shows that $p^{(0)}$ is invariant with time and hence it can be written as $p^{(0)} = p_0^{(0)}$.

The degradation reached a saturation limit with all the enzymes in the temperature range investigated. A possible explanation for this can be due to the saturation of enzyme active sites by the polymer molecules. This leads to the reduction in activity of the enzyme with time, denoted by equation (4). To confirm this, after a saturation M_n was reached, experiments were carried out with a fresh batch of enzyme. The polymer degraded further with the new batch of enzyme. In a similar experiment, the enzyme that was used for a reaction was used for degrading fresh polymer sample. This showed that there was no degradation of the polymer even after long reaction periods. These observations confirm enzyme deactivation. Hence, substituting equation (4) in equation (10) yields

$$\frac{dq^{(1)}}{dt} = k_s a_0 \exp(-k_d t) x_s p^{(0)} \tag{12}$$

Solving equation (12) with initial condition, $q^{(1)}(t=0) = 0$ gives

$$\frac{q^{(1)}(t)}{p_0^{(1)}} = \frac{k_s a_0 x_s}{k_d M_{n0}} (1 - \exp(-k_d t)) \tag{13}$$

where $M_{n0} = \frac{p_0^{(1)}}{p_0^{(0)}}$ is the initial M_n of the polymer at time t=0. Using the limiting condition that the enzyme is inactive at long periods yields,

$$q^{(1)}(t \to \infty) = q_\infty^{(1)} = \frac{k_s a_0 x_s p_0^{(1)}}{k_d M_{n0}} \tag{14}$$

By using a normalization defined by $q(t) = \frac{q^{(1)}(t)}{q_\infty^{(1)}}$, equations (13) and (14) can be reduced to

$$1 - q(t) = \exp(-k_d t) \tag{15}$$

Equation (15) shows that k_d can be obtained from the semi-logarithmic

plot between [1-q (t)] and t. The value of k_s can now be obtained from equation (14) by incorporating the k_d value from the semi-logarithmic plot. Using these values of k_s and k_d, the time evolution of the experimental $\frac{q^{(1)}(t)}{p_0^{(1)}}$ is predicted from equation (13).

RESULTS AND DISCUSSION

Copolymer characterization

Figure 1 depicts the FTIR spectrum of the copolymer from 4000-400 cm^{-1}. A strong absorption band in the region 2947-2830 cm^{-1} signifies the stretching vibration of the carbon-hydrogen in $-CH_3$, $-CH_2$ and $-CH$ groups. Another strong absorption at 1736 cm^{-1} can be assigned to the carbonyl (C=O) stretching vibration of the methacrylate. The acrylate C–O stretching vibration is observed as a band in the region 1280-1230 cm^{-1}. Absorptions around 1452 cm^{-1} and 1383 cm^{-1} denote the C–H deformation of $-CH_2$ and $-CH_3$ groups. The $-O-CH_2$ stretch of an aliphatic ester is observed at 1060 cm^{-1}. In the finger print region, a strong absorption at 750 cm^{-1} shows the rocking of the $-(CH_2)_n-$ chain for $n \geq 4$, indicating the presence of the methylene links found in soybean oil peroxide. Thus the FTIR spectrum reveals the proper grafting of MMA units onto soybean oil peroxide.

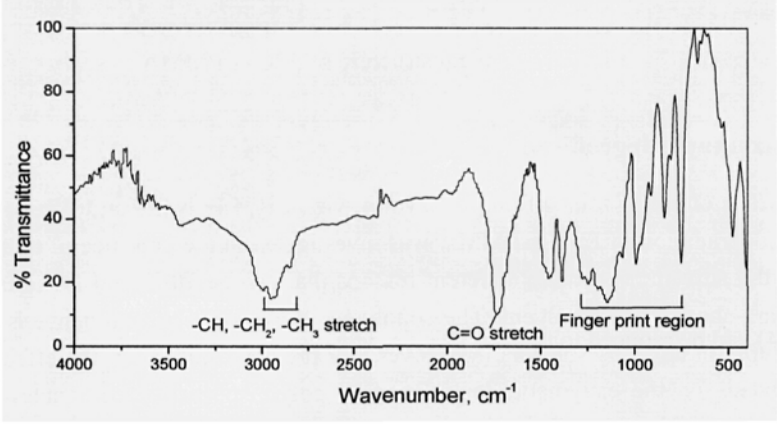

Fig. 1: FT-IR spectrum of PSO-g-PMMA.

The ^{1}H NMR peak assignment for the various groups in the copolymer was carried out, as reported by Çakmaklı et al. /18/. The relative composition of the individual monomer units in the copolymer was calculated from the relative area of the peaks at 3.7 and 4.2 ppm, which correspond to $-COOCH_3$ of MMA and $-CH_2$ of the triglyceride, respectively. The percentage inclusion of the polymeric soybean oil in the copolymer was thus found to be 8.12 %.

The MWD of the copolymer showed a single peak, with M_n of 85,000 and PDI of 1.45. Therefore, based on the above characterizations, the structure of PSO-g-PMMA with one soybean oil backbone, containing the glycerides of oleic, linoleic and α-linolenic acid can be represented as shown in Figure 2.

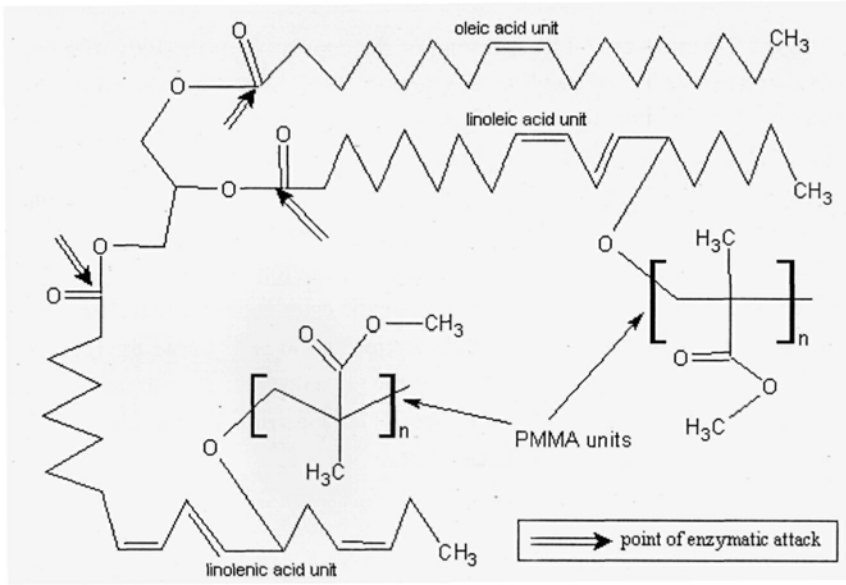

Fig. 2: Chemical structure of PSO-g-PMMA.

Enzymatic degradation

The effect of four different enzymes, viz., CR, LP, N435 and PP, on the biodegradation of PSO-g-PMMA was investigated in the presence of toluene as the solvent. There are different reasons that can be attributed for toluene being chosen as the solvent. The copolymer is hydrophobic and hence is not soluble in aqueous solvents. Moreover, our previous studies on the effect of solvents on the enzymatic degradation of poly(bisphenol-A carbonate) /7/, poly(ε-caprolactone) /8/, and poly(alkyl)acrylates /24/ indicated that toluene is one of the preferred solvents, as the enzyme activity is dependent on both

the viscosity and polarity of the solvent. Higher viscosity affects the transport of the polymer to the enzyme active site and polar solvents significantly desorb the bound water on the enzyme, thereby leading to lower enzymatic activity /25/.

Figure 3 shows the time evolution of GPC chromatogram, when PSO-g-PMMA was degraded at 60 °C, in presence of CR. It is evident that low molecular weight products are formed with M_n of 600. As time progressed, the height of this peak increased signifying the increase in mass concentration of the low molecular weight products. At the end of 15 days, their concentration was constant, without any change in M_n. Also, there is an observable reduction in area of the polymer peak signifying the reduction in polymer mass concentration. These confirm that the copolymer degrades by chain end scission, i.e., by the unzipping of the monomeric and oligomeric units from the chain end.

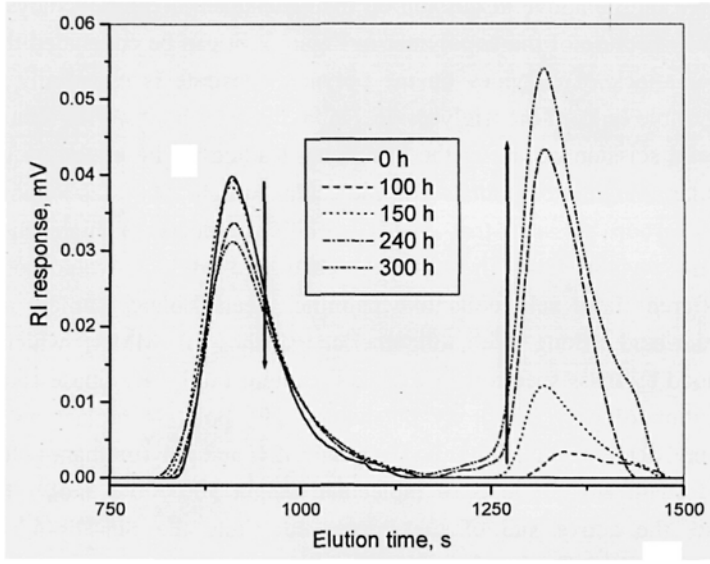

Fig. 3: Time evolution of the GPC chromatogram for the degradation of PSO-g-PMMA in the presence of CR at 60 °C

The above observations suggest that the lipases which are employed here for the degradation are active at specific sites. A three dimensional structural study of the lipases indicated that they share a common motif, known as α/β-hydrolase fold /26/. The catalytic activity of a lipase can be attributed to the

process called interfacial activation. This involves the change in conformation of the enzyme from the 'closed' to the 'open' structure. The switching mechanism for the two conformations is due to a helical segment called a 'flap' or 'lid'. It is the hinge type motion of the lid that leads to the accessibility of the active site to the solvent, thereby leading to adsorption and hence the formation of transition complex between the polymer and enzyme, due to hydrophobic interactions. This is generally known as an acyl-enzyme intermediate, which for *Candida antarctica* is tetrahedral in structure /27/. The degradation of the polymer from the enzyme intermediate occurs 'exo' to the polymer chain, as the enzyme cannot penetrate into the randomly coiled polymer chain. This leads to the formation of chain end products by the unzipping of the monomer, oligomer or side groups from the enzyme bound active sites. Thereafter, due to the motion of solvent molecules, the polymer is desorbed from the enzyme.

Based on the above discussion on the mechanism of lipase catalysis and from the structure of the copolymer in Figure 2, it can be concluded that the point of attack of enzymes on the polymer substrate is essentially at the hydrolyzable links of the triglyceride, i.e., at the ester bonds. This means that chain end scission occurs at the backbone, leading to the evolution of low molecular weight compounds (of molecular weight 600), as opposed to pendant group scission that yields specific products of high and low molecular weights /28/. Therefore, the chain end products would comprise the different fatty acid units like palmitic, stearic, oleic, linoleic and α-linolenic acid, along with oligomeric branches of MMA, which was confirmed by mass spectrometric analysis. Interestingly, the lipase catalyzed degradation of poly(3-hydroxy butanoate) /29/, poly(ε-caprolactone) /6,8/, poly(vinyl acetate) /11/, poly(alkyl)acrylates /24/ and poly(urethane) /30/ also yielded chain end products of molecular weight 500-700 g mol^{-1}. This is because, the active site of the lipases constitute the Ser-His-Asp triad sequence in the α/β-hydrolase fold. Extensive crystallographic studies /31/ prove that, in lipases, a narrow and deep channel of 10 Å × 4 Å width and 12 Å depth leads to the 'open' configuration of active site. The entrance of the active site is surrounded by a large hydrophobic surface of 450 $Å^2$ surface area. The shape of the channel accounts for the stereospecificity of the enzyme. Therefore, it can be envisioned that the length of the polymeric chain end that is bound to the active site of the enzyme through the channel is limited to the dimensions of the channel. Accounting for the many

conformations which a polymer chain assumes, it can be expected that only chain ends of molecular weight 500-700 g mol^{-1} could be accommodated inside the channel. Therefore, the molecular weight of the chain end product obtained for the degradation of different polymers with different lipases depends only on the active site of the enzyme and independent of the polymer or its chain length.

The enzymatic degradation of the PSO-g-PMMA was also investigated at different temperatures from 40 to 70 °C. Figures 4, 5 and 6 show the kinetics of degradation of the copolymer with all the enzymes at 50, 60 and 70 °C, respectively, and figure 7 shows the degradation of the copolymer at 40 °C with PP. Figures 4a, 5a, 6a and 7a represent the linear semilog plot of $[1-q(t)]$ with time. k_d for all the systems was obtained from the slope given by equation (15). Figures 4b, 5b, 6b and 7b represent the variation of mass fraction of the specific product with time, given by equation (13). Using the limiting condition given by equation (14), k_s was determined and incorporated in equation (13) to predict the experimental data without any fitting parameters. A unit activity ($a_0 = 1$) of the enzymes was assumed. It is clear that the model fits the experimental data remarkably well and the regression coefficients were close to 0.99. The rate coefficients, k_d and k_s for the degradation of PSO-g-PMMA with all the enzymes are depicted in Figs. 8 and 9, respectively. The order of magnitude of the rate coefficients agree well with our previous studies on the lipase catalyzed degradation of poly(vinyl acetate) /9/, poly(bisphenol-A carbonate) /7/, and poly(alkyl)acrylates /24/. A careful comparison of the rate coefficients at different temperatures for all the enzymes suggest that CR, LP and N435 are more active at 60 °C, while PP exhibits maximum activity at 50 °C. Recently, an equilibrium model has been proposed and validated for the dependence of enzyme activity on temperature /32/. According to this mechanism, enzymes lose their activity at high temperatures and assume an inactive form, which is in reversible equilibrium with the active form. It is this inactive form that undergoes irreversible thermal inactivation and attains a thermally denatured state. This confirms that the optimum temperature at which the enzyme activity is maximum is independent of the polymer and depends only on the conformation of the enzyme. Thus, the optimal temperatures observed in this study are consistent with the earlier works /7,9,24/ on the degradation of different biodegradable polymers.

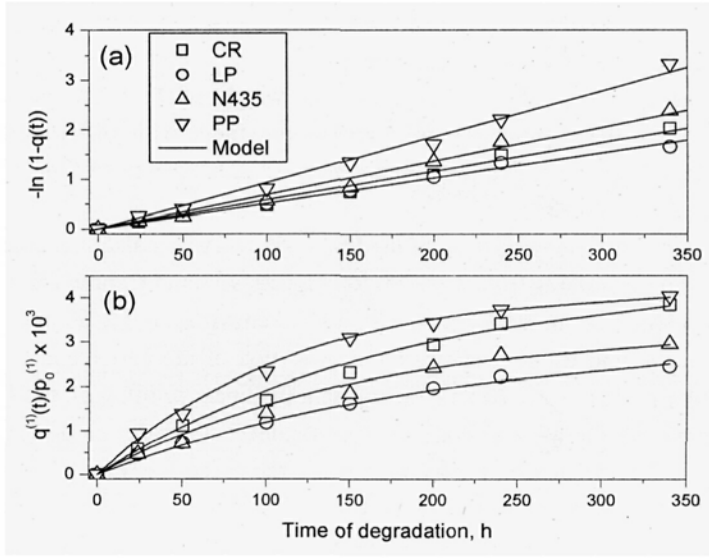

Fig. 4: Variation of (a) –ln [1 - $q(t)$] and (b) mass fraction of specific products with degradation time for the degradation of PSO-g-PMMA in presence of different enzymes at 50 °C.

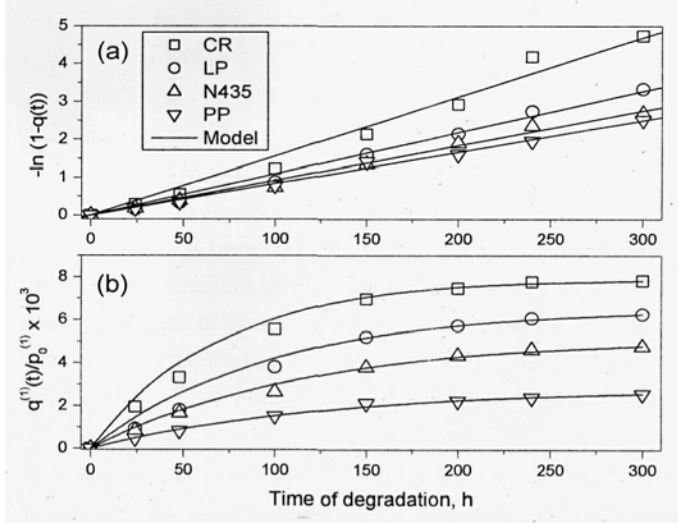

Fig. 5: Variation of (a) -ln[1 - $q(t)$] and (b) mass fraction of specific products with degradation time for the degradation of PSO-g-PMMA in presence of different enzymes at 60 °C.

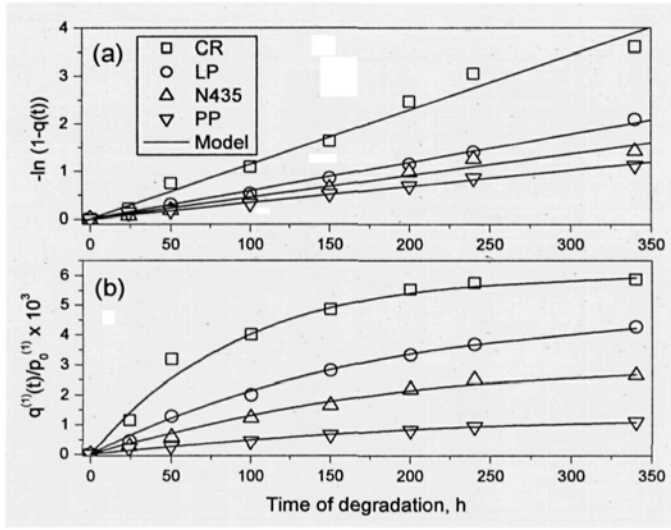

Fig. 6: Variation of (a) –ln [1 - $q(t)$] and (b) mass fraction of specific products with degradation time for the degradation of PSO-g-PMMA in the presence of different enzymes at 70 °C.

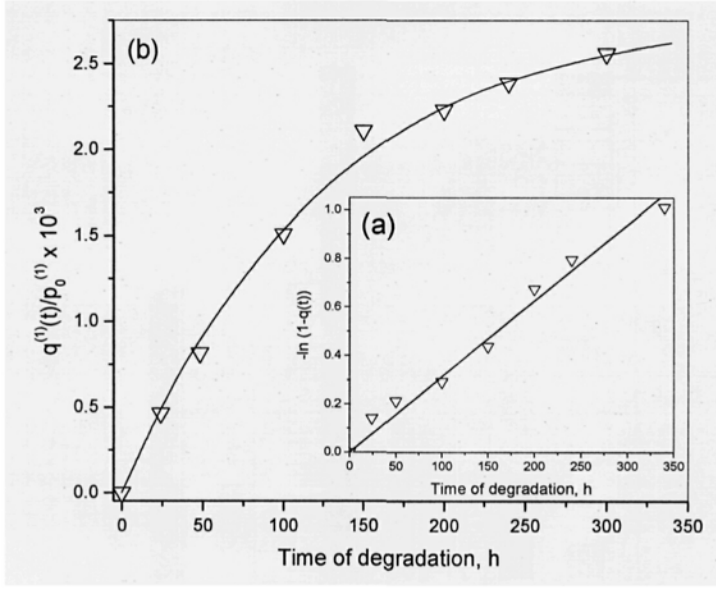

Fig. 7: Variation of mass fraction of specific products with degradation time for the degradation of PSO-g-PMMA in presence of PP at 40 °C. [Inset: Linear variation of –ln [1-$q(t)$] with time/

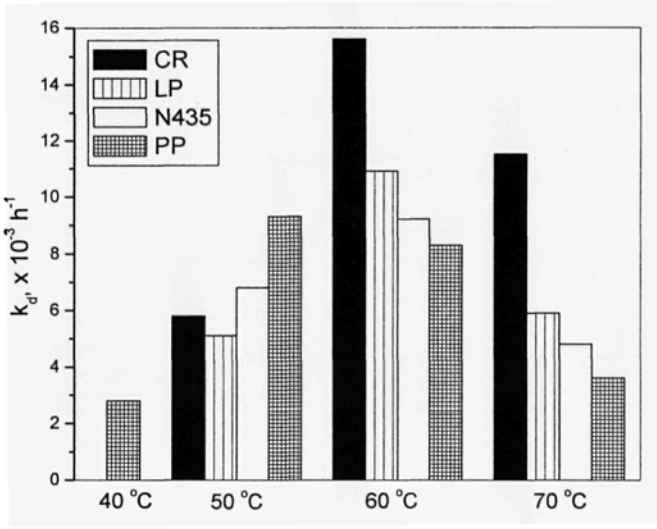

Fig. 8: Variation of k_d with temperature for the degradation of PSO-g-PMMA in the presence of different enzymes.

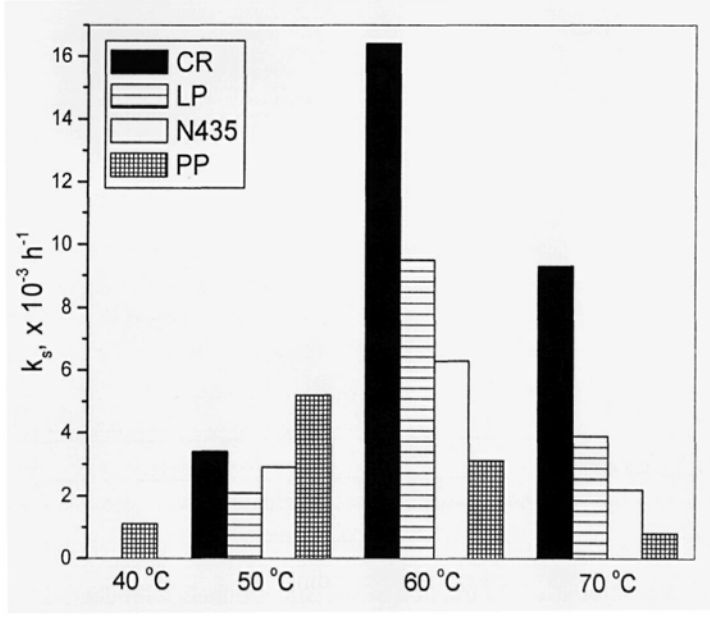

Fig. 9: Variation of k_s with temperature for the degradation of PSO-g-PMMA in the presence of different enzymes.

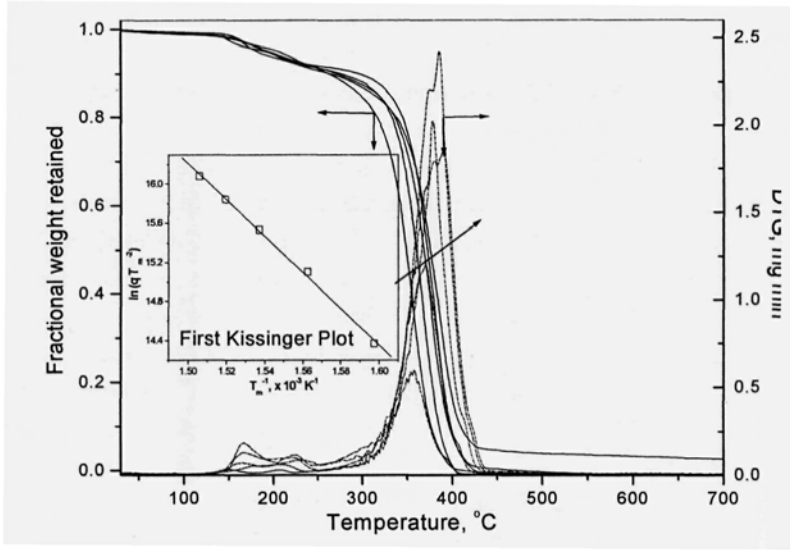

Fig. 10: TG-DTA of PSO-g-PMMA at different heating rates; the arrow indicates increasing heating rate, viz., 4.4, 8.8, 13, 17.5, 22 °C min ^{1}. [Inset: First Kissinger plot/

Among the different enzymes employed in this study, CR showed the maximum k_s, followed by LP, N435 and PP at 60 °C and 70 °C, and PP exhibited the maximum k_s at 50 °C. It is also interesting to note that k_d is higher than k_s for all the systems except for CR at 60 °C. This shows that catalysis by CR results in a net specific chain end scission coefficient, which can be defined as the ratio of k_s and k_d, greater than unity compared to all other systems. The difference in activity among the lipases considered in this study stems from the fact that different enzymes adopt different conformations to different side groups that are bound to the active site. This is a possible reason why *Candida antarctica* exhibits lower activity for large triglyceride substrates /31/. This phenomenon was also exhibited by poly(vinyl acetate), where longer and shorter side chains were hydrolyzed to different extents by different enzymes /9/.

In order to gain a better understanding about the stability of PSO-g-PMMA, pyrolytic degradation of PSO-g-PMMA was performed at different heating rates of 4.4, 8.8, 13, 17.5 and 22 °C min^{-1}. From Figure 10, it can be deduced that there are two distinct regions of weight loss observed in the

TG/DTA of the copolymer at different heating rates. The initial weight loss of about 5-10% in the region 150-250 °C can be attributed to the decomposition of backbone soybean oil in the copolymer. The second region occurs in the range 350-400 °C, and corresponds to the total loss of PMMA units. The first Kissinger technique /33/ was adopted to find the activation energy (E) and frequency factor (Z) of thermal degradation, corresponding to the degradation of PMMA units in the copolymer. This method involves monitoring the temperature at which the weight loss rate is maximum at all heating rates. The inset of figure 10 depicts the first Kissinger plot. By assuming the order of degradation to be unity, E and Z were found to be 154 kJ mol^{-1} and 3.08×10^{10} s^{-1}, respectively. This value of activation energy of the copolymer is lesser than that of PMMA, which is 166 kJ mol^{-1} /34/. Hence it is clear that grafting a vegetable oil reduces the thermal stability of PMMA units in the copolymer, thereby making PMMA more susceptible to pyrolysis at lower temperatures. This is also supported by the reduction in glass transition temperature of the copolymer compared to that of PMMA /18/. Nevertheless, owing to the high temperatures, energy and atmospheric pollution due to the evolution of harmful gases involved in pyrolysis, enzymatic degradation of the graft copolymer system in solution, proves to be a promising alternative for the degradation of synthetic polymers, which accumulate in the ecosystem as recalcitrants. Nearly complete degradation can be achieved by subjecting the copolymer to many cycles of enzymatic treatment, which breaks down the 'weak' links of the oil, thereby destabilizing the synthetic polymer matrix.

CONCLUSIONS

In the current research work, we have synthesized PSO-g-PMMA by initially photopolymerizing soybean oil in an ultraviolet reactor, to form the polymeric peroxide, and then grafting MMA chains onto the soybean oil units. The formation of the graft copolymer was confirmed by FTIR, ^1H NMR and GPC studies. The degradation of this copolymer was carried out in the presence of lipases, viz., CR, LP, N435 and PP at different temperatures. The enzymes degraded the copolymer by chain end scission, which was confirmed by the evolution of low molecular weight compounds in GPC. A kinetic model based on continuous distribution kinetics was proposed and the

rate coefficients for the chain end scission and enzyme deactivation were evaluated. The model prediction of the experimental data of the mass fraction of the chain end product was good and the rate coefficients reflected all the salient features of the degradation process. The activity of the enzymes at 60 and 70 °C for the degradation of the copolymer followed the order CR>LP>N435>PP, and that at 50 °C, it was PP>CR>N435>LP. Pyrolytic degradation of the copolymer showed activation energy of 154 kJ mol^{-1}, which was lower than that of PMMA. This suggests that grafting reduces the thermal stability compared to that of the homopolymer.

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