Effect of solvents on the enzyme mediated degradation of copolymers

Materials Research Express

PAPER

Effect of solvents on the enzyme mediated degradation of copolymers

RECEIVED
11 March 2015

REVISED
6 July 2015

ACCEPTED FOR PUBLICATION
14 August 2015

PUBLISHED
11 September 2015

Aditi Banerjee[1], **Kaushik Chatterjee**[1] **and Giridhar Madras**[2]

[1] Department of Materials Engineering, Indian Institute of Science, Bangalore 560012, India
[2] Department of Chemical Engineering, Indian Institute of Science, Bangalore 560012, India

E-mail: giridhar@chemeng.iisc.ernet.in

Keywords: population balance equations, polymer degradation, distribution kinetics

Abstract

The biodegradation of polycaprolactone (PCL), polylactic acid (PLA), polyglycolide (PGA) and their copolymers, poly (lactide-co-glycolide) and poly (D, L-lactide-co-caprolactone) (PLCL) was investigated. The influence of different solvents on the degradation of these polymers at 37 °C in the presence of two different lipases namely Novozym 435 and the free lipase of porcine pancreas was investigated. The rate coefficients for the polymer degradation and enzyme deactivation were determined using continuous distribution kinetics. Among the homopolymers, the degradation of PGA was nearly an order of magnitude lower than that for PCL and PLA. The overall rate coefficients of the copolymers were higher than their respective homopolymers. Thus, PLCL degraded faster than either PCL or PLA. The degradation was highly dependent on the viscosity of the solvent used with the highest degradation observed in acetone. The degradation of the polymers in acetone was nearly twice that observed in dimethyl sulfoxide indicating that the degradation decreases with increase in the solvent viscosity. The degradation of the polymers in water-solvent mixtures indicated an optimal water content of 2.5 wt% of water.

1. Introduction

Biodegradable polymers have a wide variety of uses ranging from food packaging to biomedical applications. Some of these biodegradable polymers such as polyurethanes, polyesters, polyanhydrides and polyacrylates also demonstrate antimicrobial properties [1, 2]. Their degradation proceeds primarily via hydrolysis forming lower molecular weight fragments. The study of the degradation of these polymer is useful for environmental applications wherein post-consumer products made of such polymers may be discarded in landfills and water bodies, and processed in waste recycling plants [1]. The stability and degradation of the polymer is also an important aspect for their application in biomedicine and the degradation of the polymer can affect cell growth, drug release, host response etc [3].

Lipase, an esterase enzyme, can cleave or hydrolyze ester bonds in polyesters such as poly(lactic acid) (PLA) and poly(ε-caprolactone) (PCL) etc [3]. Polymer degradation can vary depending on the matrix, the solvent used [4] and nature of polymer/enzyme [5]. While PCL can be used for drug delivery [6], the degradation of PCL and other polyesters mainly occurs on their surface as their hydrophobicity limits the interaction with the hydrophilic enzyme. Poly (lactide-co-glycolide) (PLGA) is also extensively used in bone tissue engineering [7], bone regeneration [8] but several factors [9] including fluid flow [10] affect the degradation of PLGA both *in vitro* and *in vivo*. The degradation of copolymers such as PLA–PCL–PLA has also been investigated [11]. Though degradation of individual polymers has been reported, the effects of various solvents on their degradation have not been reported.

The degradation of polymers has been extensively investigated under melt, thermal and catalytic conditions [12]. However, these processes have high heat transfer resistance, high melt viscosity, and also result in undesirable by-products [13]. Degradation in solution is an alternate methodology wherein all products are in a

single liquid phase of much lower viscosity that enables better heat transfer resulting in enhanced reaction rates [14], and often resulting in a modified product profile [15].

Lipases exhibit stability and activity even in organic solvents [16] presumably because a thin layer of water is bound to the enzyme that allows retention of the enzyme's native conformation [17]. Thus lipases can be potentially used in the enzymatic degradation of polymers in organic solvents. The synthesis of polymers using enzymes as catalysts has been proposed as a benign process [18] involving trans-esterification that involves the ester group cleavage in the polymer chain. Thus the enzymatic degradation (hydrolysis) of polyesters in non-aqueous such as organic media is important [18]. In this regard, an one-pot degradation−polymerization of biodegradable polymers has been proposed with lipase as the catalyst [19]. In this process, the degradation of the polymer is carried out in non-aqueous media and then the solvent is removed and the oligomer is polymerized. This repeated cycle of polymerization followed by degradation results in an environmentally benign process of polymer recycling [20]. This process requires optimization with regard to the lipase and the solvent to be used.

Sivalingam *et al* [21] have reported the influence of different solvents on PCL degradation but the effect of solvents on other polymers has not been reported. Thus the main objective of this study was to investigate the influence of various solvents and solvent–water combinations on the degradation of polymer and their copolymers in the presence of lipases. The degradation kinetics have been studied and kinetic parameters for polymer degradation and enzyme deactivation were determined based on continuous distribution kinetics.

2. Experimental

2.1. Materials

Polycaprolactone (PCL), polylactic acid (PLA), polyglycolide (PGA), poly (lactide-co-glycolide) (PLGA), poly (DL-lactide-co-caprolactone) (PLCL) and free lipase (porcine pancreas) were obtained from Sigma Aldrich (USA). Commercial immobilized lipase, Novozym 435 was obtained from Novozym Inc.. All other chemicals, such as dimethyl sulphoxide (DMSO), 1, 4-dioxane, dimethyl formamide (DMF), dichloromethane (DCM), tetrahydrofuran (THF) and acetone were obtained from S.D. Fine Chemicals (India).

2.2. Degradation experiments

Enzymatic degradation and the solvent effect on the degradation of different polymers (PCL, PGA, PLA, PLGA, PLCL) were carried out in five different solvents (DMSO, DMF, DCM, dioxane and acetone) in the presence of Novozym 435 and porcine pancreas lipase at 37 °C. 10 mL of polymer solution (2 g L^{-1}) with 1 g L^{-1} of each lipase was taken in reaction vials kept at constant temperature in an incubator-shaker. The temperature of the incubator was controlled with a variation of ±1 °C. Samples of 500 μL were taken at time intervals for analysis in gel permeation chromatography (GPC). No degradation was observed when experiments were also conducted without any enzyme.

2.3. GPC analysis

The samples were analyzed with a GPC system that consists of a Waters pump, 100 μl sample loop, three GPC columns of different pore sizes, and a refractive index detector (Waters 2410). THF was used as the eluent with a flow rate of 0.9 mL min^{-1}. The universal calibration curve was used that was based on polystyrene standards and converted using the Mark–Houwink equation.

3. Theoretical model

The polymer is cleaved at specific chain positions by the enzymes and produces oligomers of molecular weight (x_a) given by

$$P(x) \xrightarrow{k_s} P\left(x - x_a\right) + Q\left(x_a\right). \tag{1}$$

$P(x)$ and $Q(x_a)$ represent the molecular weights of polymer and degraded specific product, whose molar concentrations are given by $p(x,\ t)$ and $q_t(t)$. Thus the population balance for the polymer and product can be expressed as [22] and given by equations (2) and (3)

$$\frac{\partial p(x,\ t)}{\partial t} = k_s\, a(t) \int_x^{\infty} p(x',\ t)\, \delta\left(x,\ \left(x' - x_a\right)\right) \mathrm{d}x' \ - \ k_s a(t) p(x,\ t), \tag{2}$$

$$\frac{\partial q(t)}{\mathrm{d}t} = \int_{x_a}^{\infty} k_s a(t) p(x',\ t) \delta\left(x_a,\ x\right) \mathrm{d}x'. \tag{3}$$

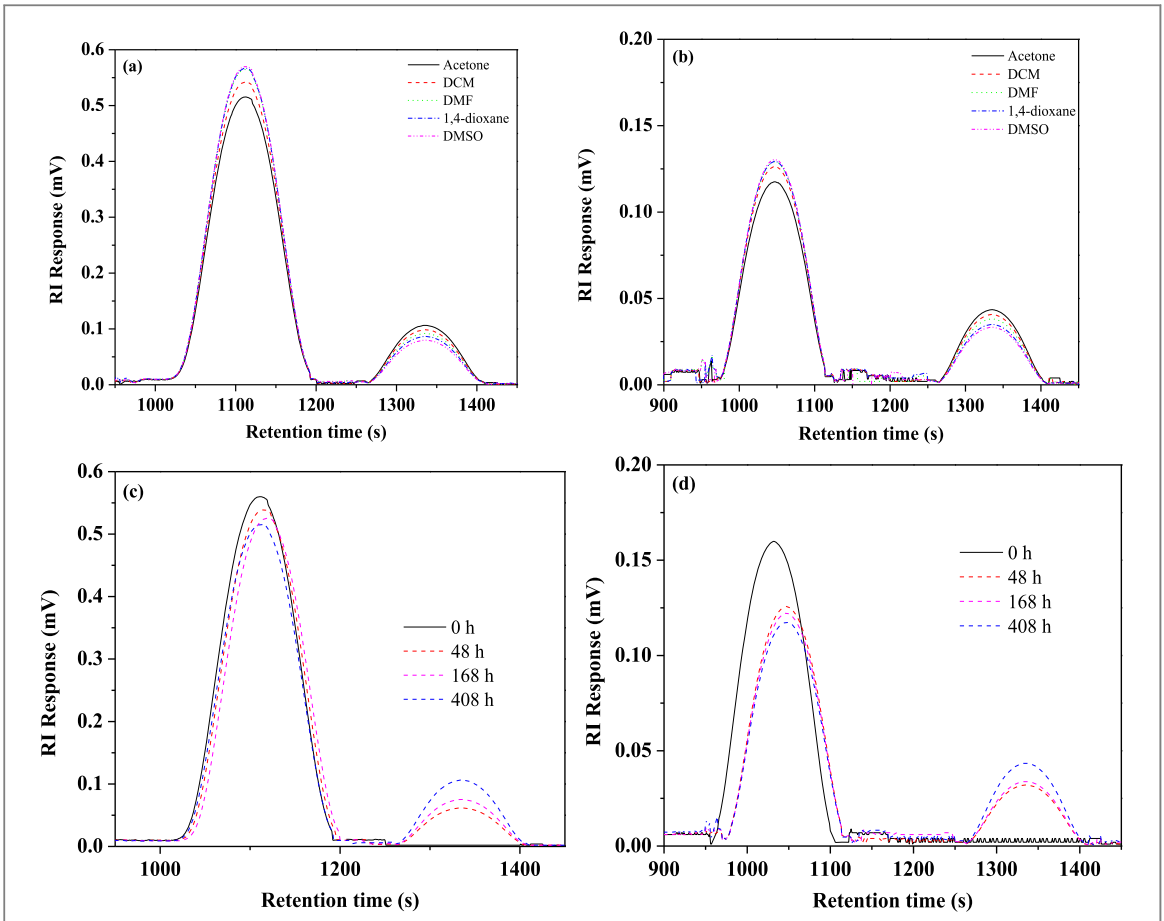

Figure 1. Gel permeation chromatographs of enzymatic degradation of (a) PLA (b) PLCL in various solvents in the presence of Novozym 435 at 408 h of degradation. Degradation of (c) PLA and (d) PLCL in the presence of Novozym 435 in acetone at varying times of degradation.

The enzyme activity can be represented as $a(t) = a_0 \exp(-k_d t)$, where a_0 represents the enzyme activity at $t = 0$ and k_d is rate coefficient of deactivation of the enzyme. The rate coefficient for polymer degradation, k_s, is independent of molecular weight [23] for specific chain scission and the moment equations for equations (2) and (3) are given by equations (4) and (5), respectively

$$\frac{dp^{(i)}}{dt} = k_s a_0 \exp\left(-k_d t\right)\left(-x_a\right)^i p^{(0)}(t) - k_s a_0 \exp\left(-k_d t\right)\sum_{t=0}^{i} p^{(i)}(t), \tag{4}$$

$$\frac{dq^{(i)}(t)}{dt} = k_s a_0 \exp\left(-k_d t\right) p^{(0)} x_a^i. \tag{5}$$

The 0th and 1st moments can be obtained by putting $i = 0$ and 1 in the equations (4) and (5). For $i = 0$, the total polymer moles ($p^{(0)}$) does not change with time i.e., $p^{(0)} = p_0^{(0)}$ and equation (5) can be solved with $i = 1$ and the initial condition of $q^{(1)}(t = 0) = 0$, to yield equation (6)

$$q^{(1)}(t) = k_s a_0 x_a p_0^{(0)} \left(\frac{1 - \exp\left(-k_d t\right)}{k_d}\right). \tag{6}$$

As $p_0^{(0)} = \frac{p_0^{(1)}}{M_{n0}}$ and at $t \to \infty$, where enzyme loses all its activity, equation (6) becomes

$$q_s^{(1)} = q^{(1)}(t \to \infty) = \frac{k_s a_0 x_a p_0^{(1)}}{k_d M_{n0}}. \tag{7}$$

The simplified mass fraction of the formed specific products can be determined from equation (8) by dividing equations (6) and (7)

Table 1. Rate coefficient for enzyme deactivation and Polymer–copolymer degradation.

		Novozym 435				Porcine pancreas lipase			
Polymer	Solvent	$q_s/p_0^{(1)}$ (x10^{-3})	k_d (x10^{-3})	k_s (x10^{-3})	k_{ov}	$q_s/p_0^{(1)}$ (x10^{-3})	k_d (x10^{-3})	k_s (x10^{-3})	k_{ov}
PCL	Acetone	66.46	4.36	42.15	9.66	13.07	6.37	11.57	1.90
	DCM	32.86	4.80	22.94	7.01	11.84	8.06	10.97	1.72
	DMF	48.20	5.82	40.80	4.78	9.45	9.94	13.66	1.37
	1, 4-dioxane	22.58	6.13	20.13	3.28	6.23	12.10	13.88	0.90
	DMSO	21.06	6.57	20.12	3.06	6.35	12.52	12.11	0.92
	0.5% water	50.41	5.21	38.20	7.33	15.30	5.90	13.13	2.22
	1% water	57.74	4.85	40.73	8.39	17.10	5.12	12.73	2.48
	2.5% water	52.63	4.73	36.21	7.65	17.62	4.87	12.48	2.56
PLA	Acetone	66.08	2.94	30.97	10.53	12.72	6.2	12.58	2.02
	DCM	32.57	5.16	26.80	7.49	11.64	8.29	15.38	1.85
	DMF	47.02	8.14	61.02	5.19	9.16	9.44	13.80	1.46
	1, 4-dioxane	22.46	9.82	35.17	3.58	6.13	10.66	10.42	0.97
	DMSO	20.65	10.8	35.55	3.29	4.72	11.81	8.90	0.75
	0.5% water	54.56	2.60	22.62	8.70	21.17	3.54	11.95	3.37
	1% water	61.99	5.62	55.54	9.88	22.43	4.04	14.45	3.57
	2.5% water	65.76	5.85	61.34	10.48	23.14	4.87	17.97	3.69
PGA	DMF	2.14	8.21	2.57	0.31	1.69	2.62	0.65	0.24
	1, 4-dioxane	1.85	10.5	2.86	0.27	1.47	3.43	0.74	0.21
	DMSO	1.02	17.39	2.62	0.15	0.50	4.65	0.34	0.07
	0.5% water	5.16	7.57	5.73	0.75	3.14	6.37	2.93	0.46
	1% water	8.90	5.94	7.76	1.30	6.28	7.51	6.92	0.92
	2.5% water	12.67	8.54	15.88	1.86	12.57	7.75	14.30	1.84
PLCL	Acetone	70.96	6.61	86.7	13.1	13.57	17.34	43.5	2.51
	DCM	59.63	9.09	100	11.0	12.44	17.61	40.52	2.30
	DMF	50.31	11.89	110	9.30	11.24	18.64	38.75	2.07
	1, 4-dioxane	31.33	14.68	85	5.79	9.82	19.74	35.83	1.81
	DMSO	27.81	15.53	79.8	5.14	7.35	21.03	28.58	1.35
	0.5% water	63.81	9.24	109	11.8	13.22	9.17	22.41	2.44
	1% water	66.99	8.91	110	12.4	14.24	9.94	26.17	2.63
	2.5% water	67.70	9.78	122	12.5	15.27	10.43	29.43	2.82
PLGA	Acetone	35.25	8.46	50.9	6.01	13.49	7.17	16.50	2.30
	DCM	32.65	9.65	53.7	5.57	11.52	7.75	15.24	1.96
	DMF	26.62	10.43	47.4	4.54	10.35	8.47	14.96	1.76
	1, 4-dioxane	20.66	11.54	40.7	3.52	6.40	9.79	10.69	1.09
	DMSO	16.45	13.18	37	2.80	4.69	11.75	9.40	0.80
	0.5% water	36.87	7.68	48.3	6.29	15.23	10.13	26.33	2.59
	1% water	37.96	8.73	56.5	6.47	18.30	10.64	33.21	3.12
	2.5% water	39.05	9.42	62.7	6.66	20.61	8.55	30.07	3.51

$$q_r(t) = \frac{q^{(1)}(t)}{q^{(1)}(t \to \infty)} = 1 - \exp\left(-k_d t\right). \tag{8}$$

The above equations represent the time evolution of the mass fraction of the specific products. These equations can be used to calculate the rate coefficient of polymer degradation, k_s and the enzyme deactivation rate coefficient, k_d. Both these rate coefficients have units of time^{-1}.

4. Results and discussion

The influence of solvents on the enzymatic degradation of all the polymers and copolymers namely PCL, PGA, PLA, PLGA and PLCL was studied at 37 °C in solution. Solvents were chosen on basis of their viscosity and polymer solubility. As a first approximation, all solvents were chosen such that the Hildebrand solubility parameters ranged from 20 to 26 MPa$^{1/2}$ to ensure that the polymers are completely soluble in the chosen solvents. This was further verified using the Hansen solubility parameters [24]. The solvents were then chosen such that the viscosities varied an order of magnitude. Thus, acetone was the least viscous solvent while dimethyl sulfoxide (DMSO) was the most viscous solvent. All other solvents had viscosities between these two solvents. The viscosities of the solvents were taken from a standard handbook [25].

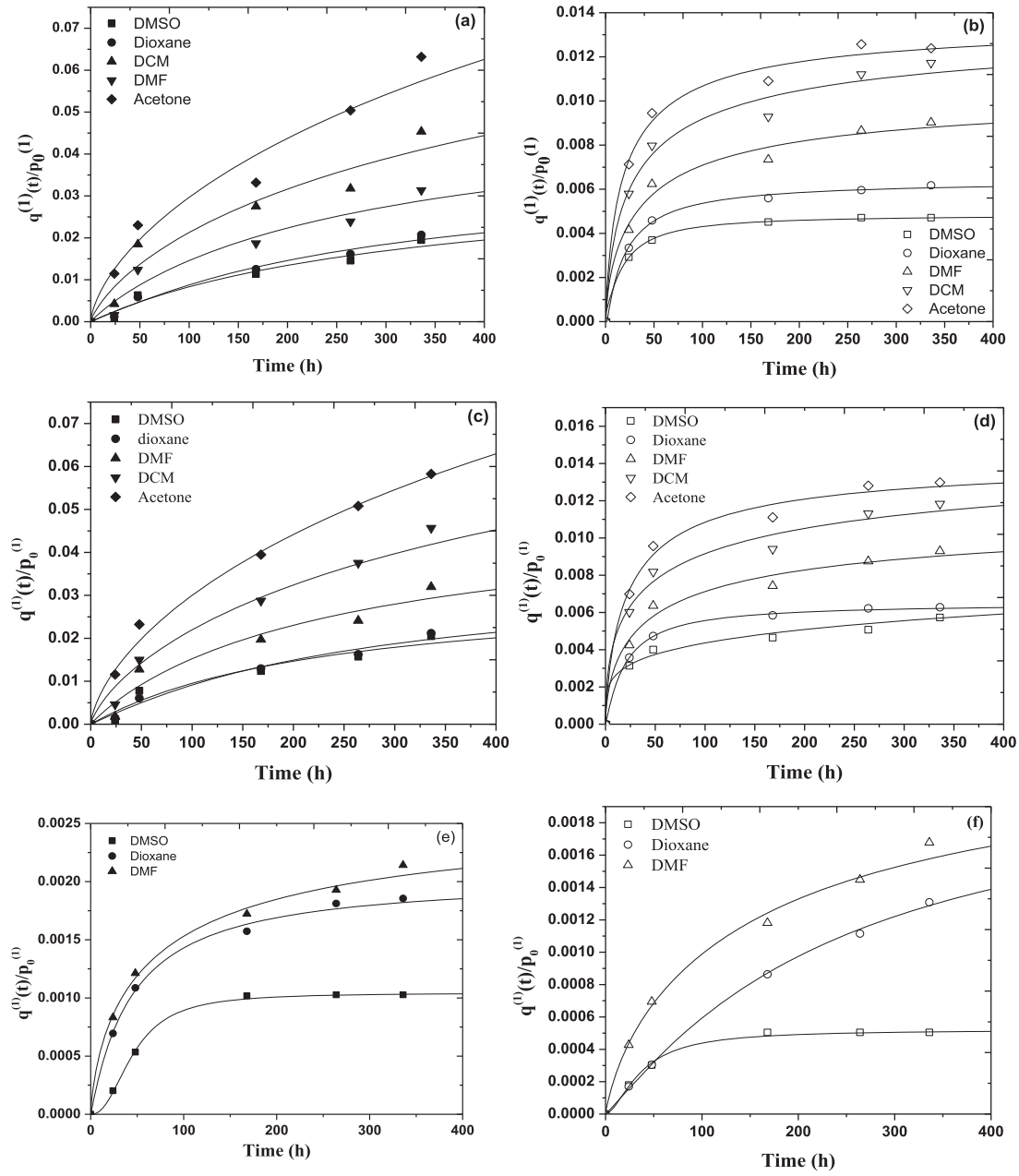

Figure 2. Mass fraction variation of specific product formed with time for various polymers in various solvents; (a) PLA with Novozym 435; (b) PLA with porcine pancreas lipase; (c) PCL with Novozym 435; (d) PCL with porcine pancreas lipase; (e) PGA with Novozym 435; (f) PGA with porcine pancreas lipase.

Figures 1(a) and (b) represent the typical molecular weight distribution by GPC analysis of PLA and PLCL in various solvents after 408 h of degradation in the presence of Novozym 435. The reaction attained steady state and no further degradation was observed after 408 h and 504 h for porcine pancreas lipase and Novozym 435, respectively. Thus it is been observed that immobilized lipase Novozym 435 has higher and longer activity than free enzyme porcine pancreas lipase. Figures 1(c) and (d) show the enzymatic degradation of PLA and PLCL in the presence of Novozym 435 in acetone at varying times of degradation. With an increase in the degradation time, the specific product peak increases and it was constant around molecular weight of 500.

Equation (8) implies that a plot of $\ln[1 - q_r(t)]$ will be linear with time and the slope can be used to determine k_d. After obtaining k_d, equation (7) can be used to determine k_s. The degradation product has a molecular weight of around 500. The parameters, $q^{(1)}(t)$ and $p_0^{(1)}$, are the mass fractions of the specific product and the polymer. These parameters can be obtained from the area under the respective peaks in the chromatograph. The quantities, $q^{(1)}(t)/p_0^{(1)}$, represent the ratio of the specific product mass fraction to the polymer mass. The values in tables for $q_s^{(1)}/p_0^{(1)}$ are based on the steady state obtained after 408 h and 504 h for

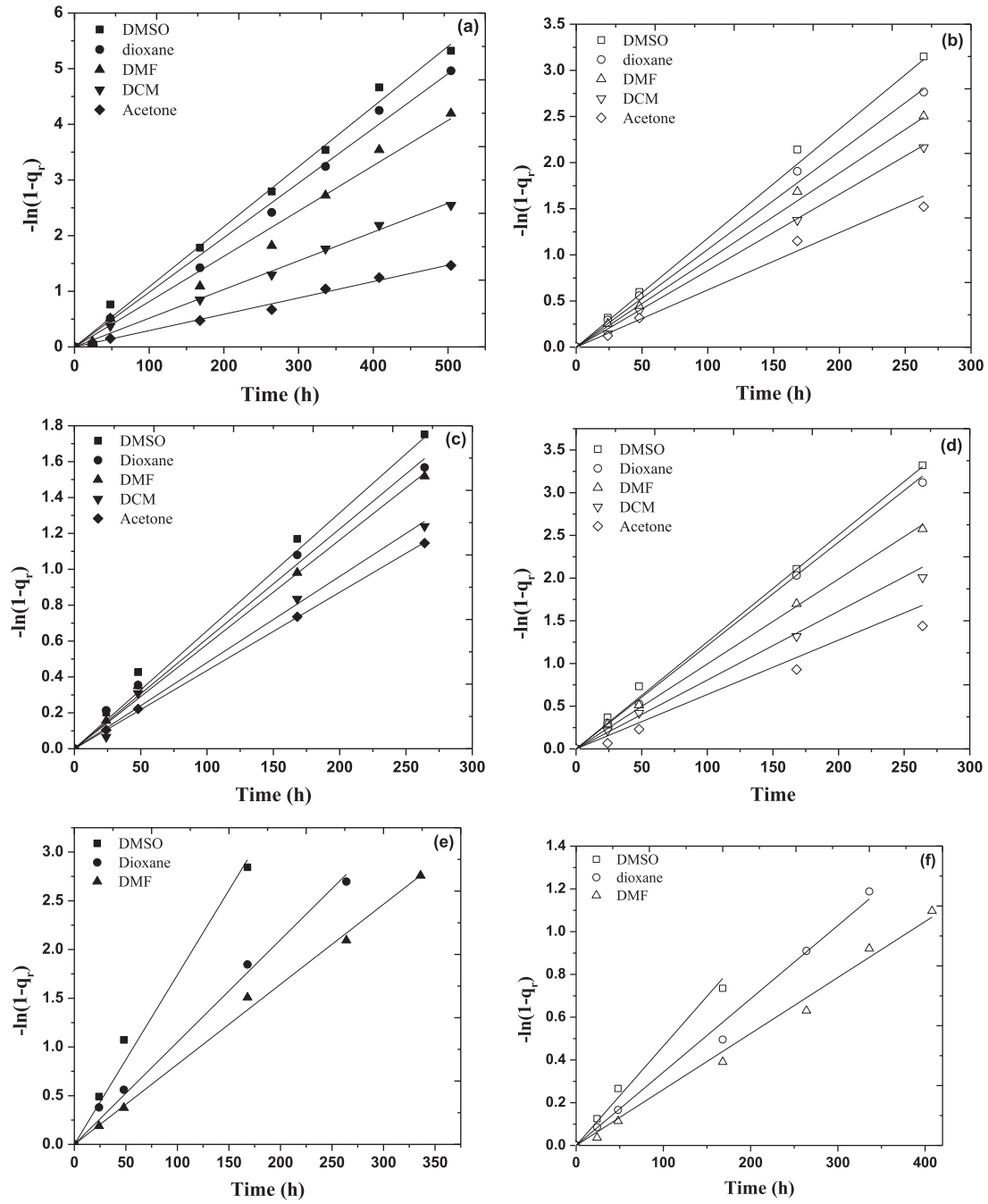

Figure 3. Variation of $\ln(1 - q_r)$ with degradation time for various polymers in different solvents. (a) PLA with Novozym 435; (b) PLA with porcine pancreas lipase; (c) PCL with Novozym 435; (d) PCL with porcine pancreas lipase; (e) PGA with Novozym 435; (f)PGA with porcine pancreas lipase.

porcine pancreas lipase and Novozym 435, respectively. The rate coefficients of polymer degradation and enzyme deactivation are listed in table 1.

Figures 2–5 show the variation of the mass fraction of the products with time. The enzyme deactivation coefficient, k_d has been calculated from the slope of semi-logarithmic plot of q_r with degradation time. Figures 3 and 5 show this plot and the value of k_d is obtained from the slope of the lines in the figures. The polymer degradation coefficient, k_s, was then calculated from equation (7) using the determined k_d value. Subsequently, the values of k_s and k_d was substituted in equation (7) and the variation of the mass fraction with time is shown in figures 2 and 4.

Degradation of PCL, PLA, PGA, and their copolymers occurs in aqueous media by the hydrolysis of ester bonds whose reaction is auto-catalyzed by carboxylic groups [26]. However, in the presence of a suitable enzyme, the degradation rate can be considerably increased. For example, it has been reported that the

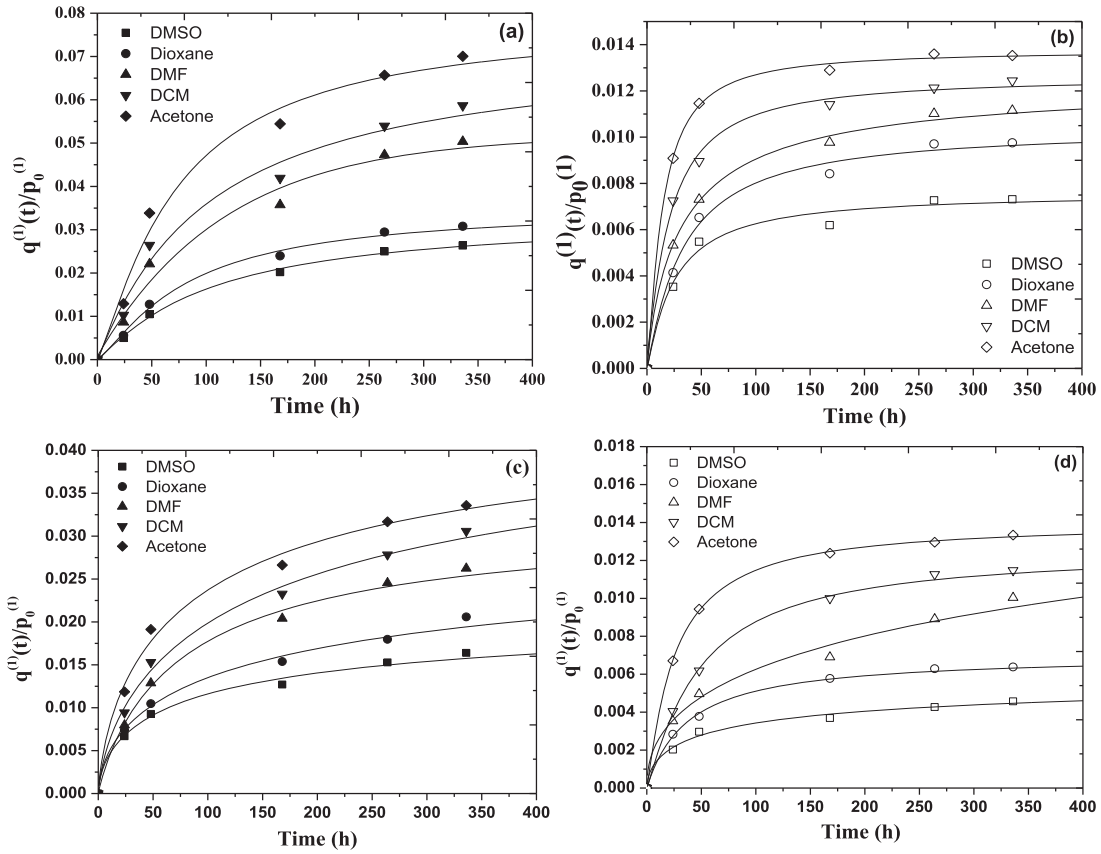

Figure 4. Mass fraction variation of specific product formed with time for various copolymers in various solvents. (a) PLCL with Novozym 435; (b) PLCL with porcine pancreas lipase; (c) PLGA with Novozym 435; (d) PLGA with porcine pancreas lipase.

degradation rate of PCL in the presence of lipase increased by two orders of magnitude when compared to the degradation rate obtained in the absence of the lipase [27]. The hydrolysis of PCL by the enzyme occurs mainly at the polymer–enzyme surface because it may be difficult for a hydrophilic enzyme to diffuse into a hydrophobic polymer like PCL. Enzyme-catalyzed degradation of crystalline and hydrophobic polymeric biomaterials follow a mechanism based on surface erosion [6] but the mechanism can depend on many factors such as homogeneity and chemical composition [2, 28].

It is most likely a small amount of water contained in the lipase and the residual water in the solvent was involved in the ester bond hydrolysis in polyesters [18]. In all cases, the peak in the GPC corresponding to the polymer decreased while the peaks due to oligomers (at number-average molecular weight of around 500) appeared. No polymer whose molecular weight was intermediate between those of the starting material and the oligomer was observed. This indicates that polymer degradation by the enzyme was quite specific. This is in direct contrast to degradation by acid catalysts wherein the degradation occurs on random points of the polymer chain [18].

The overall rate coefficient, k_{ov}, which is dimensionless, can be determined from k_s/k_d. The rate coefficient follows the order: PLCL > PLGA > PLA > PCL > PGA. Thus the overall rate coefficient of the copolymer, PLCL, was higher than of both PCL and PLA. Similarly, the degradation of the copolymer, PLGA, was higher than that of the homopolymers, PLA and PGA. However, there was no significant difference in the rate coefficients between PLA and PCL. PLCL exhibited at least 10% increased rate over PCL while PLGA also exhibited at least 10% higher rate compared to PLA.

The solvent viscosity has a significant effect on overall degradation of PCL, PLA, PLCL, PGA and PLGA can be observed in figure 6. The degradation of PLA, PLCL and PLGA follows a similar order in various solvents and is acetone > DCM > DMF > 1-4 dioxane > DMSO. Considering PGA is not soluble in acetone and DCM, PGA also follows the same order. The overall rate coefficient of PLCL was higher than of both PCL and PLA. Similarly, the degradation of PLGA was higher than that of PLA and PGA. The overall rate coefficient, k_{ov}, of degradation of the polymers in acetone is nearly twice of that observed in DMSO. The difference of the rate coefficients obtained in DCM and acetone is not significant. Similarly, the difference of the rate coefficients obtained in dioxane and DMSO is not significant. However, in all the cases, the solvent viscosity affects polymer

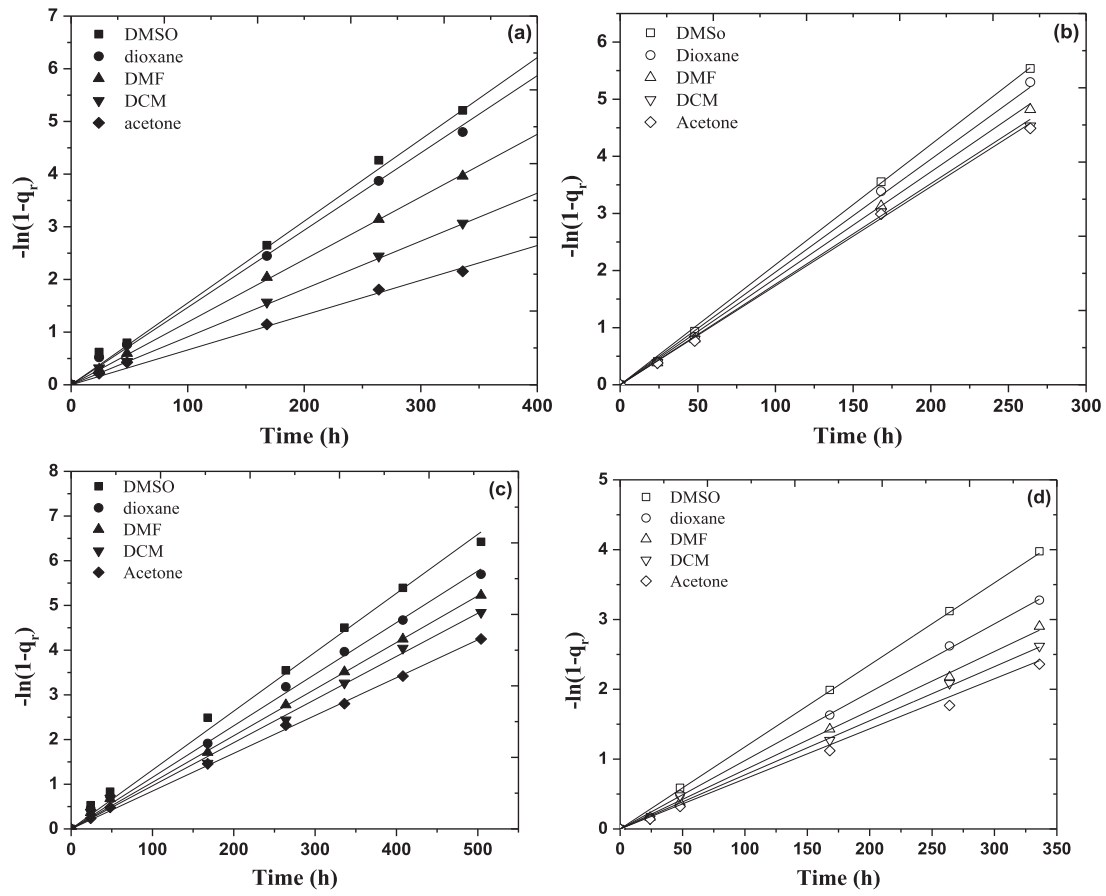

Figure 5. Variation of $-\ln(1 - q_r)$ with degradation time for various copolymers in different solvents. (a) PLCL with Novozym 435; (b) PLCL with porcine pancreas lipase; (c) PLGA with Novozym 435; (d) PLGA with porcine pancreas lipase.

degradation inversely and the polymer degradation rate reduces with an increase in the solvent viscosity. This is due to contact of polymer and enzyme in solvents with high viscosity. As the viscosity increases, the transport properties reduce resulting in lower transport of the enzyme to polymer resulting in reduced polymer degradation [23].

Hydrophobic solvents affects the hydration layer around the enzyme resulting in a conformational change. Thus lipases that are in contact with a water–hydrocarbon interface have higher activity than that observed in an anhydrous solvent [29]. To examine the effect of water on the enzyme activity and the degradation of the polymers, these polymers were degraded in solvent (DMF)–water mixtures with different proportions of water. DMF has been chosen as the solvent for these experiments because all the polymers dissolve in DMF. When the mass fraction of water is more than 2.5%, PLCL and PLGA precipitate from the solution. Thus this provides an upper limit for the water addition. Table 1 shows the kinetic parameters for the degradation of various polymers at various water–DMF ratios. From figure 7, it can be observed that the overall degradation coefficient increases with an increase in the water ratio. It can be observed that the rate coefficient nearly doubles in the presence of 2.5% water when compared to the system without water. However, k_{ov} becomes nearly constant after an initial increase with a further increment of water content. In case of enzymatic degradation of the polymer in organic solvents, the enzyme is saturated by an organic layer resulting in hydrophobicity of the enzyme resulting in lower transport properties and lesser degradation. Though excessive water can deactivate the enzyme, an optimal amount of water surrounding the enzyme ensure optimal activity. Previous studies have shown that the maximum degradation of PCL occurred at an optimum value concentration of 8.7 wt.% of water in acetone [21] and the degradation decreased at higher concentrations of water. In this case, it is observed that the degradation did not significantly increase after 1% water and it is expected that the degradation may decrease with excessive water. However, experiments were not conducted above 2.5% as the polymers are not completely soluble in DMF–water mixtures of more than 2.5% water. However, these studies have shown that the presence of water in organic solvents does significantly influence the degradation.

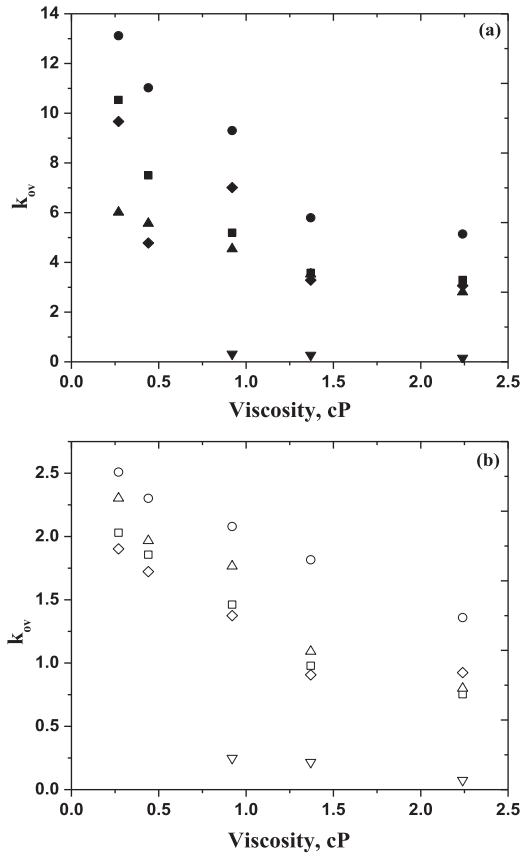

Figure 6. Effect of solvent viscosity on k_{ov} catalyzed by (a) Novozym 435, legend: ■, PLA; •, PLCL; ▲, PLGA;▼,PGA;◆, PCL; and (b) porcine pancrease lipase, legend:□, PLA; ○, PLCL; Δ, PLGA; ∇, PGA; ◊, PCL.

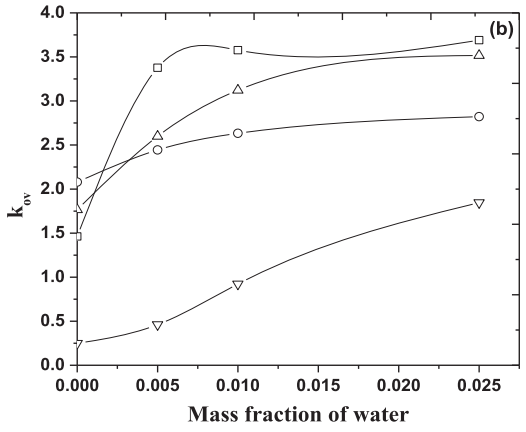

Figure 7. Variation of k_{ov} with the mass fraction of water in water–DMF mixture; (a) Novozym 435, legend: ■, PLA; •, PLCL;▲,PLGA; ▼,PGA; ◆, PCL and (b) porcine pancrease lipase, legend:□, PLA; ○, PLCL; Δ, PLGA; ∇, PGA; ◊, PCL.

5. Conclusions

The enzymatic degradation of three polymers (PCL, PLA and PGA) and two copolymers (PLCL and PLGA) was studied in various solvents in the presence of two lipases, Novozym 435 and porcine pancreas lipase. The immobilized enzyme Novozym 435 showed better activity than the free enzyme porcine pancreas lipase. Various kinetic parameters such as the polymer degradation coefficient and enzyme deactivation coefficient were determined from continuous distribution modeling. The overall rate coefficient of PLCL was higher than of both PCL and PLA. Similarly, the degradation of PLGA was higher than that of PLA and PGA. The influence of solvents on the degradation of these polymers was also investigated. It was observed that the highest degradation

was observed in acetone and the lowest in DMSO. Thus, the activity of enzyme and polymer degradation decreased with increasing viscosity of the solvent. The influence of water on polymer degradation was also studied by degrading the polymer with different DMF–water mixtures and the optimal concentration of water was determined.

Acknowledgments

The authors thank the Department of Science and Technology (DST), India for financial support. KC acknowledges the Ramanujan Fellowship from DST and the corresponding author thanks DST for the JC Bose fellowship.

References

[1] Jamshidian M, Tehrany E A, Imran M, Jacquot M and Desobry S 2010 Poly-lactic acid: production, applications, nanocomposites, and release studies *Comprehensive Rev. Food Sci. Food Saf.* **9** 552–71
[2] Azevedo H S and Reis R L 2005 *Biodegradable Systems in Tissue Engineering and Regenerative Medicine* (Boca Raton, FL: CRC Press) pp 177–201
[3] Liu L, Li S, Garreau H and Vert M 2000 Selective enzymatic degradations of poly(l-lactide) and poly(ε-caprolactone) blend films *Biomacromolecules* **1** 350–9
[4] Gan Z, Liang Q, Zhang J and Jing X 1997 Enzymatic degradation of poly(ε-caprolactone) film in phosphate buffer solution containing lipases *Polym. Degrad. Stab.* **56** 209–13
[5] Darwis D, Mitomo H, Enjoji T, Yoshii F and Makuuchi K 1998 Enzymatic degradation of radiation crosslinked poly(ε-caprolactone) *Polym. Degrad. Stab.* **62** 259–65
[6] Chawla J S and Amiji M M 2002 Biodegradable poly(ε-caprolactone) nanoparticles for tumor-targeted delivery of tamoxifen *Int. J. Pharm.* **249** 127–38
[7] Gentile P, Chiono V, Carmagnola I and Hatton P 2014 An overview of poly(lactic-co-glycolic) acid (PLGA)-based biomaterials for bone tissue engineering *Int. J. Mol. Sci.* **15** 3640–59
[8] Lanao R P F, Jonker A M, Wolke J G C, Jansen J A, van Hest J C M and Leeuwenburgh S C G 2013 Physicochemical properties and applications of poly(lactic-co-glycolic acid) for use in bone regeneration *Tissue Eng.* B **19** 380–90
[9] Tracy M 1999 Factors affecting the degradation rate of poly(lactide-co-glycolide) microspheres *in vivo* and *in vitro Biomaterials* **20** 1057–62
[10] Agrawal C M, McKinney J S, Lanctot D and Athanasiou K A 2000 Effects of fluid flow on the *in vitro* degradation kinetics of biodegradable scaffolds for tissue engineering *Biomaterials* **21** 2443–52
[11] Huang M-H, Li S and Vert M 2004 Synthesis and degradation of PLA–PCL–PLA triblock copolymer prepared by successive polymerization of ε-caprolactone and dl-lactide *Polymer* **45** 8675–81
[12] Lucas N, Bienaime C, Belloy C, Queneudec M, Silvestre F and Nava-Saucedo J-E 2008 Polymer biodegradation: mechanisms and estimation techniques—a review *Chemosphere* **73** 429–42
[13] Murakata T, Wagatsuma S, Saito Y, Suzuki T and Sato S 1993 Effect of solvent on thermal degradation of poly(p-methylstyrene) *Polymer* **34** 1431–5
[14] Madras G, Chung G Y, Smith J M and McCoy B J 1997 Molecular weight effect on the dynamics of polystyrene degradation *Ind. Eng. Chem. Res.* **36** 2019–24
[15] Guy L and Fixari B 1999 Waxy polyethylenes from solution thermolysis of high density polyethylene: inert and H-donor solvent dilution effect *Polymer* **40** 2845–57
[16] Cambou B and Klibanov A M 1984 Preparative production of optically active esters and alcohols using esterase-catalyzed stereospecific transesterification in organic media *J. Am. Chem. Soc.* **106** 2687–92
[17] Klibanov A M 1989 Enzymatic catalysis in anhydrous organic solvents *Trends Biochem. Sci.* **14** 141–4
[18] Kobayashi S, Uyama H and Takamoto T 2000 Lipase-catalyzed degradation of polyesters in organic solvents. a new methodology of polymer recycling using enzyme as catalyst *Biomacromolecules* **1** 3–5
[19] Takamoto T U and Kobayashi, S. H 1999 An one pot synthesis for polymer recycling *Polym. Prepr. Japan* **48** 1057
[20] Kobayashi S 1999 Enzymic polymerization-synthesis of artificial polymers catalyzed by natural polymers *High Polym. Japan* **48** 124
[21] Sivalingam G, Chattopadhyay S and Madras G 2003 Solvent effects on the lipase catalyzed biodegradation of poly (ε-caprolactone) in solution *Polym. Degrad. Stab.* **79** 413–8
[22] Sivalingam G, Chattopadhyay S and Madras G 2003 Enzymatic degradation of poly (epsilon-caprolactone), poly (vinyl acetate) and their blends by lipases *Chem. Eng. Sci.* **58** 2911–9
[23] Sivalingam G and Madras G 2004 Dynamics of lipase catalyzed enzymatic degradation of poly(bisphenol-A carbonate) *J. Appl. Polym. Sci.* **91** 2391–6
[24] Hansen C 2007 *Hansen Solubility Parameters: A User's Handbook* vol 2 (Boca Raton, Florida: CRC Press)
[25] Wohlfarth C 2009 *Viscosity of Pure Organic Liquids and Binary Liquid Mixtures* vol 25 (Berlin: Springer)
[26] Hakkarainen M, Albertsson A-C and Karlsson S 1996 Weight losses and molecular weight changes correlated with the evolution of hydroxyacids in simulated *in vivo* degradation of homo- and copolymers of PLA and PGA *Polym. Degrad. Stab.* **52** 283–91
[27] Wu C, Jim T F, Gan Z, Zhao Y and Wang S 2000 A heterogeneous catalytic kinetics for enzymatic biodegradation of poly (ϵ-caprolactone) nanoparticles in aqueous solution *Polymer* **41** 3593–7
[28] You Y, Min B-M, Lee S J, Lee T S and Park W H 2005 *In vitro* degradation behavior of electrospun polyglycolide, polylactide, and poly (lactide-co-glycolide) *J. Appl. Polym. Sci.* **95** 193–200
[29] Ito T, Kikuta H, Nagamori E, Honda H, Ogino H, Ishikawa H and Kobayashi T 2001 Lipase production in two-step fed-batch culture of organic solvent-tolerant *Pseudomonas aeruginosa* LST-03 *J. Biosci. Bioeng.* **91** 245–50