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ORIGINAL PAPER

The effect of aliphatic esters on the formation and degradation behavior of PLGA-based in situ forming system

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Abstract The purpose of this study was to investigate the effect of three aliphatic esters, ethyl heptanoate, methyl heptanoate, and ethyl nonanoate on the in vitro degradation behavior of in situ forming systems. In situ forming implants based on 33% (w/w) poly (lactide-co-glycolide) (PLGA)/57% (w/w) *N*-methyl-2-pyrrolidone (NMP)/10% (w/w) esters were prepared after injection of the final formulation in phosphate buffer solution (pH 7.4, 0.2 M) at 37 °C. The influence of additives on the implants formation, morphology, and also on their in vitro degradation behaviors over a period of 45 days was investigated. The degraded matrices were evaluated to determine morphological analysis by SEM and ^{1}H-NMR study. The solution of degradation medium was studied to indicate NMP removal and altering acidity. The results showed that the additives generated a high porous structure and caused the fast phase inversion. However, the ^{1}H-NMR spectra indicated that ester additives remained in the matrices during degradation periods. The results of the acidity study showed that the degradation rates in the matrices containing esters were higher than the control matrix. In addition, it is shown that esters with lower molecular weight have affected the polymer degradation more efficiently than higher molecular weight esters.

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Keywords In situ forming systems · Biodegradation · Solvent removal · Morphology · Poly (lactide-co-glcolide) · Aliphatic esters

Introduction

In the past few years, in situ forming systems have been used for various biomedical applications, including drug delivery, cell encapsulation, and tissue repair [1]. In the drug delivery field, several marketed parenteral systems such as microspheres, implants and in situ forming delivery systems had been developed. The in situ forming systems are prepared by different methods, one of which is the phase separation by polymer precipitation. The system is composed of a water-insoluble polymer dissolved in a water-miscible solvent. After the polymer solution is injected via a syringe into the body, the solvent diffuses out of the polymer while water permeates in the polymer matrix. Immediately, the insoluble polymer precipitates and the solid polymer matrix are formed due to phase separation [2–6]. For drug delivery application, the active drugs are added to the polymer solution to produce a homogeneous solution or a dispersion which depend upon the solubility of the drug. It is necessary to understand polymer properties, matrix morphology, and formation processes in order to improve matrix performance in drug delivery [3].

Dunn et al. [7] had described a biodegradable injecting drug delivery system. The copolymer family of poly (lactide-co-glycolide) (PLGA) was used to prepare this system. PLGA copolymers act as matrix material from which the drug substance is released through diffusion and during the degradation process by hydrolysis [8, 9]. Moreover, in situ forming performance is affected by their morphology that depends on phase inversion conditions such as polymer type, additives, coagulation bath, and polymer solution composition [3].

The additives in such systems would influence formation of implant and degradation of the polymer, thereby; the drug release rates. The effects of additives on the drug release process have been investigated [10–15]. It has been found that water-soluble or insoluble additives significantly altered drug release behavior from a controlled drug delivery system. The choice of additives used as a modifying agent depends on the types and ratios of polymer/solvent of the systems. Some researchers have used dimethyl citrate, triethyl citrate, ethyl heptanoate, glycerin, and hexanediol as additives [16, 17].

Solution gelation rate, morphology of matrix, the amount of water take up and diffusion coefficient in an in-situ forming drug delivery system are under influence of the additive [18]. Additives may be used to vary the water uptake characteristics of these systems [19, 20], or other components may be added to alter the matrix formation characteristics [21]. Also the addition of some organic additives may improve the injectability of the final solution followed by reducing solution viscosity.

Bakhshi et al. [2] and Astaneh et al. [3] have studied the effect of additives, mainly ethyl heptanoate, glycerol, and ethyl benzoate, on the in situ formation and their ability of the drug release. They correlated the drug release behavior of the system with its morphology. Since the matrix morphology is affected by phase separation rate; they had used different type of concentration of the additives. Their

results suggested that additives which slow down the rate of solidification due to low affinity between solvent and non-solvent, lead to a more dense structure and hence reduce the initial solvent removal rate and the drug release.

Our aim in this study is to prepare an injectable PLGA-based in situ forming system including one of aliphatic esters, e.g., ethyl heptanoate, methyl heptanoate, and ethyl nonanoate. These substances have chemical structure similar to PLGA. Ethyl heptanoate (EH) or ethyl enanthate, methyl heptanoate (MH) or methyl enanthate, ethyl nonanoate (EN) or ethyl pelargonate are colorless liquids having sweet odor and water-insoluble esters that are used as additives. These chemical compounds have usually been used in flavoring alcoholic beverages [22]. Figure 1 shows the chemical structure of esters. We have focused our study on the deeper understanding of degradation process without using any drugs to omit the drug function. However, morphology and chemical composition of matrices during degradation time are reported.

Experimental

Material

PLGA Resomer RG 504 [poly (DL-lactide-co-glycolide) 50:50, IV = 0.45–0.60 dL/g] was purchased from Boehringer Ingelheim (Ingelheim, Germany), NMP, ethyl heptanoate, methyl heptanoate, and ethyl nonanoate were obtained from Merck (Darmstadt, Germany). All chemicals were used as received without further purification.

Preparation of in situ forming system

Polymer solutions were prepared from PLGA in NMP with and without esters (Table 1) in several glass vials. The polymer solutions were mixed using a

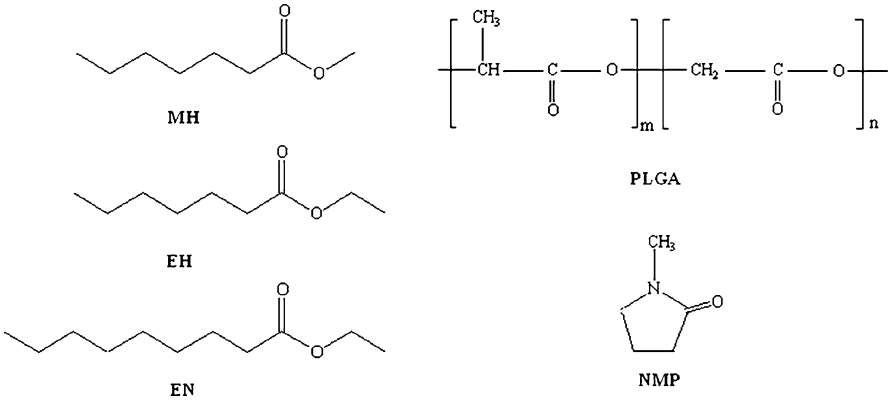

Fig. 1 Chemical structure of PLGA, NMP, ethyl heptanoate (EH), methyl heptanoate (MH), and ethyl nonanoate (EN)

Table 1 Compositions of the prepared solution

Formulation	Polymer (%w/w)	NMP (%w/w)	Ester type	Ester (%w/w)
F_1	33	67	–	–
F_2	33	57	EH	10
F_3	33	57	MH	10
F_4	33	57	EN	10

EH ethyl heptanoate, *MH* methyl heptanoate, *EN* ethyl nonanoate

laboratory shaker (100 rpm, 12 h) and were sonicated for 5 min to achieve a homogeneous solution.

A 0.2 g portion of the prepared solution was injected into three separate glass vials containing 20 mL phosphate buffer solution (pH 7.4, 0.2 M) as degradation medium using medical syringes. The vials were kept in a laboratory oven at 37 °C. In order to follow the performance and morphology of in situ formed system, the samples were stored in buffer for the appropriate times.

Solvent removal study

To analyse the released NMP, a novel high performance liquid chromatography (HPLC) method with good precision was developed using a high performance liquid chromatography (Agilent® HPLC (1200 series) system) operated by reversed phase Waters C-18 column (150 mm × 4.6 mm, 5 μm) at 30 °C, isocratic elusion of a mobile phase composed of 68:32 volume ratio trifluoroacetic acid (0.1% v/v) and acetonitril. The UV detector, flow rate and condition of injection volume were 220 nm, 0.5 mL/min, and 20 μL, respectively.

Scanning electron microscopy (SEM)

For the characterization of the cross-section morphology by the scanning electron microscopy (Cambridge S360), the samples were removed from buffer solution and dried for 24 h in laboratory oven at room temperature, frozen in liquid nitrogen, broken, mounted into the sample holder, gilded, and examined with SEM.

Nuclear magnetic resonance (NMR)

Samples were removed from the degradation medium, dried, and dissolved in DMSO-d6 to assign with a Brucker-Advance 400 MHz instrument at room temperature.

Results and discussion

NMP removal percentage

The removal of NMP from PLGA matrix occurs at once with diffusion of water into matrix which determines the final matrix morphology. Phase inversion is the

response of injected solution into aqueous medium. Presence of additive influences the rate of phase inversion. Matrices morphology investigation is necessary to understand matrices transport characteristics. By increasing the affinity between the solvent and non-solvent, phase inversion is performed faster and as a result a thin layer at the surface of the matrices and finger-like holes in the cross-section of the matrices are formed. On the contrary, delayed precipitation causes the formation of thicker skins at the surface and sponge-like structure at the matrices cross-section [21].

The water influx rate and the gelation rate are important kinetic parameters during the systems formation. The water influx rate refers to the diffusion of water from biological fluids and subsequent accumulation within the injected polymer solution [21]. Due to more water penetration into the matrices which have more porosity, the degradation rate takes place in them more rapidly. In the first step of this study, the removed NMP from matrices was evaluated by HPLC. Table 2 compares the removed NMP percentage from PLGA matrices to buffer solution during 4 days of degradation time. After this time, NMP removal percentage is 50% for control matrix (F_1) and 100, 95, and 66% for matrices containing EH (F_2), MH (F_3), and EN (F_4), respectively.

Results show that the additives increased the release of NMP from matrices. Also, this value was exceeded more for F_2 and F_3 in comparison with F_4.

It seems possible that the nature of the chemical groups at the polymer chain-ends can affect the release of NMP from matrices. The literature has underlined the impact of the nature of the polymer chain-ends in drug delivery systems [23]. In the case of PLGA Resomer RG non-H type, hydroxycarboxylic acid ester is used as chain length controller for generating alkyl ester as the end groups [24]. In our study, it has been expected that the matrices prepared from PLGA Resomer RG 504 (a non-H type) have hydrophobic character.

A slow NMP removal for control sample (F_1) is observed due to hydrophobicity of PLGA Resomer RG 504 causing a decline in water penetration. There are several inter molecular interactions between components of additive-loaded matrices. Figure 2 shows schematic diagram of the interaction between components of system during

Table 2 Released NMP % during in situ forming system solidification

Sample	Time	Released NMP %
F_1	15 min	13
	2 days	50
	4 days	51
F_2	15 min	14
	2 days	80
	4 days	100
F_3	15 min	9
	2 days	77
	4 days	95
F_4	15 min	41
	2 days	50
	4 days	66

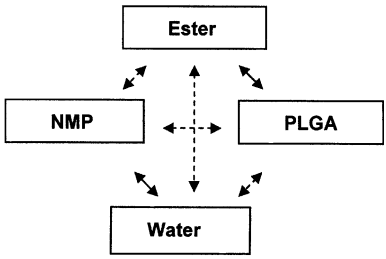

Fig. 2 Schematic diagram of the interaction between components of system during matrix solidification (*solid line*: strong affinity, *dotted line*: low affinity)

matrix solidification. NMP has good miscibility with water, but esters by having hydrophobic characteristic; have low solubility with water. PLGA and each of additives have strong affinity due to the similarity of their chemical structure (shown by solid line). Hence, during matrix solidification, the NMP can readily be transferred to the external media due to more interaction between PLGA and esters and reducing the affinity of ester/PLGA with NMP (shown by dotted line). As a result, matrices containing esters show fast phase inversion and more NMP removal percentage.

Morphology study

Figure 3 represents the cross-section morphology of the matrices at 3 and 7 days after solidification. There are differences between control matrix and other matrices containing additives. The morphology of the control sample (Fig. 3a) shows a homogenous appearance with small pores so-called "sponge-like structure". As seen, the morphology images of cross-section of ester-loaded matrices (Fig. 3c, e, g) show irregular macro voids and larger pores in comparison with control sample. Mainly, during matrix formation, slow release rate of solvent forms sponge-like structure, and high release rate of solvent causes formation of finger structure.

For additive-loaded matrices, tendency to precipitation and separation of PLGA by removal of NMP are faster. As mentioned above, addition of esters as a hydrophobic component having chemical structure similar to PLGA increases the exchange rate of solvent and water, causing formation of matrices with a large pore structure. The results of morphology study are in good agreement with results obtained in NMP removal percentage section.

After 7 days of degradation process (Fig. 3b, d, f, h), the matrices undergo more degradation reaction and the pores become larger. However, erosion in some of the edges of matrices (mainly F_2 and F_3) appears which proves degradation progress.

NMR study

^{1}H-NMR studies were used to determine the chemical composition of matrices during degradation time (Fig. 4). The ^{1}H-NMR spectrum of polymer matrix showed typical signals attributed to PLGA and residue esters. The resonance signal at 1.45 ppm (a) for methyl groups of the lactic acid units and at 4.8 ppm (b) corresponding to the methylene groups of glycolic acid and at 5.2 ppm (c) relates to methine groups of lactic acid were appeared. The presence of ester in the matrices was confirmed by the peaks 0.85 (d), 1.3–1.4 (e), 2.3 (f), 4.2 (g) ppm. Signals

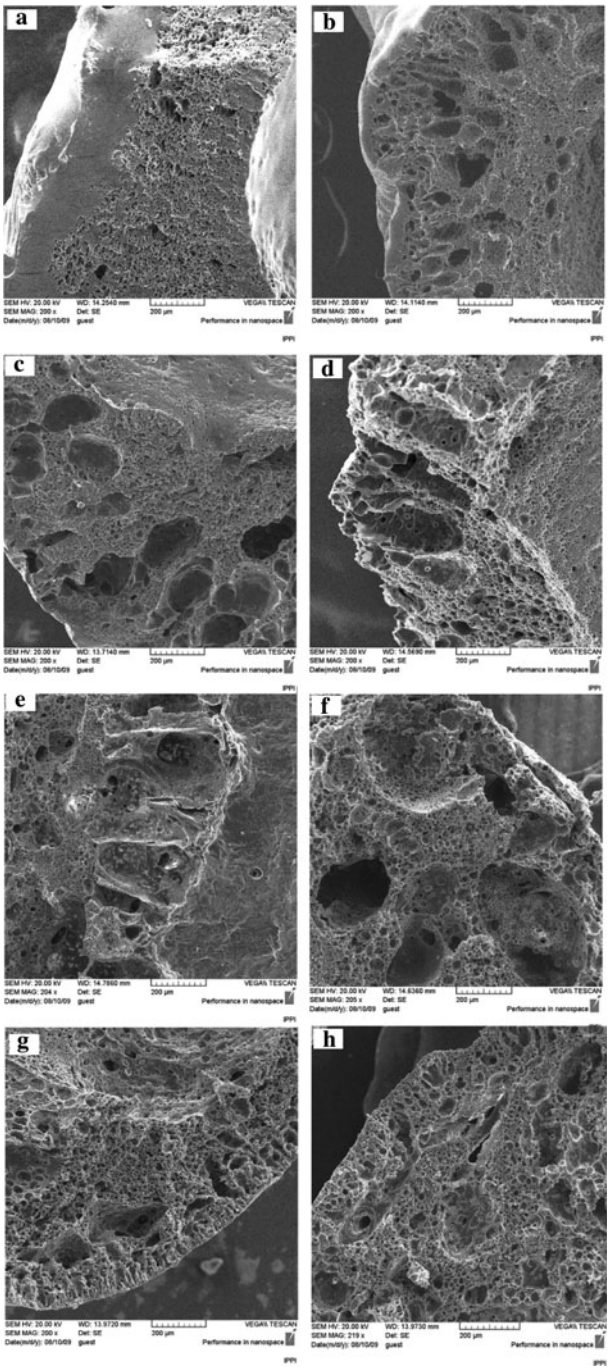

Fig. 3 Cross-section morphology of different matrices (F_1: **a**, **b**; F_2: **c**, **d**; F_3: **e**, **f**; F_4: **g**, **h**) 3 and 7 days after solidification (×200). **a**, **c**, **e**, and **g** for 3 days; **b**, **d**, **f**, and **h** for 7 days

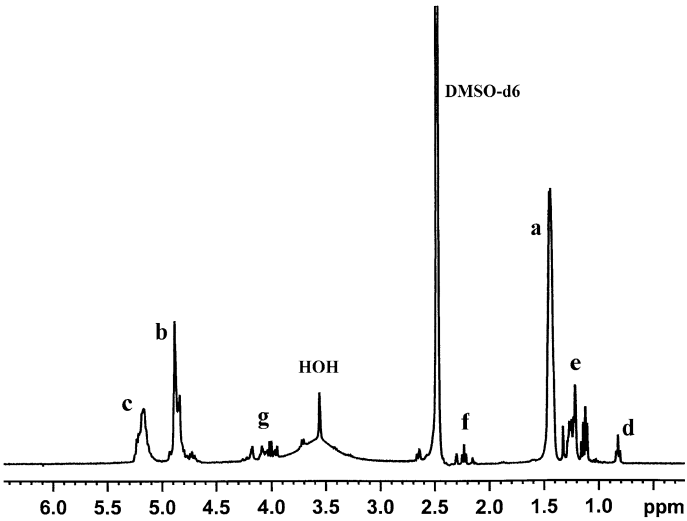

Fig. 4 ^{1}H-NMR spectrum of PLGA matrix containing MH

attribute to DMSO and water were observed in 2.5 (h) and 3.3 (i) ppm. As a result, the data show that ester remains in the matrix during degradation.

Residual ester can affect the degradation behavior of polymer. Researchers believe that the presence of low molecular weight substances or solvents make the polymer main chain more flexible which effect the degradation process [25]. As a result, water penetration into solid matrix containing esters would be faster than for control matrix (F_1), thereby; degradation rate will occur rapidly. Among the studied additives, methyl heptanoate causes more flexibility in polymer main chain due to its lower molecular weight. Hence, matrix containing methyl heptanoate is believed to show the most rapid degradation rate among the studied samples.

Difference between degradation rate of matrices containing EH (F_2), MH (F_3), and EN (F_4) were evaluated using ^{1}H-NMR spectra (Fig. 5). These changes were studied using methylene group signal of glycolide end unit (4.67–4.76 ppm), which is caused by degradation of PLGA estric bond. The ratio between a signal area from glycolic end unit and that from the methylene group of glycolic acid (4.76–4.95 ppm) was used for quantitative evaluation of degradation behavior. The values were calculated as: 0.1377, 0.09659, and 0.06717 for F_3, F_2, and F_4, respectively. This observation confirms that PLGA degradation in the presence of MH, EH, and EN proceeds in the following manner:

$$\mathrm{MH} > \mathrm{EH} > \mathrm{EN}.$$

Degradation study

Poly (lactide-co-glycolide) matrix undergoes hydrolytic degradation in contact with water. The monomer or oligomer carboxylic acid end groups (lactic and glycolic

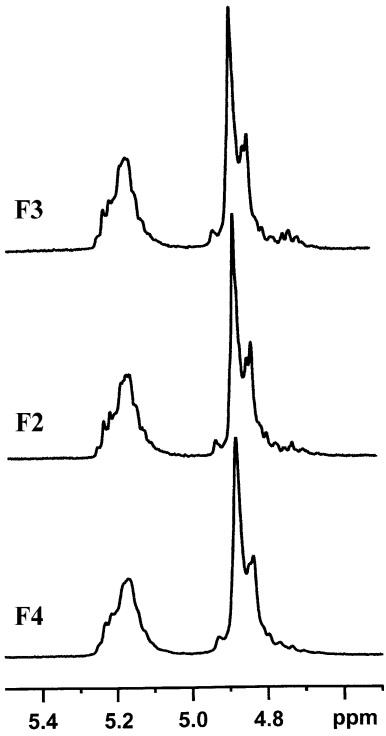

Fig. 5 Comparison of ^{1}H-NMR spectra of PLGA matrix containing MH (F_3), EH (F_2), and EN (F_4)

acid) were produced by the hydrolytic cleavage of ester bond which induce a decrease in internal pH of the matrix. These alterations further accelerate degradation in the central region. For this cause, the polymer degradation profile looks similar to a sigmoidal curve [3].

As shown in Fig. 6, variation of pH due to PLGA degradation in buffer solution with time shows different passes for the control sample and ester-loaded matrices. Decline in pH at each time especially between days 18th and 35th as an indication of degradation rate, shows the same trend observed from NMR (e.g., line [a] in Fig. 6).

Conclusion

The effect of EH, MH, and EN as additives with chemical structure similar to PLGA on preparation and degradation rate of the PLGA-based in situ forming systems has been demonstrated. It was found that the NMP was removed rapidly from additive-loaded matrices during solidification in comparison with a matrix without additive (control matrix). The SEM results suggested a fast phase inversion for additive-loaded matrices. The morphology of these matrices is very porous rather than sponge-like structure seen for control matrix. The pH measurements of degradation mediums show that the additive-loaded matrices are degraded more than control

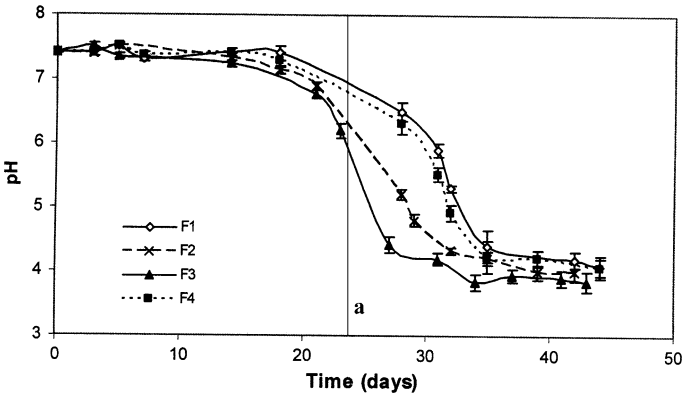

Fig. 6 pH changes profiles during degradation of in situ forming system

sample due to more water penetration. The NMR result also confirms the presence of esters in matrices during degradation time.

References

1. Ruel-Gariepy E, Leroux JC (2004) In situ-forming hydrogels—review of temperature-sensitive systems. Eur J Pharm Biopharm 58:409–426
2. Bakhshi R, Vasheghani-Farahani E, Mobedi H, Jamshidi A, Khakpour M (2006) The effect of additives on naltrexone hydrochloride release and solvent removal rate from an injectable in situ forming PLGA implant. Polym Adv Technol 17:354–359
3. Astaneh R, Erfan M, Barzin J, Mobedi H, Moghimi HR (2008) Effects of ethyl benzoate on performance, morphology, and erosion of PLGA implants formed in situ. Adv Polym Technol 27:17–26
4. Hatefi A, Amsden B (2002) Biodegradable injectable in situ forming drug delivery systems. J Control Release 80:9–28
5. Kranz H, Bodmeier R (2007) A novel in situ forming drug delivery system for controlled parenteral drug delivery. Int J Pharm 332:107–114
6. Korber M, Bodmeier R (2008) Development of an in situ forming PLGA drug delivery system: I. Characterization of a non-aqueous protein precipitation. Eur J Pharm Sci 35:283–292
7. Dunn RL, English JP, Cowsar DR, Vanderbelt DP (1990) Biodegradable in situ forming implants and methods of producing the same. US Patent 4,938,763
8. Siepmann J, Gopferich A (2001) Mathematical modeling of bioerodible, polymeric drug delivery systems. Adv Drug Deliv Rev 48:229–247
9. Houchin ML, Topp EM (2008) Chemical degradation of peptides and proteins in PLGA: a review of reactions and mechanisms. J Pharm Sci 97:2395–2404
10. Chandrashekar BL, Zhou M, Jarr EM, Dunn RL (2000) Controlled release liquid delivery compositions with low initial drug burst. US Patent 6,143,314
11. Jain RA, Rhodes CT, Railkar AM, Malick AW, Shah NA (2000) Controlled release of drugs from injectable in situ formed biodegradable PLGA microspheres: effect of various formulation variables. Eur J Pharm Biopharm 50:257–262
12. Mobedi H, Nekoomanesh M, Orafaei H, Mivehchi H (2006) Studying the degradation of poly-(L-lactide) in presence of magnesium hydroxide. Iran Polym J 15:31–39
13. Houchin ML, Neuenswander SA, Topp EM (2006) Effect of excipients on PLGA film degradation and on the stability of an incorporated peptide. J Control Release 117:413–420
14. Ara M, Watanabe M, Imai Y (2002) Effect of blending calcium compounds on hydrolytic degradation of poly (DL-lactic acid-co-glycolic acid). Biomaterials 23:2479–2483

15. Siegel SJ, Kahn JB et al (2006) Effect of drug type on the degradation rate of PLGA matrices. Eur J Pharm Biopharm 64:287–293
16. Takenaga M, Yamaguchi Y et al (2002) A novel sustained-release formulation of insulin with dramatic reduction in initial rapid release. J Control Release 79:81–91
17. Dunn RL, Tipton AJ (1997) Polymeric compositions useful as controlled release implants. US Patent 5,702,716
18. Zare M, Mobedi H et al (2008) Effect of additives on release profile of leuprolide acetate in an in situ forming controlled release system: in vitro study. J Appl Polym Sci 107:3781–3787
19. McHugh AJ (2005) The role of polymer membrane formation in sustained release drug delivery systems. J Control Release 109:211–221
20. Brodbeck KJ, Pushpala S, McHugh AJ (1999) Sustained release of human growth hormone from PLGA solution depots. Pharm Res 16:1825–1829
21. Graham PD, Brodbeck KJ, McHugh AJ (1999) Phase inversion dynamics of PLGA solutions related to drug delivery. J Control Release 58:233–245
22. Hawley's Condensed Chemical Dictionary (11th edn). Van Nostrand Reinhold Co., NY, NY, pp 224, 555, 567
23. Schwach G, Oudry N, Delhomme S, Luck M, Lindner H, Gurny R (2003) Biodegradable microparticles for sustained release of a new GnRH antagonist. Part I: screening commercial PLGA and formulation technologies. Eur J Pharm Biopharm 56:327–336
24. http://www.boehringer.com/. www.resomer.com. Boehringer Ingelheim Pharma GmbH & Co. KG. Accessed 20 Oct 2009
25. Zhang X, Wyss UP, Pichora D, Goosen MFA (1994) Investigation of poly(lactic acid) degradation. J Bioact Compat Polym 9:80–100