International Journal of Pharmaceutics 371 (2009) 33–39

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Injectable *in situ* forming depot systems: PEG-DAE as novel solvent for improved PLGA storage stability

K. Schoenhammer[a,b], H. Petersen[a], F. Guethlein[a], A. Goepferich[b,*]

[a] *Novartis Pharma AG, Technical Research and Development, 4056 Basel, Switzerland*
[b] *Department of Pharmaceutical Technology, University of Regensburg, 93040 Regensburg, Germany*

ARTICLE INFO

Article history:
Received 10 October 2008
Received in revised form 9 December 2008
Accepted 11 December 2008
Available online 24 December 2008

Keywords:
Poly(D,L-lactide-*co*-glycolide)
Degradation
Stability
In situ forming depot

ABSTRACT

Injectable *in situ* forming depots (ISFD) that contain a peptide or a protein within a polymeric solution comprise an attractive, but challenging application system. Beyond chemical compatibility, local tolerability and acute toxicity, an important factor for an ISFD is its storage stability as a liquid. In this study, poly(D,L-lactide-*co*-glycolide) (PLGA) degradation in the presence of poly(ethyleneglycol) (PEG) as biocompatible solvent was investigated as a function of storage temperature and water content. The PLGA molecular weight (M_w) was determined by gel permeation chromatography (GPC), and monitored by NMR during degradation.

Rapid PLGA degradation of 75% at 25 °C storage temperature was shown to be the result of a transesterification using conventional PEG as solvent. A significant improvement with only 3% M_w loss was obtained by capping the PEG hydroxy- with an alkyl- endgroup to have poly(ethyleneglycol) dialkylether (PEG-DAE). The formation of PEG-PLGA block co-polymers was confirmed by NMR, only for PEG300. Reaction rate constants were used to compare PLGA degradation dissolved in conventional and alkylated PEGs. The degradation kinetics in PEG-DAE were almost completely insensitive to 1% additional water in the solution. The transesterification of the hydroxy endgroups of PEG with PLGA was the major degradation mechanism, even under hydrous conditions. The use of PEG-DAE for injectable polymeric solutions, showed PLGA stability under the chosen conditions for at least 2 months. Based on the results obtained here, PEG-DAE appears to be a promising excipient for PLGA-based, parenteral ISFD.

1. Introduction

Polymeric delivery systems pose the attractive capability to control the release of drug substances to obtain defined blood levels over a specified time. In several cases this capability would provide a significant advantage. For instance, permanent medication thereby often benefit from long-term delivery systems to improve patient compliance. For permanent medication and several other applications (Vert, 2007) in humans (Hatefi and Amsden, 2002; Sultana et al., 2006) and in animals (Matschke et al., 2002), the need for appropriate depot systems exists. *In situ* forming depots comprise a specific class of polymeric delivery systems, that possess the advantages of a straightforward manufacturing even for sensitive molecules and ease of application as a liquid, which solidifies after application by phase separation (Packhaeuser et al., 2004; Packhaeuser and Kissel, 2007). When based on a polymer such as poly(D,L-lactide-*co*-glycolide)s (PLGA), the depot is biodegradable in vivo (Nair and Laurencin, 2007). Currently, there are two injectable *in situ* forming depots on the market: Atridox® and Eligard®. Both products were developed based on the Atrigel technology of Dunn et al. (Ravivarapu et al., 2000). This technology employs PLGA dissolved in N-methyl-2-pyrrolidinone (NMP), which is a water miscible solvent, and a drug powder suspended in this solution prior to application.

The major prerequisite characteristics of the solvent of an *in situ* depot system include good solubility properties for the polymer, chemical compatibility, biocompatibility and overall stability. Additionally, a suitable solvent for subcutaneous (s.c.) or intramuscular (i.m.) injection should be minimally irritating to the injection site, and it and its metabolic products should not have deleterious side-effects on the organism. The ICH classification of solvents in pharmaceutical products narrows the use by the permitted daily exposure (PDE) of listed excipients. In 2002, toxicological results led to a decrease of the PDE for NMP from 48.4 to 5.3 mg/day (International Conference on Harmonisation, 2005a,b). Water miscible solvents such as NMP or dimethylsulfoxide (DMSO) and hydrophobic solvents like ethyl benzoate or triacetin have also been investigated for their tolerability and phase inversion dynamics for parenteral injectable devices (Graham et al., 1999; Brodbeck et al., 1999; Kranz and Bodmeier, 2008). Mainly, the

* Corresponding author. Tel.: +49 941 943 4843; fax: +49 941 943 4807.
E-mail address: achim.goepferich@chemie.uni-regensburg.de (A. Goepferich).

0378-5173/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2008.12.019

biocompatibility of those solvents has been studied in recent years (Royals et al., 1999). Surprisingly however, polymer–solvent interactions have rarely been addressed, although it is known that biodegradable polymers such as PLGA can interact significantly with nucleophiles (Lucke and Gopferich, 2003; Na et al., 2003; Murty et al., 2005).

Low molecular weight PEGs seem to have promising solubility properties for use with PLGAs, and they have previously been incorporated into parenteral formulations at concentrations up to 50–65% (Powell et al., 1998; Strickley, 1999). Despite these beneficial properties, low molecular weight PEGs also possess primary hydroxy groups that may lead to undesirable interactions with PLGAs. Therefore, we have studied and compared the effect of common PEGs (with –OH endgroups) and PEGs with alkyl endgroups on the stability of PLGA-based injectable *in situ* forming depots.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-*co*-glycolide) with 50% D,L-lactide and 50% glycolide was purchased from Boehringer Ingelheim Pharma KG (Ingelheim, Germany). The inherent viscosity of a 0.1% solution of $PLA_{50}GA_{50}12$ in chloroform was 0.16–0.24 dL/g (weight average M_w = 12000 Da), of Resomer RG 504H ($PLA_{50}GA_{50}48$) 0.45–0.60 dL/g (M_w = 48000 Da), and of poly(D,L-lactide) Resomer R 202H ($PLA_{100}15$) 0.20–0.23 dL/g (M_w = 15000 Da). Poly(ethyleneglycol) 300 (PEG300) with an average molecular weight of 300 Da, poly(ethyleneglycol) 250 dimethyl ether (PEG-DAE) with a molecular weight of 250 Da, and dichloromethane (DCM) were purchased from Sigma–Aldrich (Steinheim, Germany). HPLC grade tetrahydrofuran (THF) was obtained from Rathburn Chemicals (Walkerburn, Scotland). Argon gas was purchased from Carbagas AG, Basel, Switzerland. All chemicals were used without further purification.

2.2. Methods

2.2.1. Preparation of pure polymer powder samples for stability studies

Polymer powder ($PLA_{50}GA_{50}12$, $PLA_{50}GA_{50}48$ and $PLA_{100}15$) as received from the supplier was filled into 1 ml glass ampoules (straight stem ampoule OPC with blue point, Schott France SAS, Pont-sur-Yonne, France) and purged with argon prior to hermetic sealing to prevent the polymer from degradation induced by humidity. The storage temperature was set to −20 °C, 5 °C, 25 °C and 40 °C over 2 months storage time. For determination of molecular weight, samples were collected at t = 0, 5, 10, 19, 31 and 64 days, dissolved in THF and analyzed by gel permeation chromatography (GPC) as described below.

2.2.2. Preparation of polymer solutions for stability studies

$PLA_{50}GA_{50}12$ and $PLA_{50}GA_{50}48$ were dissolved to 20% (w/w) in PEG300 and PEG-DAE. $PLA_{100}15$ was dissolved to 20% in PEG-DAE only, because of its poor solubility in PEG300. The polymer was completely dissolved in the solvents after 24 h stirring with a magnetic stirrer (IKA Labortechnik RET basic, Staufen, Germany) at room temperature. 150–200 mg of the solutions were filled in 1 ml ampoules, purged with argon prior to filling and purged again with argon prior to hermetic sealing. The ampoules were stored at −20 °C, 5 °C, 25 °C and 40 °C and samples were taken at t = 0, 5, 10, 19, 31 and 64 days, dissolved in THF and polymer molecular weight (M_w) was determined by GPC.

To elucidate the influence of residual water in the solvents on polymer stability PEG300 and PEG-DAE were spiked with approximately 1% water. $PLA_{50}GA_{50}12$ and $PLA_{50}GA_{50}48$ were dissolved by magnetic stirring in the spiked solvents with a concentration of 20% (w/w), while again, $PLA_{100}15$ was dissolved only in spiked PEG-DAE to 20% (w/w). The spiked solutions were also filled in ampoules, purged twice with argon and stored hermetically sealed at the same temperatures as described above. Samples for GPC analysis were taken at the same sampling points.

2.2.3. Determination of water content

Water content of the solvents PEG300 and PEG-DAE was measured by Karl-Fisher titration with a KF Coulometer Mettler DL 37 (Mettler-Toledo AG, Greifensee, Switzerland). Solvent as received from the supplier and solvent spiked with approximately 1% water were injected (accurately to 0.0001 g) directly into the titration vessel containing 100 ml analyte and 5 ml katholyte (Hydranal Coulomat-AG and Hydranal Coulomat-CG, Sigma–Aldrich, Steinheim, Germany). The quantitative determination of water was based on the Karl-Fisher reaction (Scholz, 1983). The water content of each sample was determined in triplicate and calculated based on the consumption of I^- oxidized to I_2, which happens in the presence of water.

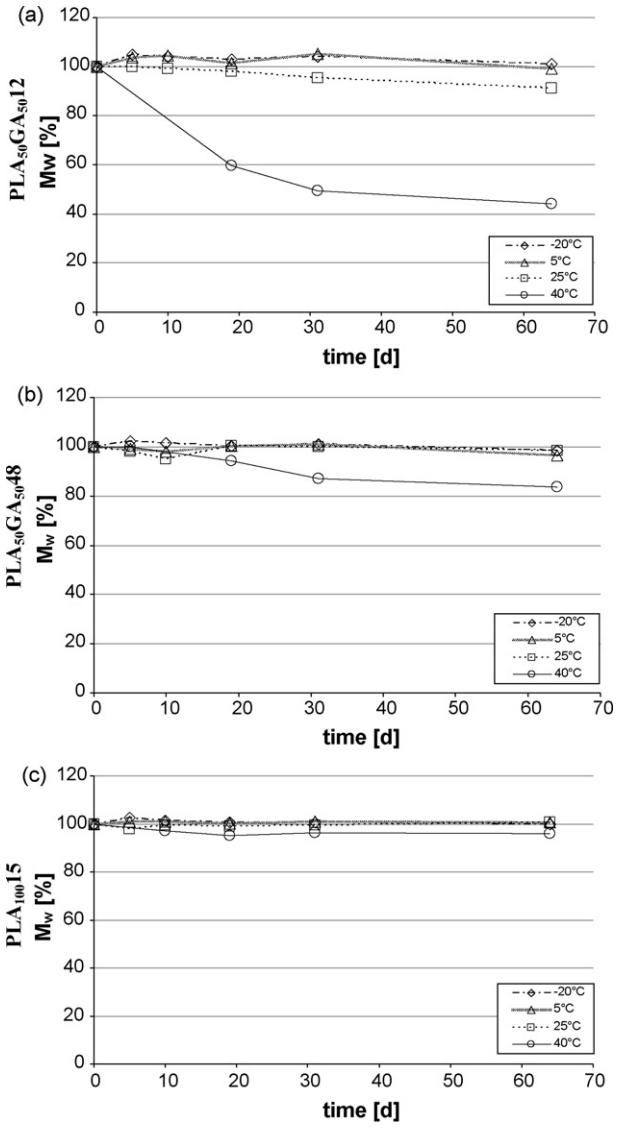

Fig. 1. Relative M_w of $PLA_{50}GA_{50}12$ (a) and $PLA_{50}GA_{50}48$ (b) and $PLA_{100}15$ (c) during storage over 2 months.

2.2.4. Gel permeation chromatography for molecular weight determination

For GPC analysis, $PLA_{50}GA_{50}12$, $PLA_{50}GA_{50}48$ and $PLA_{100}15$ powder and solutions were diluted to approximately 3 mg/ml polymer with THF 4 h prior to measurement. GPC analysis was carried out in a Waters 2695 series liquid chromatograph coupled with a 2414 refractive index detector. 100 μl were injected into a series of four columns (PLGel pre-column 5 μm 50 mm × 7.5 mm, 3 PLGel High performance GPC columns, length: 300 mm, diameter: 7.5 mm, gel pore size: 10000 Å, 500 Å, 100 Å, Polymer laboratories, Varian Incorporated, Amherst MA, USA) at an oven temperature of 35 °C. THF was used as mobile phase at a flow rate of 0.8 ml/min. For calibration, 8 polystyrene standards (M_w = 504.50 kDa, 197.30 kDa, 70.95 kDa, 30.23 kDa, 20.65 kDa, 10.68 kDa, 2.97 kDa, 0.58 kDa) from polymer laboratories (Varian Incorporated, Amherst MA, USA) were used. Sample injection was performed in duplicate, and weight average molecular weight (M_w) was calculated with the Empower software (Waters version 2002, Milford, USA). The molecular weights of the samples were calculated relative to the M_w at t = 0.

2.2.5. ^1H NMR

^1H NMR spectra of $PLA_{50}GA_{50}12$–PEG300 and $PLA_{50}GA_{50}12$–PEG-DAE solutions were recorded after 0, 21 and 64 days of storage at 25 °C. 2.1– 2.4 mg of the PLGA solutions was diluted with 100 μl DMSO-d_6 and transferred directly into a 1.7 mm NMR tube (Bruker, Faellanden, Switzerland). Spectra were taken at room temperature with a Bruker DMX 500 (Bruker, Faellanden, Switzerland) at a field frequency of 500 MHz, using a 1.7 mm TXI probe. The measurements were carried out with an acquisition time of 1.638 s, a pulse repetition time of 2.638 s, a 30° pulse width, and 10 kHz spectral width. The chemical shift was referred to the solvent peak of DMSO-d_6 (δ = 2.5). A two-dimensional heteronuclear single quantum coherence spectra (HSQC) was taken to identify the exact correlations between ^{13}C and ^1H.

3. Results and discussion

3.1. Storage stability of solid PLGA and PLA powder

To investigate possible temperature or water influenced polymer degradation in solution, first pure PLGA and PLA powder in the absence of solvent were studied for degradation when stored at different temperatures. The average M_w of $PLA_{50}GA_{50}12$ (Fig. 1a) and $PLA_{50}GA_{50}48$ (Fig. 1b), $PLA_{100}15$ (Fig. 1c) was determined over 2 months storage time. Since hydrolytic degradation of PLGAs with carboxy instead of alkyl endgroups was described to be faster (Nair

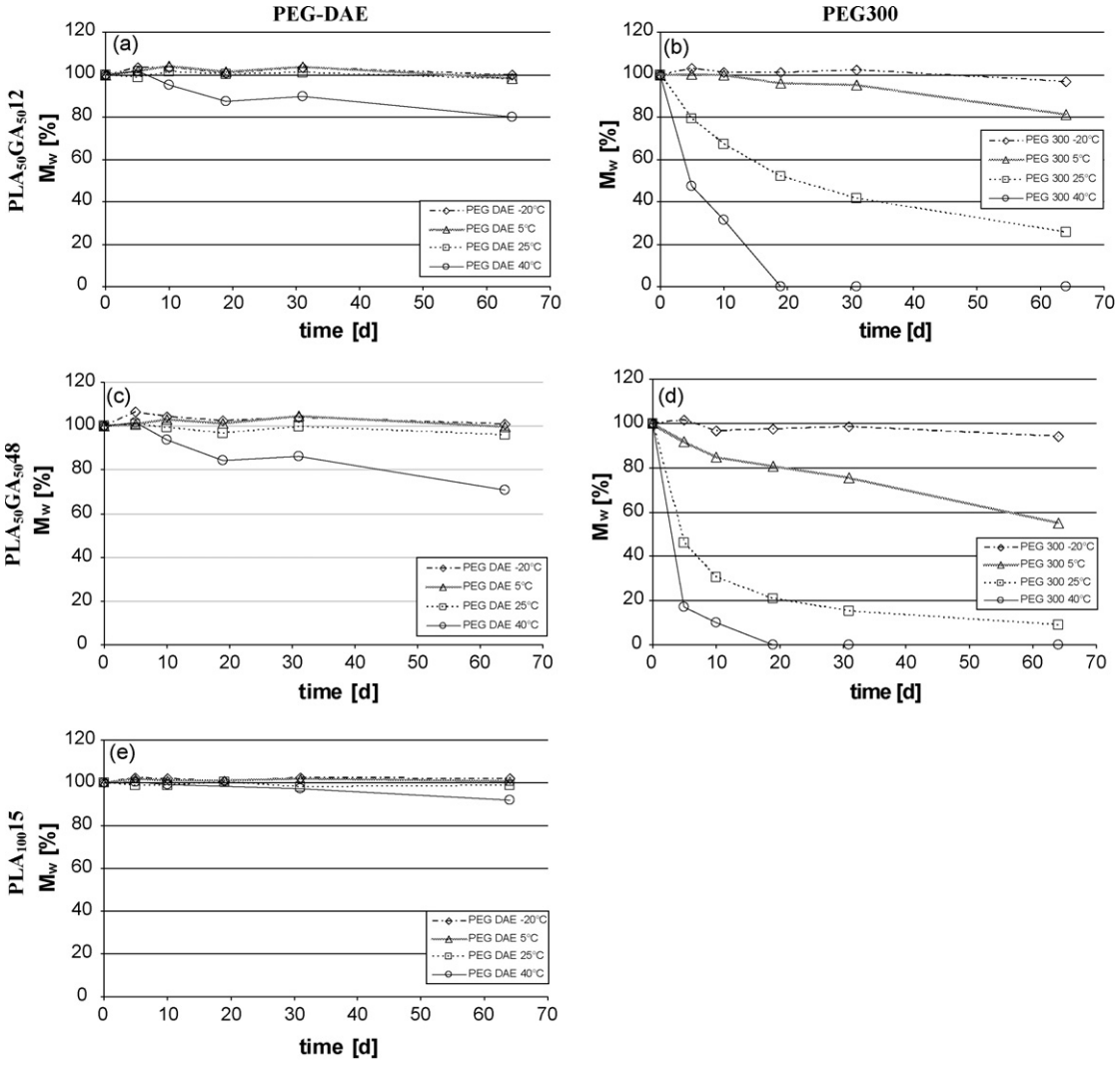

Fig. 2. Relative M_w of $PLA_{50}GA_{50}12$ in PEG-DAE (a), PEG300 (b) and $PLA_{50}GA_{50}48$ in PEG-DAE (c), PEG300 (d) and $PLA_{100}15$ dissolved in PEG-DAE (e) during storage over 2 months.

and Laurencin, 2007), the investigated polymers were chosen with free carboxy endgroups for this study. Slower degradation rates for $PLA_{100}15$ relative to both $PLA_{50}GA_{50}$s were expected (Gopferich, 1997).

A decrease to less than 50% M_w of its initial value for $PLA_{50}GA_{50}12$ (Fig. 1a) was observed over 2 months storage time at 40 °C. A slight degradation to approximately 90% of the initial M_w value was determined for $PLA_{50}GA_{50}12$ stored at 25 °C over two months. $PLA_{50}GA_{50}12$ stored at −20 °C and 5 °C over 2 months did not show a significant degradation compared to the initial M_w reference at $t = 0$.

$PLA_{50}GA_{50}48$ (Fig. 1b) with an initial molecular weight four times higher than $PLA_{50}GA_{50}12$ showed slower degradation at the same storage temperatures. A decrease in average M_w to 84% of the initial value was detected over 2 months observation time stored at 40 °C. No significant decrease in average M_w could be determined when stored at −20 °C, 5 °C and 25 °C over 2 months.

In contrast to the fast degradation of $PLA_{50}GA_{50}12$ and $PLA_{50}GA_{50}48$, $PLA_{100}15$ (Fig. 1c) showed only 4% degradation after 2 months stored at 40 °C. The change in average M_w of PLA_{100} over 2 months storage at −20 °C, 5 °C and 25 °C was negligible.

Our experiments confirmed that degradation of both $PLA_{50}GA_{50}$s was more strongly affected by temperature than $PLA_{100}15$, resulting in an improved storage stability of $PLA_{100}15$ compared to $PLA_{50}GA_{50}12$ and $PLA_{50}GA_{50}48$. Slower degradation with increase in polymer M_w was determined by comparing $PLA_{50}GA_{50}12$ and $PLA_{50}GA_{50}48$. The content of lactide and glycolide monomers and oligomers in the polymer alone with residual water may also influence the degradation kinetics (Schliecker et al., 2003). The effect of temperature on hydrolytic degradation of pure polymer powder was considered as the base level of the two $PLA_{50}GA_{50}$ and PLA_{100} degradation when using solvents.

Table 1
Solvent water content determined by Karl-Fisher titration.

Solvent	Pure solvent	Spiked solvent
PEG300	0.06%	1.04%
PEG-DAE	0.03%	1.12%

3.2. Stability of PLGA and PLA solutions in PEG and PEG-DAE

The degradation of PLGA and PLA in the two PEGs was also investigated as a function of temperature. While $PLA_{50}GA_{50}12$ and $PLA_{50}GA_{50}48$ were dissolved in PEG300 (Fig. 2b and d) and PEG-DAE (Fig. 2a and c), $PLA_{100}15$ was dissolved in PEG-DAE for aforementioned solubility issues (Fig. 2e). The water content of 0.06% for PEG300 and 0.03% for PEG-DAE (Table 1) was determined by Karl-Fisher titration.

$PLA_{50}GA_{50}12$ in PEG300 undergoes fast degradation during the first 30 days at 25 °C and 40 °C (Fig. 2b). The solution of $PLA_{50}GA_{50}12$ in PEG300, stored at 25 °C over 2 months, degraded to 20% of its initial M_w value during this time period. When the same solution was kept at 40 °C, the M_w was under the detection limit of the method

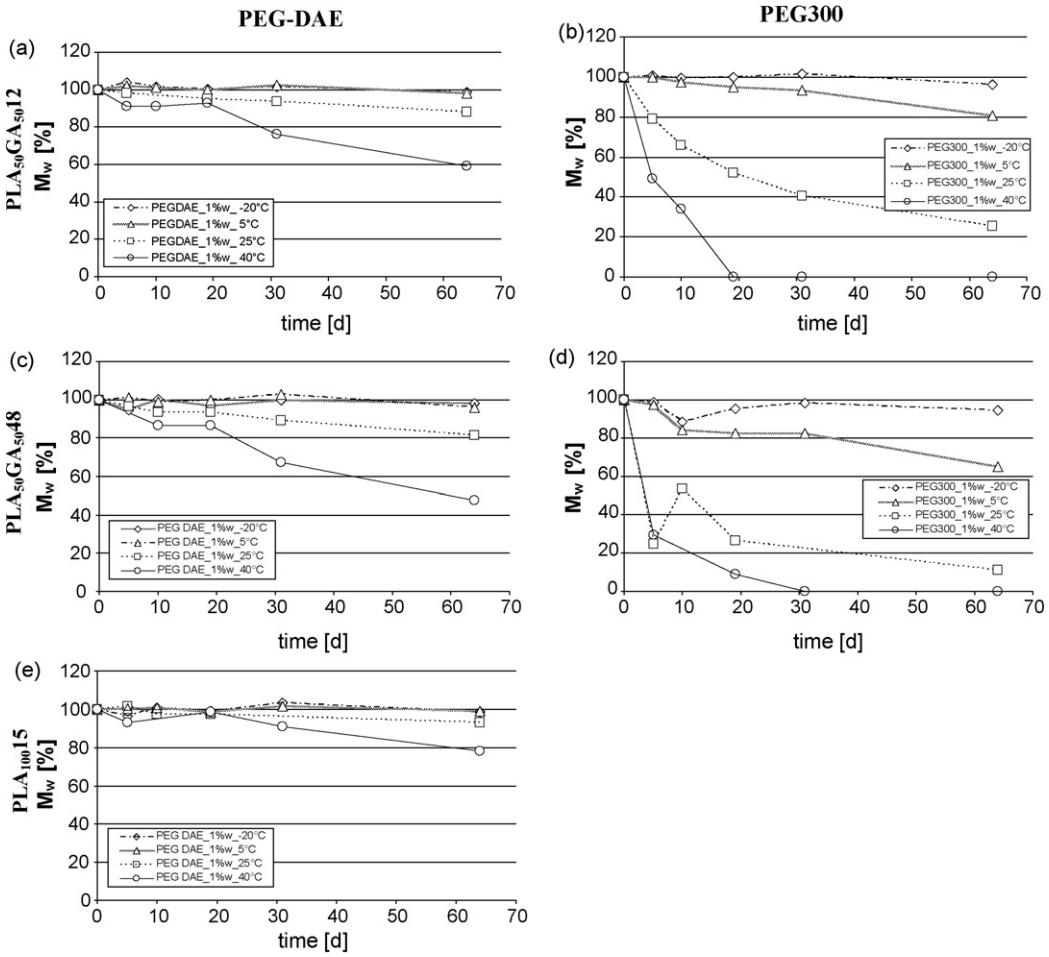

Fig. 3. Relative M_w of $PLA_{50}GA_{50}12$ in PEG-DAE (a), PEG300 (b) and $PLA_{50}GA_{50}48$ in PEG-DAE (c), PEG300 (d) and $PLA_{100}15$ dissolved in PEG-DAE (e), all spiked with 1% water during storage over 2 months.

after 20 days. In general, our observations concur with the study of Dong et al. (2006), which showed a fast PLGA degradation when dissolved in PEG400.

Compared to the PEG300 solution an improved storage stability of $PLA_{50}GA_{50}12$ dissolved in PEG-DAE (Fig. 2a) was observed. Only at 40 °C slight polymer degradation was monitored over the time of observation. The improvement of $PLA_{50}GA_{50}$ storage stability was confirmed with a solution of $PLA_{50}GA_{50}48$ in endcapped PEG-DAE (Fig. 2c). No degradation of $PLA_{100}15$ dissolved in PEG-DAE (Fig. 2e) was observed; this was in sharp contrast to the fast degradation seen for $PLA_{50}GA_{50}$-PEG300 solutions (Fig. 2b). Increased storage temperature (40 °C) showed no significant impact on $PLA_{100}15$ degradation in solution (Fig. 2e).

When compared to the profile of pure $PLA_{50}GA_{50}12$ powder (Fig. 1a), the degradation profile of $PLA_{50}GA_{50}12$ in PEG300 (Fig. 2b) displays significantly faster degradation during storage. This was also observed when comparing $PLA_{50}GA_{50}48$ dissolved in PEG300 (Fig. 2d) with the $PLA_{50}GA_{50}48$ powder (Fig. 1b) stored at the same conditions. From this data, it can be concluded additional degradation mechanisms are active when the polymers are in solution.

Compared to the PEG300 solutions, the degradation of $PLA_{50}GA_{50}12$ and $PLA_{50}GA_{50}48$ dissolved in PEG-DAE (Fig. 2a and c) was substantially reduced. Since the only difference between these two investigated solvents, was the endgroup of the PEGs, the chemical reactivity of the endgroup appears to significantly impact polymer stability. We therefore hypothesized that a chemical reaction was taking place between our polymers and PEG300 in solution. Since we speculated that it could either be a hydrolytic degradation caused by residual water or a transesterification reaction of PEG hydroxy endgroups with PLGA ester bonds, we investigated the impact of water on the reaction kinetics.

3.3. Effect of water on stability of PLGA and PLA solutions

The influence of 1% water in the solvents on polymer degradation was studied by dissolving $PLA_{50}GA_{50}12$ in PEG300 (Fig. 3b) and PEG-DAE (Fig. 3a) and $PLA_{50}GA_{50}48$ in PEG300 (Fig. 3d) and PEG-DAE (Fig. 3c) spiked with water. $PLA_{100}15$ was dissolved again in spiked PEG-DAE only (Fig. 3e), because of its low solubility. The water content of 1.04% for PEG300 and 1.12% for PEG-DAE (Table 1) was determined by Karl-Fisher titration. All polymer solutions were again analyzed by GPC to track the molecular weight of the polymers during storage.

The presence of water did not seem to have a significant effect on $PLA_{50}GA_{50}12$ (Fig. 3b) and $PLA_{50}GA_{50}48$ (Fig. 3d) degradation, as the fast loss of M_w at 25 °C and 40 °C seen during the first 30 days for the spiked PEG300 was similar to the PLGA degradation profile in the unspiked solvent (Fig. 2b and d).

A slight effect of additional water on PLGA degradation was visible when comparing the degradation profiles obtained for unspiked (Fig. 2a and c) and with water spiked PEG-DAE solutions (Fig. 3a and c). After 2 months storage time $PLA_{50}GA_{50}12$ showed 10% at 25 °C and 20% at 40 °C increased relative loss in average M_w of water spiked to unspiked samples. The degradation for $PLA_{50}GA_{50}48$ in the solutions with additional water was increased by 15% at 25 °C and 32% at 40 °C relative to the solutions with low water content. Even for $PLA_{100}15$ (Fig. 3e), an increase in degradation from 5% at 25 °C to 14% at 40 °C was observed in water spiked samples.

Overall, the water content in PLGA-PEG300 solutions does not appear to be a key factor for the degradation of PLGA. Only when slower polymer degradation occurred as in solutions of PLGA in PEG-DAE is the effect of hydrolysis caused by additional water detectable (de Jong et al., 2001). In the USP monography for PEGs,

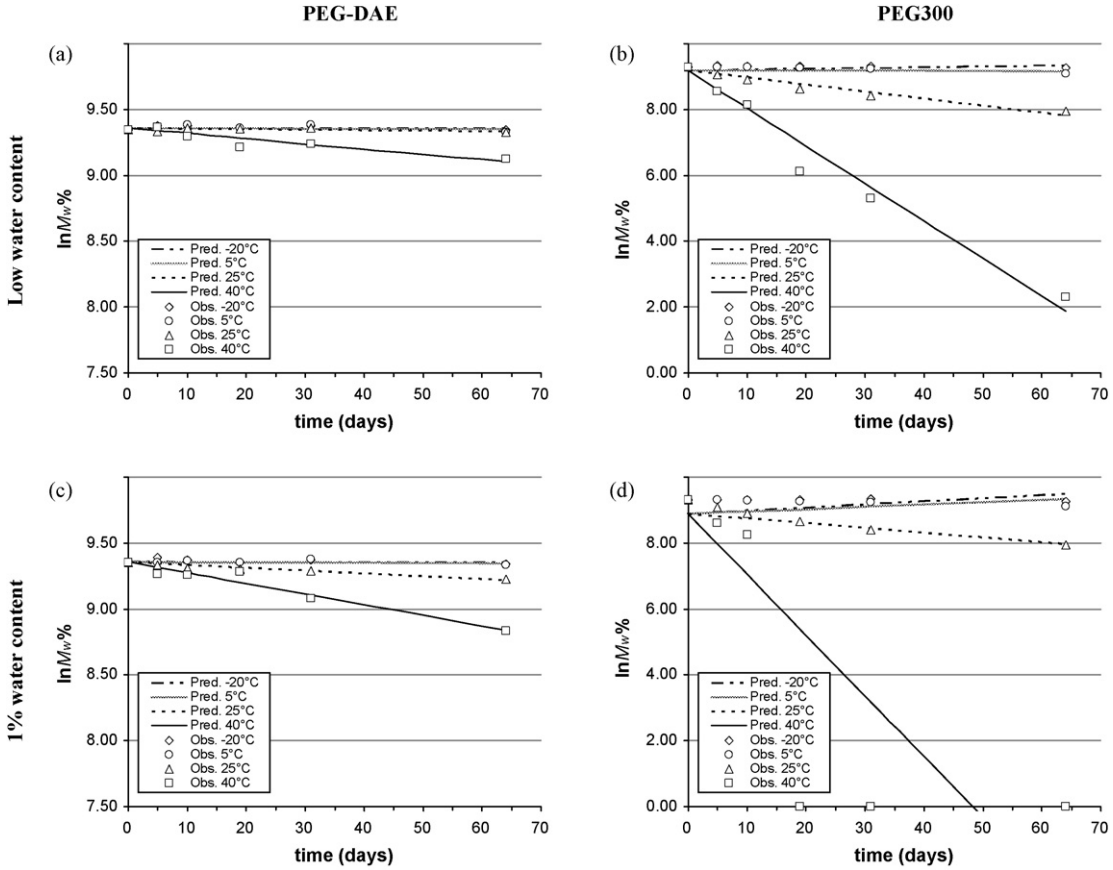

Fig. 4. Linear fit of ln M_w% versus time of $PLA_{50}GA_{50}12$ dissolved in PEG-DAE (a), PEG300 (b) and in PEG-DAE (c), PEG300 (d) both spiked with 1% water.

Table 2
Degradation rate constants ($\ln M_w\% \times 10^{-3}$, 1/day) after 2 months storage at 4 different temperatures.

Polymer	Solvent	Water	−20° C	5° C	25° C	40° C
$PLA_{50}GA_{50}12$	PEG300	No	2.43	−0.23	−21.18	−114.20
	PEG-DAE	No	0.00	0.13	−0.44	−3.98
$PLA_{50}GA_{50}48$	PEG300	No	5.91	−2.73	−39.79	−120.31
	PEG-DAE	No	0.21	−0.01	−0.93	−5.80
$PLA_{50}GA_{50}12$	PEG300	Yes	9.50	6.82	−14.24	−184.28
	PEG-DAE	Yes	0.05	−0.1	−2.16	−8.12
$PLA_{50}GA_{50}48$	PEG300	Yes	5.91	−2.25	−34.11	−188.52
	PEG-DAE	Yes	−0.14	−0.27	−3.14	−11.42

quantities of up to 2% water are accepted (USP30-NF25, 2008). Using PEG-DAE as a solvent for PLGAs still needs to be further investigated, but this study demonstrates that the lowest possible water content should be utilized. The potential water uptake of the solvent is an important factor for the stability of later products.

3.4. Determination of degradation rates

For direct comparison, degradation rate constants of water spiked and unspiked solutions were calculated from the slope of a semi logarithmic plot of weight-averaged molecular weight versus time (International Conference on Harmonisation, 2008). The slopes are shown in Fig. 4, while Table 2 displays the determined values. Depending on the storage temperature, the values for the degradation rate constants for $PLA_{50}GA_{50}12$ dissolved in PEG300 decreased from 2.43 at −20 °C to −114.20 at 40 °C (Fig. 4b). A similar decrease from 5.91 at −20 °C to −120.31 at 40 °C was observed for $PLA_{50}GA_{50}48$ in PEG300. Instead of an expected constant of 0 at −20 °C, the positive values for the degradation rates stem from variation in determined M_w. The values obtained concur in general with a fast degradation observed by Dong et al. (2006) of PLGA dissolved in PEG400. Overall, lower degradation rate constants were observed for PLGA-PEG-DAE solutions at equivalent storage temperatures. The constants for $PLA_{50}GA_{50}12$ in PEG-DAE at −20 °C were 0, decreasing in value to −3.98 at 40 °C (Fig. 4a). A slight effect of temperature on $PLA_{50}GA_{50}48$ degradation is reflected in the reaction rate constants of 0.21 at −20 °C and −5.80 at 40 °C.

The PEG300 solutions spiked with water showed reaction rate constants of −184.28 and −188.52 at 40 °C for $PLA_{50}GA_{50}12$ (Fig. 4d) and $PLA_{50}GA_{50}48$, respectively. Improved PLGA stability in PEG-DAE was still observed in the presence of additional water. The degradation rate constants for $PLA_{50}GA_{50}12$ (−8.12 at 40 °C) (Fig. 4c) and $PLA_{50}GA_{50}48$ (−11.42 at 40 °C) in PEG-DAE were only slightly lower than for the comparable unspiked samples.

The degradation rate constants for $PLA_{50}GA_{50}12$ and $PLA_{50}GA_{50}48$ in PEG300 were of the same order of magnitude as values published by Dong et al. (2006) for Resomer RG 503H in PEG400. The mentioned value in literature at 40 °C was −58.0, which is comparable with −114.20 ($PLA_{50}GA_{50}12$) and −120.31 ($PLA_{50}GA_{50}48$). The differences may stem from the molar amount of reactive hydroxy groups, which was lower in PEG400 than in PEG300 due to its lower molecular weight, leading to slower degradation rates of PLGA in PEG400 when compared to those in PEG300.

3.5. Identification of PLGA-PEG300 reaction by ^1H NMR

To prove that a transesterification is the basic degradation mechanism of PLGA in PEG300, ^1H NMR analysis was performed concurrently with the stability study of $PLA_{50}GA_{50}12$ solutions in

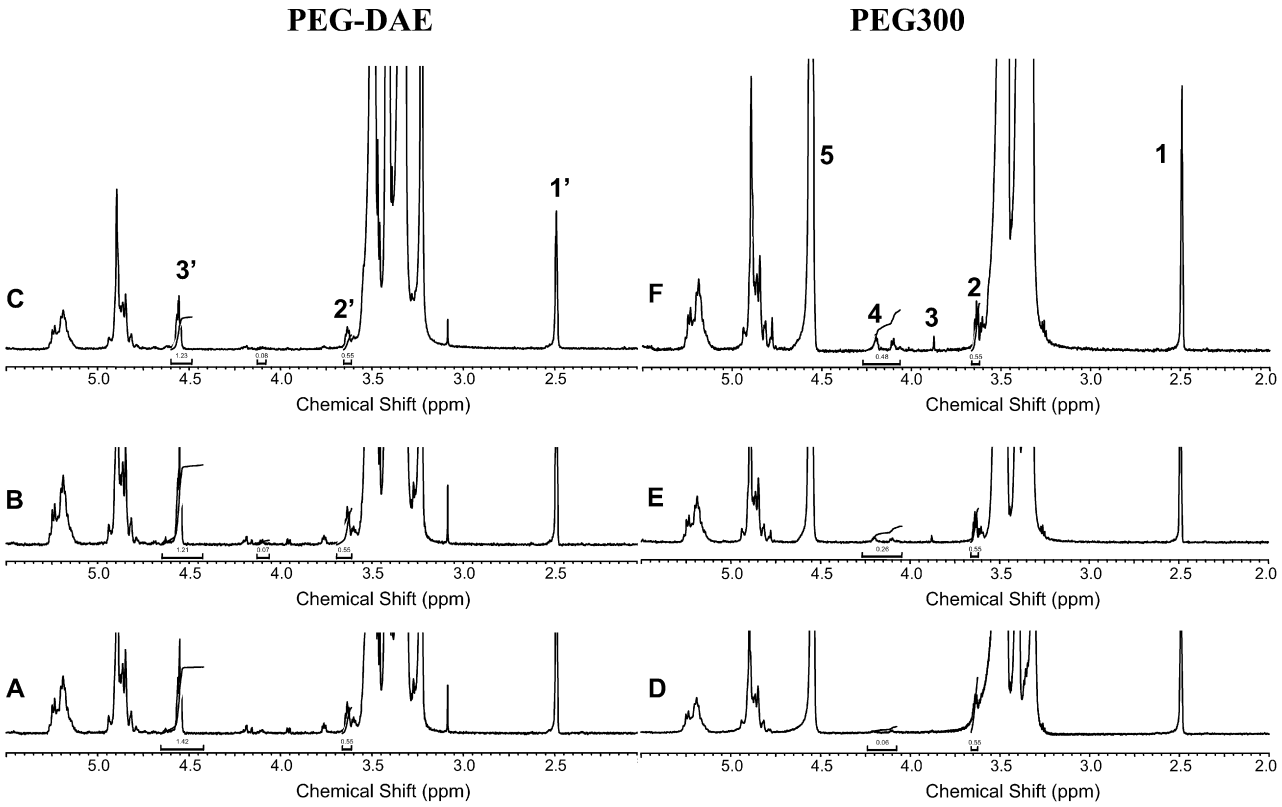

Fig. 5. Increasing signals of $PLA_{50}GA_{50}12$–PEG300 esters at $t = 0$ (D), 21 days (E) and 2 months (F) in ^1H NMR spectra compared to $PLA_{50}GA_{50}12$–PEG-DAE solutions both at $t = 0$ (A), 21 (B), 64 days (C), stored at 25 °C.

Table 3
^{1}H NMR spectra with $PLA_{50}GA_{50}12$–PEG300 ester signal (t = 64 days) at 4.25 ppm.

Peak no.	Chemical shift (ppm)	Corresponding protons
1, 1′	2.50	DMSO
2	3.60	^{13}C-SB (PEG300)
2′	3.60	^{13}C-SB (PEG-DAE)
3	3.85	CH_3-C**H$_2$**-COOH
3′	4.55	CH_3-O**H**
4	4.20	COO-C**H$_2$**-PEG300
5	4.55	PEG-O**H**

PEG300 and PEG-DAE at 3 sampling points. The spectra are shown in Fig. 5, while Table 3 relates the most important signals to their corresponding structures.

The spectra of $PLA_{50}GA_{50}12$ dissolved in PEG-DAE and stored at 25 °C taken at t = 0 (Fig. 5A), 21 days (Fig. 5B) and 64 days (Fig. 5C) were essentially identical and show, therefore, no sign of reaction products in the solution. Signals of possible side products such as methanol (4.55 ppm) and vinyl-rests (6.55 ppm) that stem from the synthesis of PEG-DAE were observed, but no increase in quantity was seen over 2 months.

In $PLA_{50}GA_{50}12$–PEG300 solutions, a weak signal between 4.00 and 4.25 ppm was detected at t = 21 days (Fig. 5E) and 2 months (Fig. 5F). Due to the time needed for the dissolution and handling of $PLA_{50}GA_{50}12$ in PEG300 at room temperature, these signals already appeared at t = 0 (Fig. 5D).

The signal at 4.25 ppm can be attributed to two hydrogen atoms of PEG300 next to an ester bond. Protons with their corresponding carbon atoms could be identified by a two-dimensional heteronuclear single quantum coherence spectra, indicating that the hydroxy endgroups of PEG300 reacted with the ester bonds of $PLA_{50}GA_{50}12$, resulting in $PLA_{50}GA_{50}12$–PEG300 esters (data not shown). In contrast, no signal at the same chemical shift was seen for $PLA_{100}15$-PEG300 mixtures or PEG300 alone, which served as a reference (data not shown). This may be due to the lower reactivity of esters between lactide acid units.

The $PLA_{50}GA_{50}12$–PEG300 esters identified by NMR confirmed that a transesterification reaction of $PLA_{50}GA_{50}12$ with the hydroxy endgroups of PEG300 took place during storage of the solution. The storage temperature of 25 °C accelerated the reaction of $PLA_{50}GA_{50}12$ and PEG300. A nucleophilic attack of the PEG hydroxy endgroup on the ester bonds in the PLGA backbone can generate PEG-PLGA esters. Differences in the reactivity between esters formed between glycolide and lactide units in PLGAs seem to be an important factor for a chemical reaction. The preferred attack of ester bonds between glycolide units or between D- and L-lactide and glycolide units by hydroxy endgroups is probable (Park, 1995). It was previously reported in literature that the attack of a nucleophile to glycolide units in PLGAs follows faster kinetics than to lactide units (Shih, 1995; Shih et al., 1996).

4. Conclusion

It was shown that block-copolymers form during degradation of PLGA in PEG300. A significant improvement of PLGA stability in solution was obtained by capping the solvent with an alkyl endgroup. Still however, hydrolytic degradation exerts strong influence on PLGA stability, and it must be strictly controlled. Alkylated PEG decreased the possibility of a reaction between nucloephilic side groups of the solvent and PLGA. The incorporation of a drug substance may also influence the PLGA degradation mechanism due to reactive side groups (Siegel et al., 2006).

Acknowledgements

Thanks for the support of M. Schuleit and Y. Duchesne for GPC measurements and L. Oberer for ^{1}H NMR analysis (Novartis Pharma AG, Basel, Switzerland).

References

Brodbeck, K.J., DesNoyer, J.R., McHugh, A.J., 1999. Phase inversion dynamics of PLGA solutions related to drug delivery: Part II. The role of solution thermodynamics and bath-side mass transfer. J. Control. Release 62, 333–344.
de Jong, S.J., Arias, E.R., Rijkers, D.T.S., van Nostrum, C.F., Kettenes-van den Bosch, J.J., Hennink, W.E., 2001. New insights into the hydrolytic degradation of poly(lactic acid): participation of the alcohol terminus. Polymer 42, 2795–2802.
Dong, W.Y., Korber, M., Lopez Esguerra, V., Bodmeier, R., 2006. Stability of poly(D,L-lactide-*co*-glycolide) and leuprolide acetate in in-situ forming drug delivery systems. J. Control. Release 115, 158–167.
Gopferich, A., 1997. Polymer bulk erosion. Macromolecules 30, 2598–2604.
Graham, P.D., Brodbeck, K.J., McHugh, A.J., 1999. Phase inversion dynamics of PLGA solutions related to drug delivery. J. Control. Release 58, 233–245.
Hatefi, A., Amsden, B., 2002. Biodegradable injectable in situ forming drug delivery systems. J. Control. Release 80, 9–28.
International Conference on Harmonisation. Guideline: Impurities: Guideline for residual solvents Q3C(R3). November 2005. 1-11-2005.
International Conference on Harmonisation. Guidelines: Quality: Q1A, Q1B, Q1C, Q1E, Q1F. November 2005. 1-11-2008.
Kranz, H., Bodmeier, R., 2008. Structure formation and characterization of injectable drug loaded biodegradable devices: in situ implants versus in situ microparticles. Eur. J. Pharm. Sci. 34, 164–172.
Lucke, A., Gopferich, A., 2003. Acylation of peptides by lactic acid solutions. Eur. J. Pharm. Biopharm. 55, 27–33.
Matschke, C., Isele, U., van Hoogevest, P., Fahr, A., 2002. Sustained-release injectables formed in situ and their potential use for veterinary products. J. Control. Release 85, 1–15.
Murty, S.B., Na, D.H., Thanoo, B.C., DeLuca, P.P., 2005. Impurity formation studies with peptide-loaded polymeric microspheres: Part II. In vitro evaluation. Int. J. Pharmaceut. 297, 62–72.
Na, D.H., Youn, Y.S., Lee, S.D., Son, M.W., Kim, W.B., DeLuca, P.P., Lee, K.C., 2003. Monitoring of peptide acylation inside degrading PLGA microspheres by capillary electrophoresis and MALDI-TOF mass spectrometry. J. Control. Release 92, 291–299.
Nair, L.S., Laurencin, C.T., 2007. Biodegradable polymers as biomaterials. Progr. Polym. Sci. 32, 762–798.
Packhaeuser, C.B., Kissel, T., 2007. On the design of in situ forming biodegradable parenteral depot systems based on insulin loaded dialkylaminoalkylamine-poly(vinyl alcohol)-g-poly(lactide-*co*-glycolide) nanoparticles. J. Control. Release 123, 131–140.
Packhaeuser, C.B., Schnieders, J., Oster, C.G., Kissel, T., 2004. In situ forming parenteral drug delivery systems: an overview. Eur. J. Pharmaceut. Biopharmaceut. 58, 445–455.
Park, T.G., 1995. Degradation of poly(lactic-*co*-glycolic acid) microspheres: effect of copolymer composition. Biomaterials 16, 1123–1130.
Powell, M.F., Nguyen, T., Baloian, L., 1998. Compendium of excipients for parenteral formulations. PDA J. Pharmaceut. Sci. Technol. 52, 238–311.
Ravivarapu, H.B., Moyer, K.L., Dunn, R.L., 2000. Parameters affecting the efficacy of a sustained release polymeric implant of leuprolide. Int. J. Pharmaceut. 194, 181–191.
Royals, M.A., Fujita, S.M., Yewey Gerald, L., Rodriguez, Jose., Schultheiss Patricia, C., Dunn Richard, L., 1999. Biocompatibility of a biodegradable *in situ* forming implant system in rhesus monkeys. J. Biomed. Res. 45, 231–239.
Schliecker, G., Schmidt, C., Fuchs, S., Wombacher, R., Kissel, T., 2003. Hydrolytic degradation of poly(lactide-*co*-glycolide) films: effect of oligomers on degradation rate and crystallinity. Int. J. Pharmaceut. 266, 39–49.
Scholz, E., 1983. Karl Fisher Titration. Springer Verlag, Berlin, Heidelberg, New York, Tokyo.
Shih, C., 1995. Chain-end scission in acid catalyzed hydrolysis of poly(-lactide) in solution. J. Control. Release 34, 9–15.
Shih, C., Waldron, N., Zentner, G.M., 1996. Quantitative analysis of ester linkages in poly(-lactide) and poly(-lactide-*co*-glycolide). J. Control. Release 38, 69–73.
Siegel, S.J., Kahn, J.B., Metzger, K., Winey, K.I., Werner, K., Dan, N., 2006. Effect of drug type on the degradation rate of PLGA matrices. Eur. J. Pharmaceut. Biopharmaceut. 64, 287–293.
Strickley, R.G., 1999. Parenteral formulations of small molecules therapeutics marketed in the United States (1999)-part I. PDA J. Pharmaceut. Sci. Technol. 53, 324–349.
Sultana, Y., Jain, R., Aqil, M., Ali, A., 2006. Review of ocular drug delivery. Curr. Drug Deliv. 3, 207–217.
USP30-NF25. NF Monographs: Polyethylene Glycol. United States Pharmacopeia. United States Pharmacopeia. 2-1-2008. USP30-NF 25.
Vert, M., 2007. Polymeric biomaterials: strategies of the past vs. strategies of the future. Progr. Polym. Sci. 32, 755–761.