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Preparation of Hybrid Fe_3O_4/Poly(lactic-*co*-glycolic acid) (PLGA) Particles by Emulsion and Evaporation Method. Optimization of the Experimental Parameters

*A. Pérez, C. Mijangos, R. Hernández**

Summary: The incorporation of iron oxide nanoparticles into poly(lactic-co-glycolic) (PLGA) particles was carried out with an emulsion and evaporation method. To optimize the mean size and the particle size distribution of the particles, the following parameters were modulated: the molecular weight and the hydrolyzation degree of the surfactant, the concentration of the aqueous phase and the stirring rate. Samples were characterized by means of scanning electron microscopy (SEM), transmission electron microscopy (TEM), dynamic light scattering (DLS) and thermogravimetric analysis (TGA).

Keywords: emulsion; evaporation; iron oxide particles; PLGA particles

Introduction

During the last years, poly(lactic acid) (PLA), poly (glycolic acid) (PGA) and more specifically its co-polymers poly(lactic-*co*-glycolic acid) (PLGA) have been investigated for applications in controlled drug delivery[1–3] due to their biocompatibility and their biodegradability.[4–6]

Different methods can be used to prepare PLGA particles: emulsion and evaporation,[7,8] emulsion and diffusion,[9] nanoprecipitation[10] and salting out.[11,12] Among these methods the emulsion and evaporation method is the most commonly employed and the easier one.[13,14] The emulsion evaporation method involves a surfactant agent like poly(vinyl alcohol) (PVA), poly (ethylen-*alt*-maleic acid) (PEMA)[15] or Span 80[16] to ensure the stability of the polymeric droplet in the liquid medium. Although a wide variety of surfactants have been reported, poly (vinyl alcohol) (PVA) is the most commonly used in the preparation of PLGA particles.[17,18]

When PLGA particles are synthesized by the emulsion and evaporation method, some amount of surfactant remains on their surface even after several washing steps. This fact could represent a drawback since chemical surface properties can be modified.[19] However, when poly (vinyl alcohol) is used as the surfactant, it remains on the surface on a very small amount which is the so called residual PVA.[20]

The incorporation of iron oxide nanoparticles (Fe_3O_4 nanoparticles) into PLGA particles provides a further functionalization for these particles to be employed in biomedical applications. As iron oxide nanoparticles are able to respond to an external permanent magnet, hybrid PLGA particles are potential candidates for magnetic controlled drug delivery[21] or for magnetic hyperthermia.[22]

In this paper, we report on the preparation of Fe_3O_4/PLGA particles by an emulsion and evaporation method using PVA as a surfactant mixed with an aqueous ferrofluid containing Fe_3O_4 nanoparticles. As a first stage, different experimental parameters including the molecular weight and hydrolyzation degree of the PVA used as surfactant, stirring rate and

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Instituto de Ciencia y Tecnología de Polímeros, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain
E-mail: rhernandez@ictp.csic.es

concentration of the aqueous solution were evaluated in order to control the particle size, particle size distribution and amount of residual PVA of the hybrid materials.

Experimental Part

Materials

The poly(lactic-*co*-glycolic acid) Resomer RG 504 (PLGA) used in this study was purchased from Boehringer-Ingelheim. According to the manufacturer, the ratio of lactic: glycolic is 50:50 and the molecular weight ranges between 38000 and 54000 g/mol. Three poly (vinyl alcohol) (PVA) with different molecular weights and hydrolyzation degrees were employed as surfactants to prepare PLGA particles and their properties are summarized in Table 1. Chloroform was purchased from SDS. Oleic-acid-coated iron oxide particles dispersed in water as a ferrofluid, NGAP FeO-05#4, were provided by Nanogap Subnmparticles, Spain. According to the manufacturer, the crystalline form is magnetite, Fe_3O_4 and the average size is 18.55 ± 2 nm. The ferrofluid has a 10% magnetite and a density of 1.08 g/mL. Milli-Q (18.3 MΩ) water was used in all experiments.

Preparation of PLGA Particles

PLGA particles were prepared by an oil-in-water (o/w) emulsion solvent evaporation method. To prepare PLGA particles by this method, an organic phase containing the polymer (PLGA) dissolved in chloroform (solvent immiscible with the aqueous phase) and an aqueous phase (continuous phase) containing the surfactant (PVA) are needed. The organic phase (5 mL) was slowly added to the aqueous phase (50 mL) under mechanical stirring (UltraTurrax T25, IKA, Germany) at a defined stirring speed. In order to remove the organic solvent, the emulsion was maintained under magnetic stirring at room temperature during 48 hours. After that, the particles were collected by centrifugation (Heraeus-Megafuge 1.0) and washed twice with distilled water to eliminate the excess of the surfactant. Finally, washed particles were freeze-dried and stored at room temperature. Samples with the same PLGA concentration (0.2 (%w/v)) and different surfactant concentration (1, 0.5, 0.25 (%w/v)) were prepared at the same stirring rate (10000 rpm). Samples were designated as PLGA_PVAC_1%, PLGA_PVAC_0.5% and PLGA_PVAC_0.25%, respectively.

Table 1.
Pharmaceutical grades of PVA used in this study.

Sample	Molecular weight (g/mol)	Hydrolyzation degree %
PLGA_PVA_A	89000–98000	99
PLGA_PVA_B	31000–50000	99
PLGA_PVA_C	31000–50000	89

PLGA particles with iron oxide nanoparticles (magnetite) were prepared by an emulsion solvent evaporation method as previously described for raw PLGA particles. PVA_C was used as the surfactant. In the aqueous phase PVA_C was dissolved at 1%wt. in water (0.5g in 50 mL of water) at 80 °C under stirring. Then, 0.25mL of magnetite nanoparticles were added to the aqueous phase. So, magnetite nanoparticles in the aqueous phase were at 0.05%wt of concentration (0.025 g in 50.25mL of water). The PVA-to-magnetite nanoparticles mass ratio is [1:0.05].

Scanning Electron Microscope (SEM) and Field Emission Scanning Electron Microscopy (FESEM)

The morphology of the polymeric particles was observed by scanning electron microscope (ESEM, XL30 Philips). Each sample was dispersed with distilled water at 0.1 mg/mL and a drop of the sample was deposited onto a cover glass. The dried sample was coated with an ultrathin coating of gold deposited on the sample by high-vacuum metallization. The morphology of the hybrid PLGA particles was investigated with a FESEM Hitachi model SU8000 with TE detector (FESEM-TE) which afforded ultra-low voltage imaging resolution of 1.3 nm at 1.0 kV operating at 25–30 kV. A drop of particles solution diluted with water was deposited on a copper grid (Electron Microscope Science, CF-400-Cu grids) which was dried at room temperature.

Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS) experiments were performed in a Malvern Zetasizer Nano S instrument equipped with a 633 nm laser diode. Previous to each measurement, the dispersed samples in water were filtered using a 0.80 μm Millex-AA filter. Measurements were done at 20 °C in water solution placed in folded capillary cells (Malvern ®) with a volume of 1mL.

Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) data was recorded on a TA Q-500 analyzer under nitrogen atmosphere at flow of 60mL/min, using platinum pans where approximately 5mg of each sample was deposited. The samples were heated from 20 °C to 800 °C at 10 °C/min.

Results and Discussion

The effect of the molecular weight (Mw) and the hydrolyzation degree of the PVA employed as the surfactant on the size and size distribution of PLGA particles, was determined for the three samples prepared using three commercial grades of PVA (PVA_A, PVA_B and PVA_C) as shown in Table 1. The concentration of PVA in the aqueous phase was 1 wt% and the concentration of PLGA in the organic phase was 0.2 wt%. The stirring rate was maintained at 10000 rpm.

The particle size as determined through DLS was 1.3 ± 0.4, 1.2 ± 0.5 and 1.1 ± 0.2 μm for samples PLGA_PVA_A, PLGA_PVA_B and PLGA_PVA_C, respectively which implies that the particle size obtained is independent from the molecular weight and the hydrolyzation degree of the PVA. However a narrower particle size distribution is obtained when PVA_C is employed as compared to the particle size distribution obtained for PVA_A or PVA_B. This is further confirmed by SEM microscopy as shown in Figure 1 which also shows lower aggregation for samples prepared with PVA_C.

As previously reported,[23] PVA interacts with PLGA through hydrophobic bonding so that a large number of hydroxyl groups of PVA are located on the surface of the particles. PVA_A and PVA_B present a higher number of hydroxyl groups because of their higher hydrolyzation degree. Therefore, the formation of inter or intra hydrogen bonds via hydroxyl groups of PVA is favoured thus promoting the aggregation among PLGA particles. On the contrary, PVA_C in which the hydrolyzation degree is lower presents a higher number of acetyl groups on the surface of the PLGA particles. These groups would hinder the formation of hydrogen bonds of the hydroxyl groups.

Moreover, Figure 1 shows the presence of film residues for the samples PLGA_PVA_A and PLGA_PVA_B indicating that during the washing process the PVA cannot be completely removed when PVA_A and PVA_B are employed as surfactants. The amount of PVA can be obtained from thermogravimetric analysis

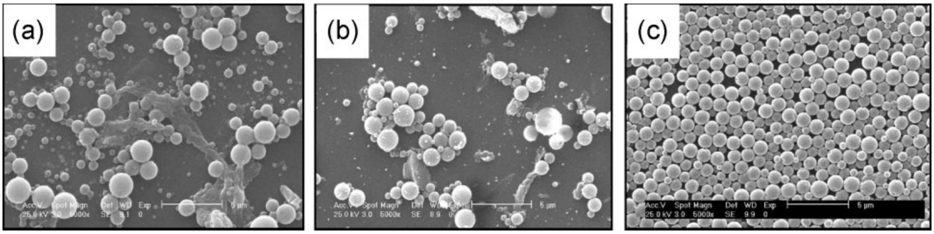

Figure 1.
SEM images: (a) PLGA_PVA_A (b) PLGA_PVA_B (C) PLGA_PVA_C.

Table 2.
Onset degradation temperature and mass loss of raw materials.

Samples	Step (I)		Step (II)	
	Temperature °C	Mass loss %	Temperature °C	Mass loss %
PVA_A	252	80.5	467	11.3
PVA_B	249	70.9	400	14.9
PVA_C	293	77.7	419	13.5
PLGA	324	97.8		

and the results corresponding to the starting polymers, PLGA and PVA are reported in Table 2.

The thermal degradation of PVA occurs in two stages. The first degradation step is due to the scissions of lateral hydroxyl groups which are able to generate intramolecular water at 252 °C for PVA_A, at 249 °C for PVA_B, and at 293 °C for PVA_C with a mass loss of 80.5%, 70.9% and 77.7% respectively. The second is associated to the decomposition of the polymer backbone for the three PVA under consideration ranging from 400 °C to 470 °C with a mass loss from 11.3% to 13.5%.[24] The degradation of PLGA proceeds in a single step at 324 °C. It is related to transesterification reactions with production of cyclic degradation products.[25]

Figure 2 shows the thermogravimetric results obtained for the three samples under study. For PLGA_PVA_A and PLGA_PVA_B, two mass loss steps are detected.

The first degradation step for PLGA_PVA_A, PLGA_PVA_B and PLGA_PVA_C corresponding to the major weight loss appears at 197 °C, 192 °C and 216 °C, respectively. The fact that the degradation temperature corresponding to PLGA_PVA_C is slightly higher than the corresponding to the samples PLGA_PVA_A and PLGA_PVA_B might be related to the shell protection provided by the acetyl groups in PLGA_PVA_C. For the three samples under study, there is a decrease of ~100 °C with respect to the first weight loss observed for raw PLGA (T = 324 °C, Table 2). This fact can be attributed to the freeze-dried process which is carried out in order to stabilize the samples and store them to avoid any degradation process. Small water crystals are formed during pre-frozen step at low temperature and disrupt the stabilizer shell around the particle which results in a break of chemical bonds. As a consequence, the thermal stability of the sample is disrupted.[26]

The second degradation step occurs at 463 °C for PLGA_PVA_A and at 399 °C for

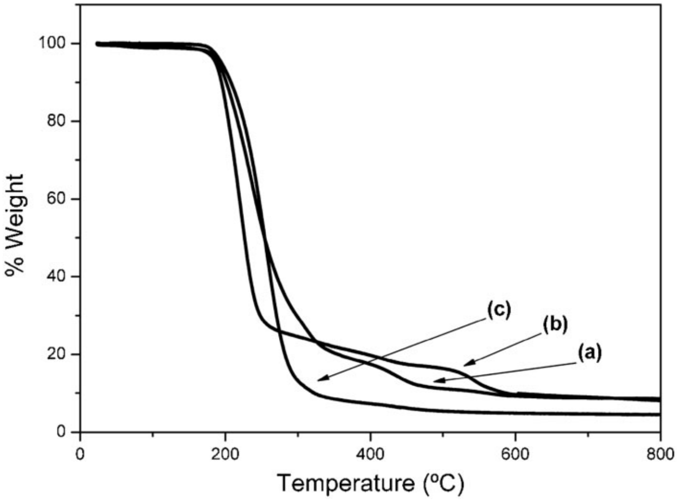

Figure 2.
Weight loss as a function of temperature for (a) PLGA_ PVA_A, (b) PLGA_PVA_B and (c) PLGA_PVA_C.

PLGA_PVA_B and can be assigned to the decomposition of the PVA backbone.[27] This step allows to determine the amount of PVA which is not eliminated during the washing process being 7.8% and 4.3% for samples PLGA_PVA_A and PLGA_PVA_B. No second degradation step is observed for the sample PLGA_PVA_C which suggests that for this sample the PVA which is not linked to the PLGA surface can be eliminated during the washing step. Therefore, only PVA linked to the PLGA spheres and that can not be detected through TGA the so called 'residual PVA' remains for the sample PLGA_PVA_C. The presence of PVA on the surface of PLGA spheres allows for the stability of aqueous dispersions of PLGA spheres up to concentrations of 5 wt%.

Considering the results obtained regarding the residual PVA and the particle size distribution for the three surfactants under study, we chose PVA_C as the surfactant to carry out the study about the effect of the concentration of the surfactant and stirring rate on the size and size distribution of PLGA particles.

Figure 3 shows the SEM images corresponding to PLGA particles prepared with different concentrations of PVA in the aqueous phase.

As can be observed, the polydispersity increases with the decrease of PVA concentration in the aqueous phase. This result could be related to the decrease in the viscosity of the aqueous phase and, as a consequence, to a decrease in the system stability due to a lower PVA concentration at constant PLGA concentration and stirring rate.[20,18] The concentration of PVA in the continuous phase promotes the coagulation of the particles[14,28] being higher when the concentration of PVA is lower, so the amount of aggregates in the sample will also be higher. Interestingly, the size of the PLGA particles does not change in the range of PVA concentrations under study which is further supported by results obtained from DLS experiments. The particle size for PLGA_PVAC_1%, PLGA_PVAC_0.5% and PLGA_PVAC_0.25% is 1.11 ± 0.27 μm, 0.89 ± 0.34 μm and 0.86 ± 0.26 μm, which represents a polydispersity of 24%, 38% and 30% respectively.

The effect of the stirring rate on the particle size and particle size distribution was studied for samples prepared at the same concentration of PVA in the aqueous phase (1 wt %) and PLGA in the organic phase (0.2 wt %). As the stirring rate increases from 4000 rpm to 10000 rpm a decrease of the particle size and particle size distribution was achieved. DLS revealed a change in the particle size from 1.9 ± 0.5 μm to 1.1 ± 0.3 μm when the stirring rate was varied from 4000 to 10000. These results were further corroborated by SEM microscopy (Figure 4).

As it has been previously reported, as the stirring rate increases, an increased shear stress is provided to the oil-in-water emulsion which results in smaller droplets formed in the process of preparation of PLGA particles.[1]

The incorporation of iron oxide particles was carried out by an emulsion and evaporation method with the experimental parameters: concentration of PVA = 1%

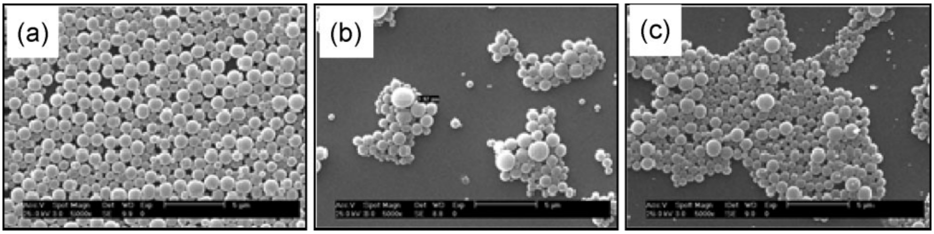

Figure 3.
Scanning electron microscopy images of (A) PLGA_PVAC_1%, (b) PLGA_PVAC_0.5% and (c) PLGA_PVAC_0.25%.

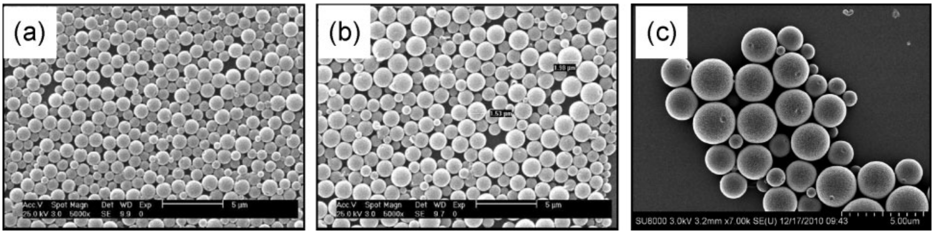

Figure 4.
Scanning electron microscopy images of PLGA particles (1 wt% Pva, 0.2 wt% PLGA) obtained at different stirring rates. a) 10000 rpm, b) 8000 rpm c) 4000 rpm.

wt, concentration of PLGA = 0.2% wt and stirring rate = 10000 rpm. Figure 5 shows the SEM and FESEM microscopy corresponding to samples PLGA_PVA_C and PLGA_PVAC_NPFe respectively.

As can be observed iron oxide NPs are well incorporated into the spherical PLGA particles. The particle size determined by DLS for the sample PLGA_PVAC_NPFe was 226.5 ± 1.4 nm which is in good agreement with the size of the particles observed in Figure 5b. It is important to note that the particle size highly decreases for the sample PLGA_PVAC_NPFe with the introduction of iron oxide particles with respect to sample PLGA_PVA_C. Given the fact that the experimental parameters employed are the same for both samples this might indicate that the iron oxide NPs exert an additional stabilization to the emulsion thus minimizing coalescence and resulting in a smaller particle size. This effect would be similar to the effect that nanoparticles exert on the so-called Pickering emulsions or solid-stabilized emulsions.

TGA results corresponding to the samples PLGA_PVA_C and PLGA_PVAC_NPFe are shown in Figure 6.

The thermal degradation of PLGA_PVA_C particles occurs in one step at T = 216 °C with a mass loss of 92.5% and it is related to the main degradation of PLGA due to transesterification reactions with production of cyclic degradation products.[25] The thermal degradation of PLGA_PVAC_NPFe also occurs in one step at a T = 263 °C with a mass loss of 66.5%. This result reveals that the incorporation of magnetite particles into PLGA spheres significantly enhances their thermal stability in agreement with previous studies.[29,30] TGA can be employed to determine the amount of magnetite particles on hybrid polymer materials as previously reported.[31] The residual weight at 800 °C increased from 4.5wt% for PLGA_PVA_C to 33.5 wt% for PLGA_PVAC_NPFe. Considering that PLGA_PVA_C almost decomposes completely at this temperature, the difference in

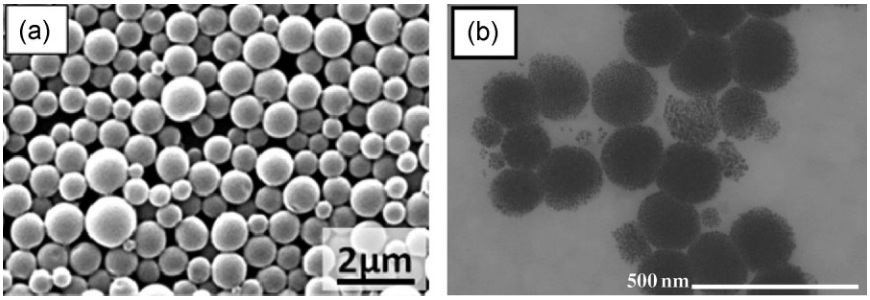

Figure 5.
(a) SEM image corresponding to PLGA_PVA_C and (b) FESEM image corresponding to PLGA_PVAC_NPFe.

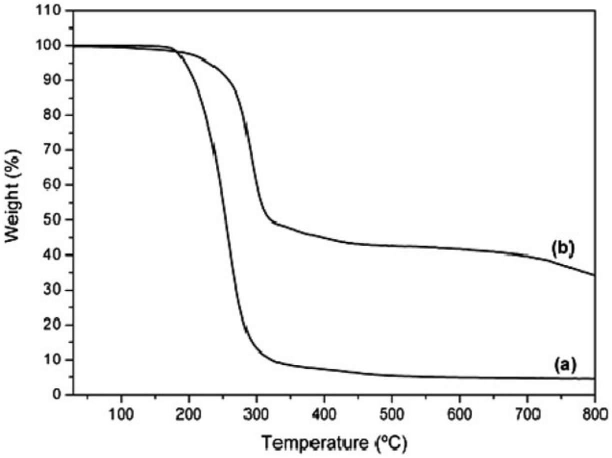

Figure 6.
Weight loss as a function of temperature for a) PLGA_PVA_C and b) PLGA_PVAC_NPFe.

residual weight between both samples can be taken as an indication of the iron oxide content in PLGA_PVAC_NPFe sample being 29 wt%.

Conclusion

This study investigates the use of an emulsion and evaporation method for preparing PLGA particles. The preparation of PLGA particles are more effectively determined by the hydrolyzation degree than by the molecular weight of the surfactant. Most favourable results regarding particle size distribution and amount of residual PVA were obtained using polyvinyl alcohol with lower molecular weight and lower hydrolyzation degree. Moreover a high stirring rate (10000 rpm) and a 1% concentration of PVA were proven as the best conditions to achieve a narrow particle size distribution. These parameters were suitable to prepare PLGA particles with iron oxide nanoparticles by emulsion and evaporation technique.The obtained hybrid PLGA particles present a higher thermal stability than raw PLGA particles. More experiments are currently into progress to assess the suitability of these materials for applications in magnetic hyperthermia.

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[1] A. Budhian, S. J. Siegel, K. I. Winey, *Int. J. Pharm.* **2007**, *336*, 367.
[2] Y. Yang, N. Bajaj, P. Xu, K. Ohn, M. D. Tsifansky, Y. Yeo, *Biomaterials* **2009**, *30*, 1947.
[3] C. M. Moraes, R. de Lima, A. H. Rosa, E. de Paula, L. F. Fraceto, *Macrom. Symp.* **2009**, *281*, 106.
[4] P. B. O'Donnell, J. W. McGinity, *Adv. Drug. Del. Rev.* **1997**, *28*, 25.
[5] M. N. V. Ravi Kumar, U. Bakowsky, C. M. Lehr, *Biomaterials* **2004**, *25*, 1771.
[6] M. L. Hans, A. M. Lowman, *Curr. Opin. Solid State Mater. Sci.* **2002**, *6*, 319.
[7] W. M. Obeidat, J. C. Price, *J. Microencapsulation* **2003**, *20*, 57.
[8] F. A. Mohammed, M. A. Hassan, *S. T. P. Pharma Sciences* **2003**, *13*, 319.
[9] D. Quintanar-Guerrero, E. Allémann, H. Fessi, E. Doelker, *Drug Dev. Ind. Pharm.* **1998**, *24*, 1113.
[10] P. Legrand, S. Lesieur, A. l. Bochot, R. Gref, W. Raatjes, G. Barratt, C. Vauthier, *Int. J. Pharm.* **2007**, *344*, 33.
[11] S. Galindo-Rodriguez, E. Allémann, H. Fessi, E. Doelker, *Pharm. Res.* **2004**, *21*, 1428.
[12] J. G. Eley, V. D. Pujari, J. McLane, *Drug delivery* **2004**, *11*, 255.
[13] V. Lassalle, M. L. Ferreira, *Macromol. Biosci.* **2007**, *7*, 767.
[14] M. N. Freitas, J. M. Marchetti, *Int. J. Pharm.* **2005**, *295*, 201.
[15] M. E. Keegan, J. L. Falcone, T. C. Leung, W. M. Saltzman, *Macromolecules* **2004**, *37*, 9779.

[16] C. Sturesson, J. Carlfors, K. Edsman, M. Andersson, *Int. J. Pharm.* **1993**, *89*, 235.

[17] P. D. Scholes, A. G. A. Coombes, L. Illum, S. S. Daviz, M. Vert, M. C. Davies, *J. Controlled Release* **1993**, *25*, 145.

[18] M. F. Zambaux, F. Bonneaux, R. Gref, P. Maincent, E. Dellacherie, M. J. Alonso, P. Labrude, C. Vigneron, *J. Controlled Release* **1998**, *50*, 31.

[19] S. C. Lee, J. T. Oh, M. H. Jang, S. I. Chung, *J. Controlled Release* **1999**, *59*, 123.

[20] S. K. Sahoo, J. Panyam, S. Prabha, V. Labhasetwar, *J. Controlled Release* **2002**, *82*, 105.

[21] H. Wang, W. Su, S. Wang, X. Wang, Z. Liao, C. Kang, L. Han, J. Chang, G. Wang, P. Pu, *Nanoscale* **2012**, *4*, 6501.

[22] R. Hernández, J. Sacristán, L. Asin, T. E. Torres, M. R. Ibarra, G. F. Goya, C. Mijangos, *The Journal of Physical Chemistry B* **2010**, *114*, 12002.

[23] H. Murakami, Y. Kawashima, T. Niwa, T. Hino, H. Takeuchi, M. Kobayashi, *Int. J. Pharm.* **1997**, *149*, 43.

[24] B. J. Holland, J. N. Hay, *Polymer* **2001**, *42*, 6775.

[25] S. D'Antone, F. Bignotti, L. Sartore, A. D'Amore, G. Spagnoli, M. Penco, *Polym. Degrad. Stab.* **2001**, *74*, 119.

[26] Y. N. Konan, R. Cerny, J. Favet, M. Berton, R. Gurny, E. Allémann, *Eur. J. Pharm. Biopharm.* **2003**, *55*, 115.

[27] Z. Peng, L. X. Kong, *Polym. Degrad. Stab.* **2007**, *92*, 1061.

[28] P. Sansdrap, A. J. Moës, *Int. J. Pharm.* **1993**, *98*, 157.

[29] A. Laachachi, E. Leroy, M. Cochez, M. Ferriol, J. M. Lopez Cuesta, *Polym. Degrad. Stab.* **2005**, *89*, 344.

[30] Z. Guo, K. Lei, Y. Li, H. W. Ng, S. Prikhodko, H. T. Hahn, *Compos. Sci. Technol.* **2008**, *68*, 1513.

[31] R. Hernández, C. Mijangos, *Macromol. Rap. Comm.* **2009**, *30*, 176.