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The effects of mPEG proportion and LA/GA ratio on degradation and drug release behaviors of PLGA-mPEG microparticles

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The purpose of this research was to evaluate the effects of mPEG proportion and LA/GA ratio on degradation and release behavior of PLGA-mPEG microparticles prepared by the emulsion evaporation method. Mometasone furoate was employed as model drug and encapsulated into five types of PLGA-mPEG microparticles in the same molecular weight (Mw), but different in mPEG proportion or LA/GA ratio. All types of PLGA-mPEG microparticles showed similar drug encapsulation efficiency and particle mean size, but PLGA-mPEG microparticles with higher mPEG proportion showed a faster Mw reduction rate, mass loss rate and size decrease rate according to the in vitro degradation experiment, and also, a faster drug release rate according to the in vitro release experiment. On the other hand, higher LA/GA ratio in PLGA chain of PLGA-mPEG causes a slower Mw reduction rate, mass loss rate, size decrease rate, and thus, a slower drug release rate.

1. Introduction

Microparticles have attracted great attention as **a** potential drug delivery system for disease treatment due to its long controlled and sustained drug release (Hanson et al. 2014; Liu et al. 2015; Oka et al. 2015; Ramazani et al. 2015; Xiong and He 2013). By now a variety of biodegradable poly (dl-lactide-co-glycolide) (PLGA) microparticles encapsulating several drug substances were commercially available, which could reduce side effects and enhance the therapeutic compliance and efficiency (Blanco-Prieto et al. 2000; Burke et al. 2004; Klose et al. 2010; Reinhold and Schwendeman 2013). PLGA, polycaprolactone (PCL) and polylactic acid (PLA) have been extensively studied as microparticles matrix due to their biocompatibility and biodegradability (Anderson and Shive 2012; Hernan Perez de la Ossa et al. 2012). Their degradation time could be varied from days to years by altering the type of polymer, the polymer molecular weight or the structure of the microspheres, and thus, adjusting the drug release behavior (Fude et al. 2005). However, the hydrophobicity of PLGA would prevent the penetration of water into the center of microparticles, forming an acidic environment due to the accumulated acidic breakdown products, which would then, cause degeneration of macromolecular drugs (Zhou et al. 2003).

In recent years, poly (dl-lactide-co-glycolide)-methoxypoly (ethyleneglycol) (PLGA-mPEG) has attracted much attention due to its amphiphilicity (Fei et al. 2014; Feng et al. 2015a), which could reduce acidic microenvironment due to easier diffusion of acidic breakdown products, protecting the drug and accelerating the diffusion of drug through the matrix (Li et al. 2008). Different types of matrix would cause a difference in drug release behavior, Mw and lactide/glycolide (LA/GA) ratio (Cui et al. 2005; Zheng 2009) were reported to affect the PLGA microparticles in vitro release rate, and the effect**s** of PCL (Yang et al. 2001) and PLGA-mPEG (Feng et al. 2015b) Mw were also discussed. Higher polymer Mw always caused a longer degradation term, slower drug release rate and prolonged release time for PCL, PLGA, PLGA-mPEG microparticles. GA showed a better degradation ability than LA and thus, higher LA/GA ratio of PLGA microparticles degraded much slower and always accompanied with a slower drug release rate.

mPEG segment was a hydrophilic part that could improve the hydrophobicity of PLGA, but the effects of mPEG proportion and LA/GA ratio on property of PLGA-mPEG were seldom adequately studied. In this study, five types of PLGA-mPEG in different mPEG proportion and LA/GA ratio were employed as matrix to fabricated drug loaded microparticles by emulsion evaporation method. The in vitro degradation and drug release behaviors were analyzed and adequately discussed to find out how the mPEG proportion and LA/GA ratio affected the microparticle**s** degradation and drug release manner. The results would be a guideline for choosing the microparticles matrix to obtain an expected drug release profile.

2. Investigations, results and discussion

2.1. Characterization of microparticles

All the types of microparticles revealed a very similar morphology, so we just showed the SEM pictures of PLGA(15000)-mPEG(5000) (LA/GA=3:1) microparticles **(Fig. 1).**

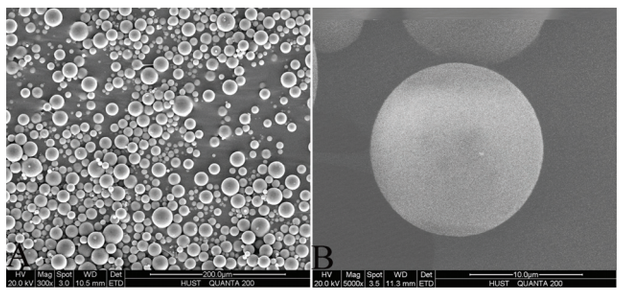

Fig. 1: Scanning electron micrographs of drug-loaded $PLGA_{(15000)}$-$mPEG_{(5000)}$ (LA/GA=3:1) microparticles: (A). 300x; (B). 5000x.

The drug-loaded particles prepared by emulsion evaporation method exhibited a spherical shape and smooth surface, with few particles broken. All the microparticles showed a drug encapsulation efficiency around 92% (Table), with little difference among them, which mean that mPEG proportion and LA/GA ratio in PLGA-mPEG had no obvious effects on drug encapsulation

process. Furthermore, the particle size analysis revealed that all the types of microparticles got a mean size around 12~13 μm, suggesting that mPEG proportion and LA/GA ratio also did not affect microparticles fabrication process.

Table: Properties of microparticles prepared by different types of PLGA-mPEG

Sample	LA/GA ratio	Encapsulation efficiency (%)	Mean size (μm)
$PLGA_{(19000)}$-$mPEG_{(1000)}$	3:1	92.33±3.71	12.33
$PLGA_{(18000)}$-$mPEG_{(2000)}$	3:1	91.40±2.07	12.09
$PLGA_{(15000)}$-$mPEG_{(5000)}$	3:1	91.82±3.14	12.47
$PLGA_{(15000)}$-$mPEG_{(5000)}$	5:1	92.26±2.83	12.55
$PLGA_{(15000)}$-$mPEG_{(5000)}$	1:1	93.48±4.25	12.58

$PLGA_{(19000)}$-$mPEG_{(1000)}$ means the Mw of mPEG chain is 1000, PLGA chain is 19000.

2.2. In vitro microparticles degradation analysis

The microparticles Mw reduction, mass loss, morphology and mean size changes were examined during the microparticles incubation in PBS in order to compare the degradation behaviors among the five types of microparticles.

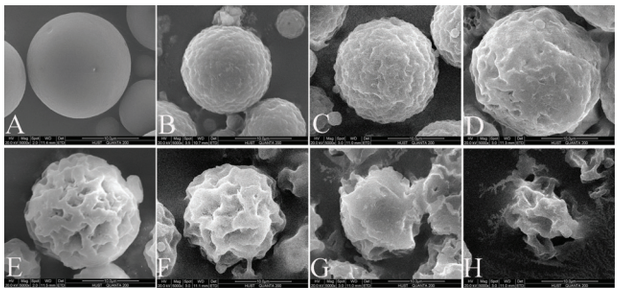

Fig. 2: SEM images of $PLGA_{(15000)}$-$mPEG_{(5000)}$ microparticles morphology change during degradation in PBS: A. original; B. 1 day; C. 4 days; D. 7 days; E. 14 days; F. 21 days; G. 28 days; H. 35 days.

Figure 2 demonstrates the microparticles morphology changes during five weeks degradation in PBS. The particles exhibited an uneven erosion layer-by-layer **with** the degradation continued, and the particle size decreased gradually and loss its spherical shape and structure in the latter degradation period. All the five types of PLGA-mPEG particles exhibited the same erosion pattern and so, we just showed one type. According to the degradation results, we found that with the mPEG proportion increased, the PLGA-mPEG microparticles exhibited a faster degradation rate, such as faster Mw reduction rate (Fig. 3), faster mass loss rate (Fig. 4) and faster particle mean size decrease rate (Fig. 5). For example, the Mw remained 73.66% of original for PLGA(19000)-mPEG(1000) microparticles after 2 weeks incubation, but 62.81% for PLGA(18000)-mPEG(2000), 59.46% for PLGA(15000)-mPEG(5000). The particles loss 16.53% of the total mass for PLGA(19000)-mPEG(1000) microparticles after 2 weeks incubation, but 21.48% loss for PLGA(18000)-mPEG(2000), 24.4% for PLGA(15000)-mPEG(5000). The particles mean size decreased to 89.5% of original for PLGA(19000)-mPEG(1000) microparticles after 2 weeks, and the value came to 86.52% and 82.35% for PLGA(18000)-mPEG(2000) and PLGA(15000)-mPEG(5000), respectively. MPEG was a hydrophilic and undegradable chain which could be fractured from PLGA-mPEG and dissolved in PBS medium. Higher mPEG proportion mean an increased hydrophilicity of PLGA-mPEG and smaller Mw of the degradable PLGA part, which could be the reason for the faster degradation and erosion rates of PLGA-mPEG microparticles.

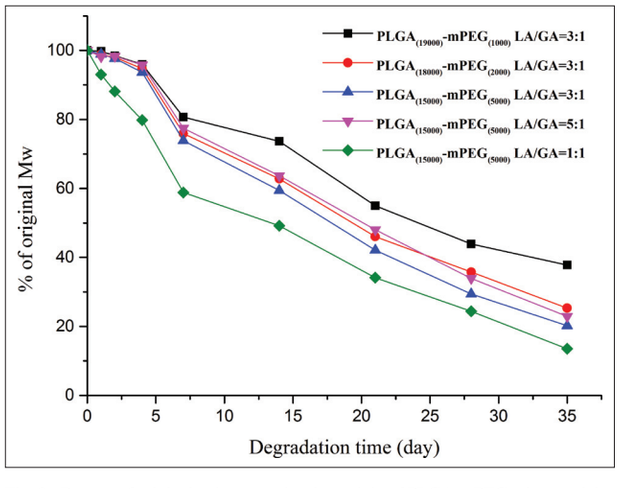

Fig. 3: Percent of original polymer molecular weight of PLGA-mPEG microparticles during degradation in PBS.

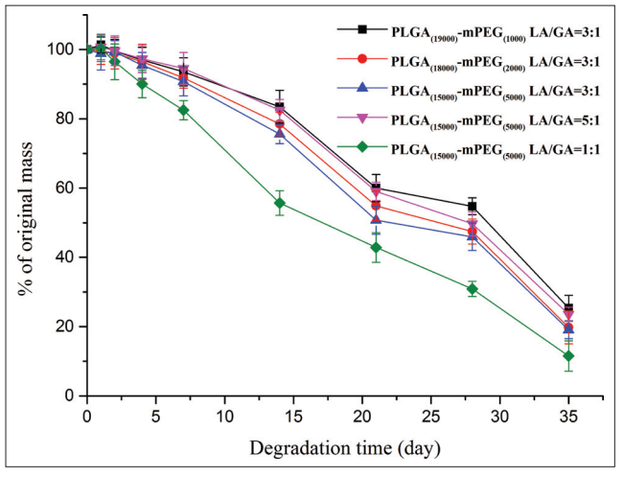

Fig. 4: Percent of original total mass of PLGA-mPEG microparticles during degradation in PBS.

Besides, through the in vitro degradation study, we found that the LA/GA ratio in PLGA-mPEG also obviously affected the particles degradation behavior. Three types of PLGA-mPEG microparticles in the same molecular, mPEG proportion, but different LA/GA ratio were compared, and the results revealed that with the LA/GA ratio decreased from 5:1 to 1:1, the particles Mw reduction rate (Fig. 3), mass loss rate (Fig. 4) and mean size decease rate (Fig. 5) got an obvious increase, and the PLGA-mPEG microparticles with LA/GA of 1:1 showed a particularly fast degradation and erosion rates, losing its particle structure after 3 weeks. As described above, in the PLGA-mPEG chain, only PLGA part degraded during incubation and thus, the PLGA-mPEG degradation rate was controlled by the PLGA degradation rate. According to the literature (Zou et al. 2012), GA part got a better degradation ability than LA, higher LA/GA ratio resulted in slower degradation rate, so, it could explained why lower LA/GA ratio in PLGA-mPEG microparticles caused a faster degradation and erosion rates.

2.3. In vitro microparticles release analysis

Figure 6 demonstrates the mometasone furoate release profile from different types of PLGA-mPEG microparticles.

The microparticles prepared by emulsion evaporation method got a drug-in-matrix blending structure and it was believed that the drug release rate mainly controlled by microparticles erosion rate (Feng et al. 2015a; Li et al. 2008). The mometasone furoate release results in our research also suggested that the drug release rate were depended on the microparticle degradation rate. Through the degradation experiment, we found that PLGA-mPEG microparticles in higher

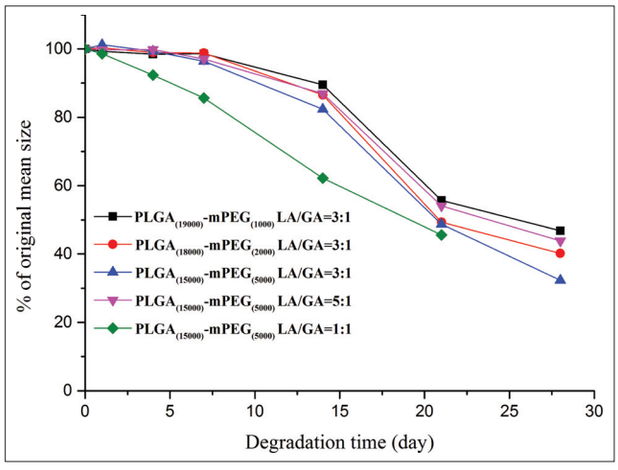

Fig. 6: In vitro drug release profile of PLGA-mPEG microparticles in PBS

mPEG proportion or smaller LA/GA ratio got a faster degradation and erosion rates, and in the release study, microparticles in higher mPEG proportion or smaller LA/GA ratio also showed a faster drug release rate. PLGA(19000)-mPEG(1000) microparticles released 55.81% of total drug after 3 weeks, and with mPEG proportion increased, the value came to 61.08% and 66.28% for PLGA(18000)-mPEG(2000) and PLGA(15000)-mPEG(5000), respectively. With the LA/GA ratio decreased from 5:1 to 1:1 in PLGA-mPEG microparticles, the release amount increased from 60.73% to 74.96% after 3 weeks incubation. Through the results, we found that the drug release rate was controlled by PLGA-mPEG microparticles degradation process, which was affected by mPEG proportion and LA/GA ratio**,** and thus, we believed that we could regulated the drug release profile by adjusting the PLGA-mPEG composition.

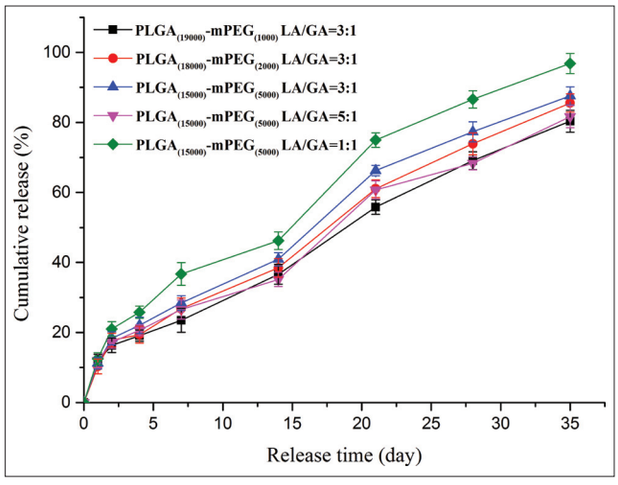

Fig. 5: Percent of original mean size of PLGA-mPEG microparticles during degradation in PBS.

2.4. Conclusion

In this study, mometasone furoate was successfully encapsulated into PLGA-mPEG microparticles **in** different mPEG proportion or LA/GA ratio. The differences of mPEG proportion and LA/GA ratio in PLGA-mPEG had no obvious effects on particle size and encapsulation efficiency. In vitro degradation study revealed that with the increase of mPEG proportion, PLGA-mPEG microparticles showed a faster degradation and size decrease rates, and thus, a faster mometasone furoate release rate. It was also found that higher LA/GA ratio in PLGA-mPEG caused a slower particle degradation rate, slower size decrease and drug release rate. We believe that the results could be a guideline for choosing a matrix to prepare microparticles obtaining a desirable degradation and release behaviors**.**

3. Experimental

3.1. Materials

Poly (dl-lactide-co-glycolide)-methoxypoly (ethyleneglycol) (PLGA-mPEG, Mw=20000) in different mPEG proportion or LA/GA ratio (Table) were purchased from Aldrich. Mometasone furoate was purchased from Kang Bao Tai Fine Chemical Co., Ltd (Wu Han, China). Poly (vinyl alcohol) (PVA, polymerization degree~1700 and hydrolysis degree~99%) from Sinopharm Chemical Reagent co., Ltd, was used as a stabilizer in the emulsion. Methylene chloride of analytical grade was purchased from China National Medicines Corporation Ltd, and used without purification.

3.2. Microparticles fabrication

Mometasone furoate was employed as model drug and encapsulated into PLGA-mPEG microparticles by emulsion evaporation method as reported (Rosca et al. 2004). A quantity of 30 mg of mometasone furoate was dissolved in 10 ml of methylene dichloride containing 1.5 g PLGA-mPEG. The solution was introduced into 30 ml PVA solution (1% w/v) and emulsified at 7000 rpm for 1 min to create the O/W emulsion, and the emulsion was then, added into 200 ml PVA solution (0.2% w/v) and stirred at 500 rpm for 2 h to evaporate the methylene **di**chloride completely under room temperature. The solidified microparticles was collected by centrifugation at 3000 rpm for 3 min under room temperature and washed three times with distilled water, and then lyophilized and stored at -5 °C.

3.3. Microparticles characterization

The microparticles morphology was examined by scanning electron microscope (SEM, Quanta 200, Holland, FEI). Microparticles was mounted onto metal stubs using a double-sided adhesive tape and then vacuum-coated with a thin layer of gold, finally, the microparticles was examined by SEM. To analyze the particle mean size, three hundred microparticles were randomly chosen from SEM micrograph and the software (Nano Measurer 1. 2) was used to calculate the particle mean size.

The mometasone furoate encapsulation efficiency was determined by high performance liquid chromatograghy (HPLC), with a mobile phase of methanol/water/acetic acid (74.8:25:0.2 vol. %) at a flow rate of 1ml/min. sample of 20 µl was injected and detected at 254 nm, with a retention time of 7.5 min. To examine the amount of mometasone furoate encapsulated, 10 mg of microparticles was dissolved in 1 ml of methylene **di**chloride and then, added 50 ml of HPLC mobile phase and stirred for 10 min. The resulting mixture was filtered through 0.45 µm nylon filter and analyzed by HPLC. The drug encapsulation efficiency was expressed as follows:

EE (%) = (Actual drug in PLGA-mPEG) / (Initial drug in PLGA-mPEG) * 100

All the measurements were run in triplicate and the data were shown as mean ± standard deviation.

3.4. In vitro degradation study

An in vitro degradation experiment was conducted to compare the degradation behavior**s** of the five types of PLGA-mPEG microparticles shown in the Table. The degradation behavior of the microparticles was evaluated by the microparticles molecular weight (Mw) reduction, total mass loss, morphology and mean size changes with time upon their degradation in PBS (pH 7.4, 0.01% sodium azide, 0.02% Tween 80). A quantity of 10 mg microparticles (twenty-four samples for each type) was incubated in 25 ml PBS under continuous shaking (50 strokes/min) at 37 °C. All the samples were centrifuged (3000 rpm, 3 min) at scheduled time point (1, 2, 4, 7, 14, 21, 28, 35 days) and 20 ml of supernatant was discarded from each sample. Three samples of each type of microparticles were randomly taken out at each time point, washed with distilled water and then lyophilized for test. For the remaining samples that were for further degradation, 20 ml of fresh medium was added back to each sample. The lyophilized PLGA-mPEG microparticles was collected for the mass loss analysis and also used to determine the Mw by Gel permeation chromatography (GPC) in an Agilent 1100 apparatus with a differential refractometer as a detector. The microparticles morphology change was analyzed by SEM and the particle mean size change was determined by the software (Nano Measurer 1. 2).

3.5. In vitro release study

The in vitro drug release test was conducted under the same conditions as the in vitro degradation study. In triplicate, 10 mg **of** mometasone furoate loaded microparticles of each type was suspended in 25 ml PBS and incubated in a thermostated shaking water bath at 37 °C under continuous agitation (50 strokes/min). At scheduled time intervals (1, 2, 4, 7, 14, 21, 28, 35 days), all the samples were centrifuged at 3000 rpm and then, 20 ml of the supernatant was withdrawn and filtered through a 0.45 µm nylon filter. The amount of mometasone furoate released was measured by HPLC and the withdrawn supernatant was replenished by the same volume of fresh medium.

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References

Anderson JM, Shive MS (2012) Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv Drug Del Rev 64: 72-82.

Blanco-Prieto MaJ, Besseghir K, Zerbe O, Andris D, Orsolini P, Heimgartner F, Merkle HP, Gander B (2000) In vitro and in vivo evaluation of a somatostatin analogue released from PLGA microspheres. J Control Release 67: 19-28.

Burke P, Klumb L, Herberger J, Nguyen X, Harrell R, Zordich M (2004) Poly(lactide-co-glycolide) microsphere formulations of darbepoetin alfa: spray drying is an alternative to encapsulation by spray-freeze drying. Pharm Res 21: 500-506.

Cui F, Cun DM, Tao AJ, Yang MS, Shi K, Zhao M, Guan Y (2005) Preparation and characterization of melittin-loaded poly (DL-lactic acid) or poly (DL-lactic-co-glycolic acid) microspheres made by the double emulsion method. J Control Release 107: 310-319.

Fei T, Yang LJ, Mo XH, Wang XL, Jun G (2014) Metronomic paclitaxel-loaded mPEG-PLA nanoparticles show enhanced anti-tumor efficacy compared to maximum tolerated dose administration. J Nanopart Res 16: 2706.

Feng SB, Nie L, Zou P, Suo JP (2015) A detailed view of PLGA-mPEG microsphere formation by double emulsion evaporation method. Chin J Polymer Sci 33: 1-9.

Feng SB, Lu F, Wang Y, Suo JP (2015a) Comparison of the degradation and release behaviors of poly(lactide-co-glycolide)-methoxypoly(ethylene glycol) microspheres prepared with single- and double-emulsion evaporation methods. J Appl Polymer Sci 132. doi: 10.1002/app.41943

Feng SB, Nie L, Zou P, Suo JP (2015b) Effects of drug and polymer molecular weight on drug release from PLGA-mPEG Microspheres. J Appl Polymer Sci 132. doi: 10.1002/APP.41431.

Fude C, Dongmei C, Anjin T, Mingshi Y, Kai S, Min Z, Ying G (2005) Preparation and characterization of melittin-loaded poly (dl-lactic acid) or poly (dl-lactic-co-glycolic acid) microspheres made by the double emulsion method. J Control Release 107: 310-319.

Hanson MC, Bershteyn A, Crespo MP and Irvine DJ (2014) Antigen delivery by lipid-enveloped PLGA microparticle vaccines mediated by in situ vesicle shedding. Biomacromolecules 15: 2475-2481.

Hernan Perez de la Ossa D, Ligresti A, Gil-Alegre ME, Aberturas MR, Molpeceres J, Di Marzo V, Torres Suarez AI (2012) Poly-epsilon-caprolactone microspheres as a drug delivery system for cannabinoid administration: development, characterization and in vitro evaluation of their antitumoral efficacy. J Control Release 161: 927-932.

Klose D, Siepmann F, Willart JF, Descamps M, Siepmann J (2010) Drug release from PLGA-based microparticles: Effects of the "microparticle:bulk fluid" ratio. Int J Pharm 383: 123-131.

Li J, Jiang G, Ding F (2008) Effects of polymer degradation on drug release from PLGA-mPEG microparticles: a dynamic study of microparticle morphological and physicochemical properties. J Appl Polymer Scie 108: 2458-2466.

Liu HJ, Chu HC, Lin LH, Hsu SY (2015) Preparation and drug release of aspirin-loaded PLGA-PEG-PLGA/montmorillonite microparticles. Int J Polymer Mat Polymer Biomat 64: 7-14.

Oka C, Ushimaru K, Horiishi N, Tsuge T, Kitamoto Y (2015) Core-shell composite particles composed of biodegradable polymer particles and magnetic iron oxide nanoparticles for targeted drug delivery. J Magnet Magnet Mat 381: 278-284.

Ramazani F, Chen W, Van Nostrum CF, Storm G, Kiessling F, Lammers T, Hennink WE, Kok RJ (2015) Formulation and characterization of microspheres loaded with imatinib for sustained delivery. Int J Pharm 482: 123-130.

Reinhold SE, Schwendeman SP (2013) Effect of polymer porosity on aqueous self-healing encapsulation of proteins in PLGA microspheres. Macromol Biosci 13: 1700-1710.

Rosca ID, Watari F, Uo M (2004) Microparticle formation and its mechanism in single and double emulsion solvent evaporation. J Control Release 99: 271-280.

Xiong LZ, He ZQ (2013) Preparation and in-vitro properties of mPEG/PLA microspheres loaded 5-fluorouracil for controlled release. Polymer-Plastics Techn Engin 52: 268-272.

Yang YY, Chung TS, Ng NP (2001) Morphology, drug distribution, and in vitro release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method. Biomaterials 22: 231-241.

Zheng W (2009) A water-in-oil-in-oil-in-water (W/O/O/W) method for producing drug-releasing, double-walled microspheres. Int J Pharm 374: 90-95.

Zhou SB, Liao XY, Li XH, Deng XM and Li H (2003) Poly-D,L-lactide-co-poly(ethylene glycol) microspheres as potential vaccine delivery systems. J Control Release 86: 195-205.

Zou P, Suo J, Nie L, Feng S (2012) Temperature-responsive biodegradable star-shaped block copolymers for vaginal gels. J Mat Chem 22: 6316.