Preparation and Evaluation of Novel Blend Microspheres of Poly(lactic-*co*-glycolic)acid and Pluronic F68/127 for Controlled Release of Repaglinide

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Received 27 December 2006; accepted 2 June 2008
DOI 10.1002/app.30173
Published online 1 December 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The objective of the present work was to study the release behavior of plain and blend microspheres (MS) of PLGA and Pluronic F68/127. In this study, a novel blend MS of poly(D,L-lactic-*co*-glycolic acid) (PLGA) and Pluronic F68/127 (PLF68/127) were prepared by the emulsion–solvent evaporation method. Repaglinide, an antidiabetic drug with a very short half-life, was successfully encapsulated into the blend MS. Various formulations were prepared by varying the ratio of PLGA and PLF68/127. Drug encapsulation up to 91% was achieved as measured by UV spectroscopy. Scanning electron microscopy showed that MS have smooth surfaces even after incorporation of PLF68/127. Particle size, as measured by using laser light scattering technique, gave an average size ranging from 12 to 47 μm. Differential scanning calorimetry (DSC) was performed to understand the crystalline nature of the drug after encapsulation into MS. DSC revealed the crystalline dispersion in the polymer matrix. In vitro release experiments performed in simulated intestinal fluid, indicated the dependence of release rate on the amount of PLF68/127 present in the MS; slow release was extended up to 153 h. Release data have been fitted to an empirical equation to compute the diffusional exponent (*n*), which indicated that the release mechanism to be non-Fickian type. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 116: 366–372, 2010

Key words: drug delivery; poly(D,L-lactic-*co*-glycolic acid); pluronic; microspheres; repaglinide; emulsion–solvent evaporation

INTRODUCTION

Blends of novel biodegradable polymers having properties distinct from the individual polymer components and that are suitable for use as carriers of pharmaceutically active agents were prepared from well-known versatile polymers such as Pluronics and poly(D,L-lactic-*co*-glycolic acid) (PLGA). Considerable research has focused on gaining a better understanding of blends of various biodegradable polymers. Blending can significantly alter the resultant properties, which depend sensitively on the mechanical properties of the component as well as the blend microstructure and the interface between the phases. Poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) have glass transition temperatures above room temperature, rendering them hard and brittle; this property of PLA and PGA is useful for very long-term drug delivery applications. To make its use for short periods of time as well as for improvement of its other properties, surface modified MS of PLGA, PLA, and PGA are observed in literature. Csaba et al.[1] reported a new type of nanoparticles consisting of PLGA and polyoxyethylene blends, which exhibit a number of features that make them a very promising nanocarrier for transmucosal DNA vaccination. Also, PLGA-based pluronic coated systems were developed for producing biologically active protein delivery profiles.[2] PLGA microcapsules of low densities (0.24 g/cm^3) with novel dimpled surfaces for pulmonary delivery of DNA (15–28% loading), were developed in pluronic-stabilized emulsions in earlier literature.[3] Polymeric MS with "open/closed" pores for sustained release of human growth hormone has been developed. Highly porous biodegradable PLGA MS were fabricated by using Pluronic F127 as an extractable porogen.[4]

A microsphere-based system has several clinical potentials in delivering small molecular weight drugs and protein pharmaceuticals over a wide time scale. The most commonly used microencapsulation procedure to load pharmaceuticals into biodegradable MS utilizes solvent emulsification/evaporation techniques.[5,6] A series of publications has dealt with the effects of formulation and process parameters

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Contract grant sponsor: University Grants Commission (UGC), New Delhi, India; contract grant number: F1-41/2001/CPP-II.

affecting microsphere characteristics such as size distribution, morphology, encapsulation efficiency, and drug release profiles.[7–12] Typical variables studied include emulsifier type and concentration, phase volume ratio, polymer type, preparation temperature, and solvent evaporation rate.

PLGA is a well-known biodegradable and biocompatible polymer with a history of safe use in human sutures, orthopaedics, bone plates, and extended-release pharmaceuticals.[13,14] It has been extensively used for developing microparticulate controlled release (CR) devices for various types of drugs as injectable depot formulations for protein and peptide drugs.[15–19] Drug release kinetics from the PLGA MS are mainly determined by both diffusion of drug through the preformed aqueous pores and interconnected channels, as well as an erosion of the polymer matrix. Thus, the drug release rate can be affected by the inherent drug properties such as molecular size and water solubility and initial microsphere morphologies such as porosity and tortuosity; on top of these effects, polymer degradation influenced by amorphous/crystallinity, hydrophilicity, molecular weight, and the presence of excipients play an important role.

Triblock copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) (PEO-*b*-PPO-*b*-PEO), available under trade name "poloxamer" or "Pluronic," are recognized as pharmaceutical multipurpose excipients capable of increasing aqueous solubility and stability of drugs.[20–22] These amphiphilic copolymers are nontoxic and commercially available in a wide range of molecular weights and architectures, which determine their hydrophilic/lipophilic properties and hence show the ability to form nanoscopic core-shell structures in water.[23] Hydrophobic cores of such structures (micelles) serve as reservoirs for hydrophobic drugs, whereas the hydrophilic shell on the interface between bulk aqueous medium and the core stabilizes the micelle. The micellar structure can enhance the solubility and stability of poorly water-soluble compounds in biological fluids.[24,25] An appropriate choice of the copolymer architecture enables the control of important pharmacokinetic characteristics of polymer-based drug formulations such as blood circulation time, drug release profile, and targeting capability.[20,26,27]

The main objectives of this study are the preparation and evaluation of plain PLGA and blend of PLGA-PLF68/127 nonporous MS for CR of antidiabetic drug such as repaglinide. In this study, PLF68/127 acts as amphiphilic filler, which enhances the release of a hydrophobic drug such as repaglinide. Repaglinide, a fast- and short-acting meglitinide analog, was chosen as the model drug since it is indicated for the development of a dosage form with increased gastro retention time (GRT). It has a very short half-life of 1 h, low bioavailability (50%), and poor absorption in the upper intestinal tract.[28,29] The in vitro release profiles of the drugs have been investigated as a function of PLF68/127 content.

EXPERIMENTAL

Materials

Repaglinide was received as gift sample from Biocon Limited, Bangalore, India. PLGA, 50 : 50, of molecular weight $\sim$ 75,000 and Pluronic F68 and F127, were purchased from Aldrich Chemical Company, Milwaukee, WI, USA. Analytical reagent grade dichloromethane (DCM) and poly(vinyl alcohol) (PVA) of molecular weight 125,000 were all purchased from S.D. Fine Chemicals, Mumbai, India. All the chemicals were used without further purification.

Preparation of MS

Blend MS of PLGA and PLF68/127 were prepared by emulsion–solvent evaporation method. PLGA, PLF68/127, and the drug (30% by weight) were dissolved in 2 mL of DCM. This solution was emulsified in 100 mL of 3% PVA solution to form the o/w emulsion using a Eurostar stirrer (IKA Labortechnik, Germany) at 800-rpm speed at room temperature for $\frac{1}{2}$ h in a hood. The microsphere solution was diluted by water and solid MS were isolated by tabletop centrifuge (Jouan, MR 23 I, France). The MS were washed 2–3 times successively with distilled water to remove the surface-adhered PVA. The MS obtained were redispersed in a small amount of distilled water and dried by lyophilization (Jouan). Different formulations were prepared by varying the amount of PLGA and PLF68/127. Totally, nine formulations were prepared and the formulation codes as well as formulation parameters are given in Table I.

The percent amount of PVA and speed of the overhead stirrer used for MS preparation was optimized before preparing all formulations. Polyvinyl alcohol stabilizes the MS during formation. When the lesser amount of PVA was used, the agglomeration of the MS was obtained in the aqueous media.

Drug content

Drug content was estimated using DCM. Microspheres ($\sim$ 10 mg) were dissolved in 50 mL of DCM and analyzed by UV spectrophotometer (Secomam, model Anthelie, France) at the λ_{max} of 243 nm. Drug encapsulation efficiency was then calculated as:

$$\% \text{ Encapsulation efficiency} = \left(\frac{\text{Drug loading}}{\text{Theoretical drug loading}}\right) \times 100. \quad (1)$$

TABLE I
Formulation Details of Drug-Loaded Microspheres Prepared from PLGA, PLF68(F1–F4), and PLGA PLF127 (F5–F8)

Formulation code	PLGA (mg)	PLF-68 (wt %)	Formulation code	PLGA (mg)	PLF-127 (wt %)
CF	300	0			
F1	300	10	F5	300	10
F2	300	20	F6	300	20
F3	300	30	F7	300	30
F4	300	40	F8	300	40

Triplicate measurements were done, but the average data (standard errors <3%) are considered. These data for various formulations are presented in Table II.

Fourier transform infrared spectral studies

Fourier transform infrared (FTIR) spectral data were taken on a Nicolet (Model Thermo 5700, Milwaukee, WI) instrument to confirm the formation of IPN structure and to find the chemical stability of the drug in the MS. FTIR spectra of placebo MS, drug-loaded MS, and pure drug were taken in the range between 4000 and 500 cm^{-1}. Samples were prepared with KBr and pellets were obtained by applying a pressure of 600 kg/cm^2 using the FTIR pellet maker.

Particle size measurements

Particle size and size distributions were measured using laser light scattering technique (Mastersizer-2000, Malvern, UK). Particle size was measured by using dry sample adopter to record volume mean diameter (V_d). Results of particle size distribution are given in Table II.

Differential scanning calorimetric studies

Differential scanning calorimetry (DSC) (Rheometric Scientific, Surrey, UK) was performed on drug-loaded MS, placebo MS, and pure drug. Samples were heated from 25 to 400°C at the heating rate of 10°C/min in a nitrogen atmosphere (gas flow rate of 20 mL/min).

TABLE II
Encapsulation Efficiency, Volume Mean Diameter, and Diffusion Exponent of Different Formulations[a]

Formulation code	% Encapsulation efficiency	Volume mean diameter (μm)	n	r[a]
CF	84	12	0.236	0.988
F1	91	15	0.383	0.992
F2	68	23	0.268	0.976
F3	54	29	0.284	0.991
F4	41	34	0.197	0.958
F5	88	19	0.347	0.969
F6	56	27	0.152	0.998
F7	45	36	0.221	0.988
F8	37	47	0.303	0.996

[a] Estimated at 95% confidence level.

Scanning electron microscopic study

SEM photographs of PLGA-Pluronic blend MS loaded with drug (Formulation F8) were taken. Microspheres were sputtered with gold to make them conducting and placed on the copper stub. Scanning was done using a JEOL model JSM-840A (Japan) instrument.

In vitro release studies

In vitro drug release from different formulations of PLGA and PLF68/127 MS were investigated in simulated intestinal fluid (SIF). These experiments were performed using a water bath with a shaker (Grant OLS200, Grant Instruments, Cambridge, UK) at the stirring speed of 100 rpm. A weighed quantity of each sample was placed in 500 mL of dissolution medium maintained at 37°C ± 0.2°C. The repaglinide concentration was determined spectrophotometrically at the λ_{max} of 243 nm. These studies were performed in triplicate for each sample, but average (with less than 3% standard errors) values were considered for data analysis.

RESULTS AND DISCUSSION

Preparation and characterization of MS

In the present study, novel PLGA and Pluronic F-68/127 blend MS for the CR of repaglinide (antidiabetic drug) were prepared by the emulsion–solvent evaporation technique. A solution of PLGA, PLF68/127, and repaglinide in DCM was poured into the agitated aqueous solution of PVA. The subsequent evaporation of DCM leads to the formation of solid MS. It was observed that no formation of pores on the surface of MS occurred. The PLF68/127 matrix acts as the hydrophilic carrier and thus controls swelling and release of the hydrophobic drug from the MS. The percent encapsulation efficiency of all the formulations varies from 37 to 91%. However, the percent

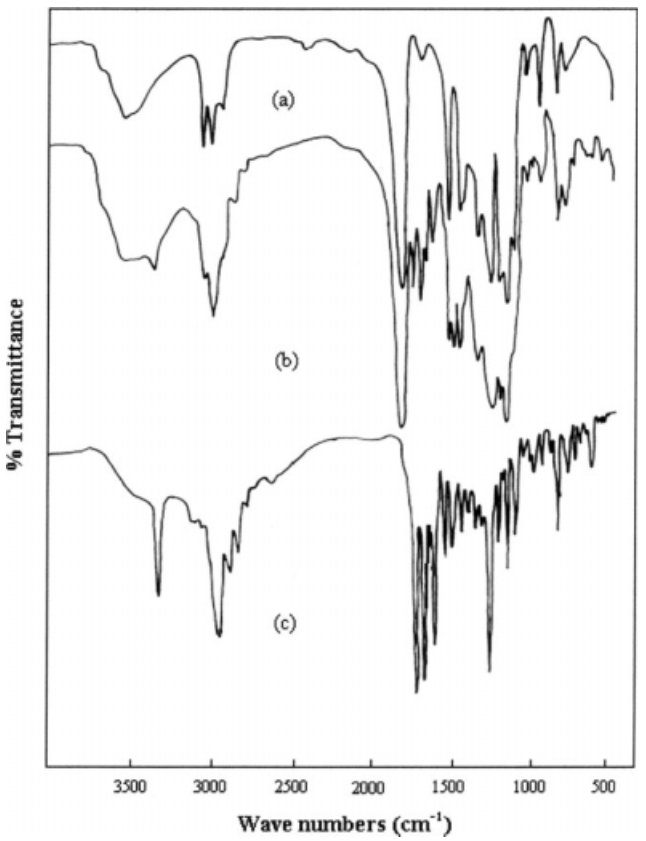

Figure 1 FTIR spectra of (a) placebo microspheres, (b) drug-loaded microspheres, and (c) pure repaglinide.

encapsulation efficiency decreased as the content of Pluronic was increased from 10 to 40%. For instance, formulation F1 has higher percent encapsulation efficiency than F2 and formulation F2 has higher percent encapsulation efficiency than F3. The formulation F3 has greater % encapsulation efficiency as compared with formulation F4. Thus the percent encapsulation efficiency follows the sequence: F1 > F2 > F3 > F4. Similar trends were also observed for formulations F5, F6, F7, and F8. This could be due to higher hydrophilic nature of Pluronics, thereby leading to the leaching of more of drug particles during microsphere preparation. The percent encapsulation efficiency data are shown in Table II.

Fourier transform infrared spectral studies

FTIR spectra of (a) placebo MS, (b) drug-loaded MS, and (c) pure repaglinide are presented in Figure 1. In the case of repaglinide, a sharp band at 3307 cm^{-1} represents N—H stretching vibrations, whereas N—H bending vibrations are represented by a band at 1566 cm^{-1}. Aromatic C—H stretching vibrations are indicated by a small band at 3030 cm^{-1}, but aromatic bending vibrations are represented by a band at 761 cm^{-1}. Aliphatic C—H stretching vibrations are indicated by bands at 2935, 2857, and 2804 cm^{-1}, whereas aliphatic C—H bending vibrations are indicated by presence of bands at 1448 and 1385 cm^{-1}. The C=O stretching vibrations of the —COOH group are indicated by a band at 1687 cm^{-1} and the —C=O stretching vibrations of secondary amide are represented by the presence of a band at 1636 cm^{-1}. The C—O—C stretching vibrations are indicated by bands at 1149, 1091, and 1041 cm^{-1}. In the case of drug-loaded MS, all the bands observed in pure drug have appeared, whereas these bands are absent in the placebo MS. This shows the absence of any chemical interactions between the drug and the polymer matrix.

Particle size analysis

Particle size data are presented in Table II. Particle size increased as the Pluronic content is increased. For instance, formulation F4 (with 40% Pluronic) has a bigger particle size than F3 (with 30% Pluronic). Similarly, formulation F3 has a larger particle size as compared to F2 and formulation F2 has a bigger particle size than F1 (i.e., the trend is F4 > F3 > F2 > F1). This could be due to the accumulation of more of Pluronic in the matrix at higher Pluronic content, thus leading to the formation of large size particles.

Differential scanning calorimetric studies

DSC thermograms of (a) placebo MS, (b) drug-loaded MS, and (c) pure repaglinide are displayed in Figure 2.

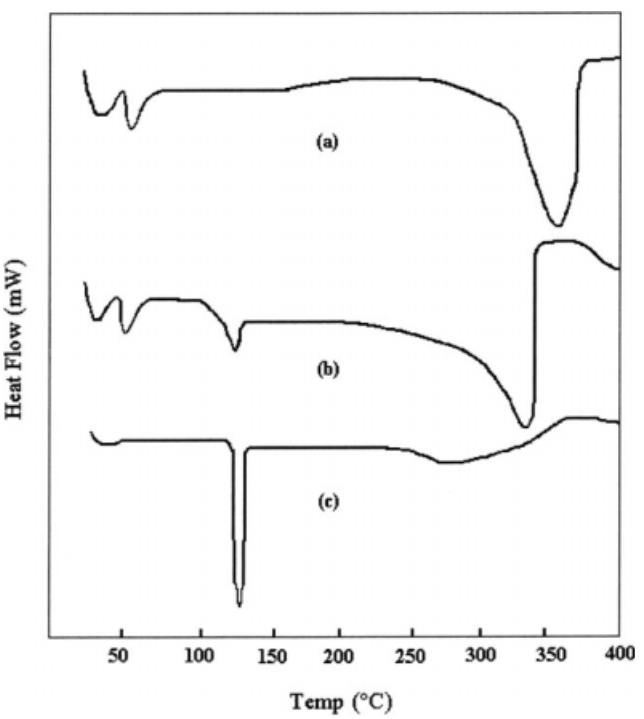

Figure 2 DSC spectra of (a) placebo microspheres, (b) drug-loaded microspheres, and (c) pure repaglinide.

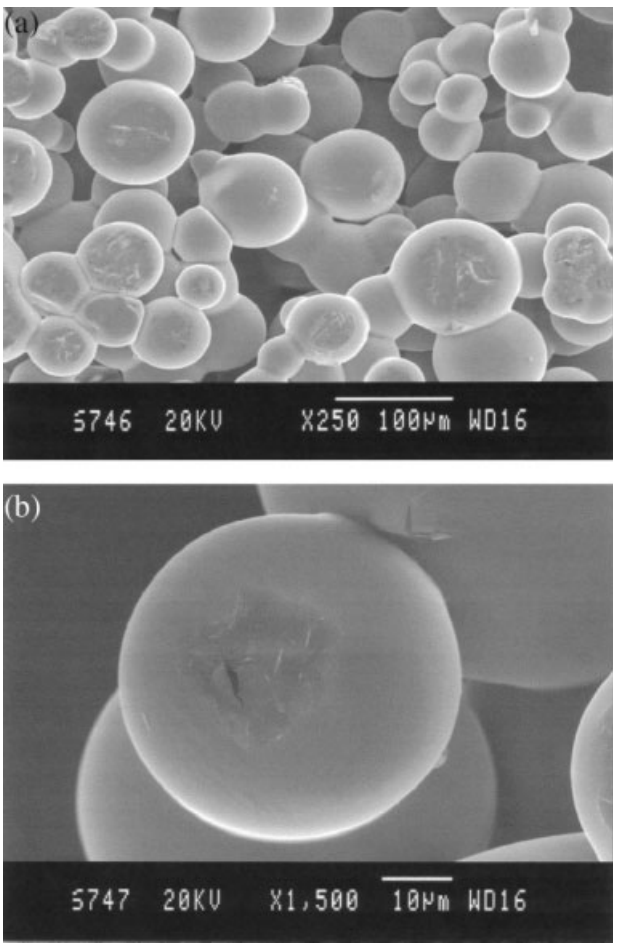

Figure 3 SEM images of (a) group of microspheres and (b) a single microsphere.

In the case of placebo MS, a peak at 52°C represents the melting endotherm for Pluronic. A peak at 58°C indicates glass transition temperature for PLGA. A broad and sharp peak at 355°C represents melting endotherm for PLGA. Thermogram of repaglinide showed a sharp peak at 132°C, indicating its melting. In the case of drug-loaded MS, a peak observed at 50°C represents melting endotherm for Pluronic. A peak at 55°C indicates the glass transition temperature for PLGA. The melting endotherm for PLGA has shifted to 340°C after encapsulation of the drug. However, a peak at 130°C corresponds to the melting endotherm for repaglinide after loading into polymer matrix. This indicates the crystalline nature of the drug after encapsulation into the polymer matrix.

Scanning electron microscopic studies

The spherical natures of MS are evident from their SEM micrographs [Fig. 3(a,b)]. As seen in the SEM micrograph (for formulation F8), the presence of Pluronic on the surface of MS is evident, but no pores are observed on their surfaces. Rupturing was observed on the surface of the MS that would have occurred during the freeze-drying of the MS. Also rupturing on the surface of the MS may happen due to dynamics of emulsification.

In vitro release studies

To understand the drug release from repaglinide-loaded blend MS of PLGA and PLF68/127, in vitro release experiments were performed in the SIF media. The results of the effect of PLF68 content in formulations F1, F2, F3, F4, and control formulation (CF) on their release rates are presented in Figure 4. The percent cumulative release is higher for F4 as compared with F3. Formulation F3 shows higher release rate when compared with F2, and similarly, F2 exhibits higher release rate than F1. The control formulation exhibits a lesser release rate than formulations F1, F2, F3, and F4. Only 36% of the drug was released from CF in 153 h, whereas it was increased to 46, 60, 69, and 75% when 10, 20, 30, and 40% of PLF68 was used for MS preparation along with PLGA, respectively (i.e., F1, F2, F3, and F4). In this process, water molecules pass through a tortuous path, but the degree of tortuosity depends on the volume fraction of the filler. Thus, the presence of PLF68 acts as a hydrophilic segment of the formulated product. Hence, the higher the pluronic content in the polymer, the higher will be the drug release rate.

The effect of PLF127 content in formulations F5, F6, F7, and F8 are compared in Figure 5. The percent cumulative release is higher for formulation F8, which is greater than F7. The formulation F7 has

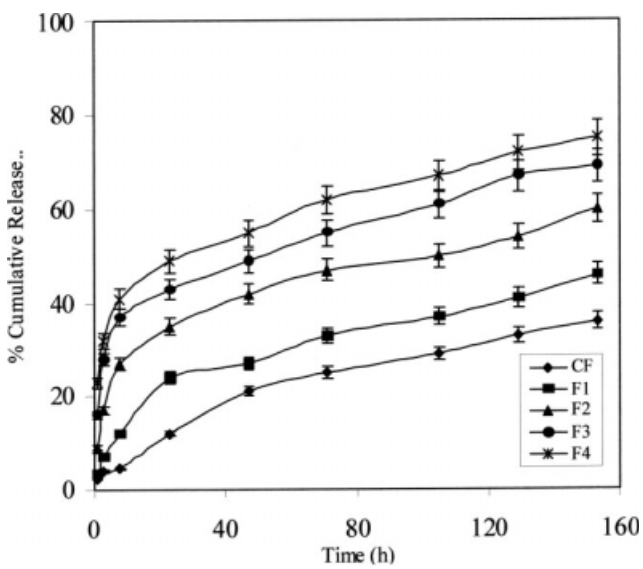

Figure 4 Effect of PLF68 on in vitro release profiles of formulations F1, F2, F3, and F4.

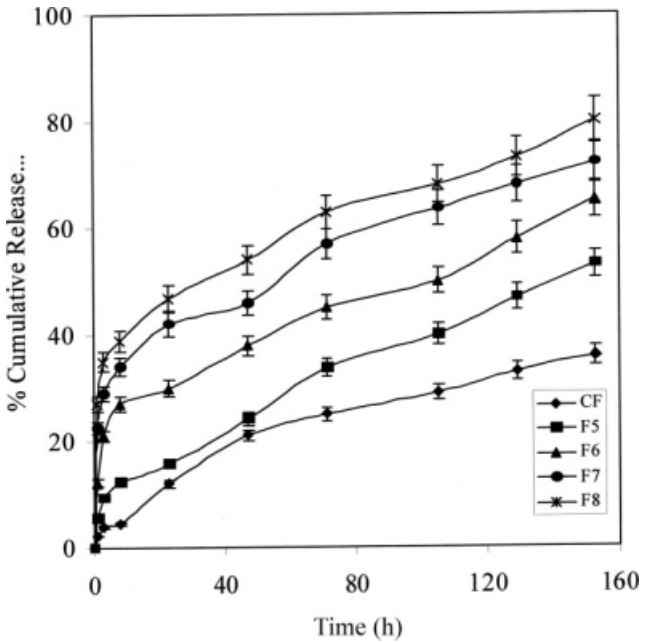

Figure 5 Effect of PLF127 on in vitro release profiles of formulations F5, F6, F7, and F8.

higher release rate than F6. In turn, formulation F6 exhibits higher release rate than F5. Therefore, as the content of PLF127 increases from 10 to 40%, the release rate also increases from 53 to 80% in 153 h because of the hydrophilic nature of PLF127. It was observed that formulations containing PLF127 coded as F5, F6, F7, and F8 exhibited higher release rates than those formulations containing PLF68 coded as F1, F2, F3, and F4. This could be due to high molecular weight of PLF127 when compared with PLF68 as well as its hydrophilic nature. Also, faster drug release rates from the blend MS prepared from PLF127 and PLGA are attributed to the bulk structure of PLF127 in MS.

The mechanism of drug release from the blend MS of PLGA and PLF68/127 have been studied by fitting the release data to an empirical equation of the type[30]:

$$\frac{M_t}{M_\infty} = kt^n, \tag{2}$$

where k is rate constant that is characteristic of drug–polymer system and n is a diffusional exponent. A value of $n = 0.5$ indicates Fickian transport; $n = 1.0$ indicates the presence of Case-II (zero order) transport. Values of n ranging between 0.5 ± 1.0 are attributed to the presence of anomalous transport. The n values calculated by using eq. (2) are included in Table II. Results for the present MS range from 0.152 to 0.383, indicating that the drug release from the MS follows; non-Fickian trend.[30] Previous studies reported in the literature also suggested similar anomalies.[31–34]

CONCLUSIONS

The present study reports the development of a novel blend MS of PLGA and Pluronic F-68/127 (PLF68/127) to study the controlled release of repaglinide using solvent evaporation method. The MS produced exhibited encapsulation efficiencies up to 91% with spherical shapes and having smooth surfaces. However, the incorporation of Pluronics could produce narrow size distributions ranging from 12 to 47 μm. The drug release mainly depends on the amount of Pluronics present in the matrix. The release rate also increases because of the hydrophilic nature of Pluronic, which enhances the release as well as solubility of hydrophobic repaglinide from the matrix. Since repaglinide has a very short half-life, it must be released at appropriate concentrations from the MS to maintain therapeutic concentrations. Hence, higher release rates were obtained in case of blend MS as compared to plain PLGA MS. The n values ranged between 0.152 and 0.383, indicating a non-Fickian release mechanism.

References

1. Csaba, N.; Sanchez, A.; Alonso, M. J. J Controlled Release 2006, 113, 164.
2. Raiche, A. T.; Puleo, D. A. Int J Pharm 2006, 311, 40.
3. Farahidah, M.; Christopher, F. V. D. W. Int J Pharm 2006, 311, 97.
4. Hong, K. K.; Hyun, J. C.; Tae, G. P. J Controlled Release 2006, 112, 167.
5. Davies, M. C.; Melia, C. D. Crit Rev Ther Drug Carr Sys 1990, 7, 235.
6. Agnihotri, S. A.; Aminabhavi, T. M. J Control Rel 2004, 96, 245.
7. Wang, J.; Schwendeman, S. P. J Pharm Sci 1999, 88, 1090.
8. Yang, Y. Y.; Chia, H. H.; Chung, T. S. J Controlled Release 2000, 69, 81.
9. Yang, Y. Y.; Chung, T. S.; Ng, N. P. Biomaterials 2001, 22, 231.
10. Arica, B.; Kas, H. S.; Orman, M. N.; Hincal, A. A. J Microencapsul 2002, 19, 473.
11. Chung, T. W.; Huang, Y. Y.; Tsai, Y. L.; Liu, Y. Z. J Microencapsul 2002, 19, 463.
12. Jiang, G.; Thanoo, B. C.; DeLuca, P. P. Pharmaceut Dev Technol 2002, 7, 391.
13. Cohen, S.; Yoshioka, Y.; Lucarelli, M.; Hwang, L. H.; Langer, R. Pharm Res 1991, 8, 713.
14. Park, T. G.; Lee, H. Y.; Nam, Y. S. J Controlled Release 1998, 55, 181.
15. Langer, R. Acc Chem Res 2000, 33, 94.
16. Gombotz, W. R.; Pettit, D. K. Bioconjugate Chem 1995, 6, 332.
17. Singh, M.; Shirley, B.; Bajwa, K.; Samara, E.; Hora, M.; O'Hagan, D. J Controlled Release 2001, 70, 21.
18. Cleland, J. L.; Johnson, O. L.; Putney, S.; Jones, A. J. S. Adv Drug Delivery Rev 1997, 28, 71.
19. O'Donnell, P. B.; McGinity, J. M. Adv Drug Delivery Rev 1997, 28, 25.
20. Kabanov, A. V.; Batrakova, E. V.; Alakhov, V. Y. J Controlled Release 2002, 82, 189.
21. Barreiro, I. R.; Bromberg, L.; Temchenko, M.; Hatton, T. A.; Alvarez, L. C.; Concheiro, A. J Controlled Release 2004, 97, 537.

22. Rowe, R. C.; Sheskey, P. J.; Weller, P. J. Handbook of Pharmaceutical Excipients, 4th ed.; Pharmaceutical Press and American Pharmaceutical Association: Bath, UK, 2003; p 447.
23. Alexandridis, P.; Holzwarth, J. F.; Hatton, T. A. Macromolecules 1994, 27, 2414.
24. Torchilin, V. P. J Controlled Release 2001, 73, 137.
25. Adams, M. L.; Lavasanifar, A.; Kwon, G. S. J Pharm Sci 2003, 92, 1343.
26. Desnoyer, J. R.; McHugh, A. J. J Controlled Release 2003, 86, 15.
27. Dufresne, M. H.; Leroux, J. C. Pharm Res 2004, 21, 160.
28. Jain, S. K.; Awasthi, A. M.; Jain, N. K.; Agrawal, G. P. J Controlled Release 2005, 107, 300.
29. Jain, S. K.; Agrawal, G. P.; Jain, N. K. J Controlled Release 2006, 113, 111.
30. Ritger, P. L.; Peppas, N. A. J Controlled Release 1987, 5, 37.
31. Han, W. W. T.; Stevens, W. F. Drug Dev Ind Pharm 2004, 30, 397.
32. Gudasi, K. B.; Vadavi, R. S.; Shelke, N. B.; Sairam, M.; Aminabhavi, T. M. React Funct Polym 2006, 66, 1149.
33. Shelke, N. B.; Sairam, M.; Halligudi, S. B.; Aminabhavi, T. M. J Appl Polym Sci 2007, 103, 779.
34. Shelke, N. B.; Aminabhavi, T. M. J Appl Polym Sci 2007, 105, 2155.