Synthesis, Characterization and Evaluation of Novel Methoxypolyethyleneglycol- *grafted*- Poly(ester-urethane)s for Controlled Release of Repaglinide

S. Vijay Kumar,[1] Namdev B. Shelke,[2] S. Prasannakumar,[1] Ajit P. Rokhade,[2] B. S. Sherigara,[1] Tejraj M. Aminabhavi[1]

[1]*Department of Industrial Chemistry, Jnana Sahyadri, Kuvempu University, Shivamoga, Karnataka 577 451, India*
[2]*Drug Delivery Division, Center of Excellence in Polymer Science, Karnatak University Dharwad, Dharwad 580 003, India*

Received 29 March 2007; accepted 29 April 2008
DOI 10.1002/app.29497
Published online 19 March 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Novel biodegradable aliphatic poly(ester-urethane)s (PEUs) based on polycaprolactone diol (PCL) and methoxypolyethyleneglycol grafted onto trimethylol propane (mPEG-*g*-TMP) were synthesized by solution polymerization technique and characterized using a variety of techniques. Microspheres ranging in size from 7 to 25 μm were prepared by the solvent evaporation technique and loaded with repaglinide up to 71 to 96%. Increasing molar ratios of mPEG-*g*-TMP propane with respect to polycaprolactone diol gave increase in particle size along with increase in % encapsulation efficiency. Surface morphology and spherical nature of the microspheres were confirmed by scanning electron microscopy (SEM). The release of repaglinide varied, depending upon the molar ratios of mPEG-*g*-TMP moieties with respect to PCL. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 251–257, 2009

Key words: polycaprolactone diol; methoxypolyethylene glycol; repaglinide; controlled release

INTRODUCTION

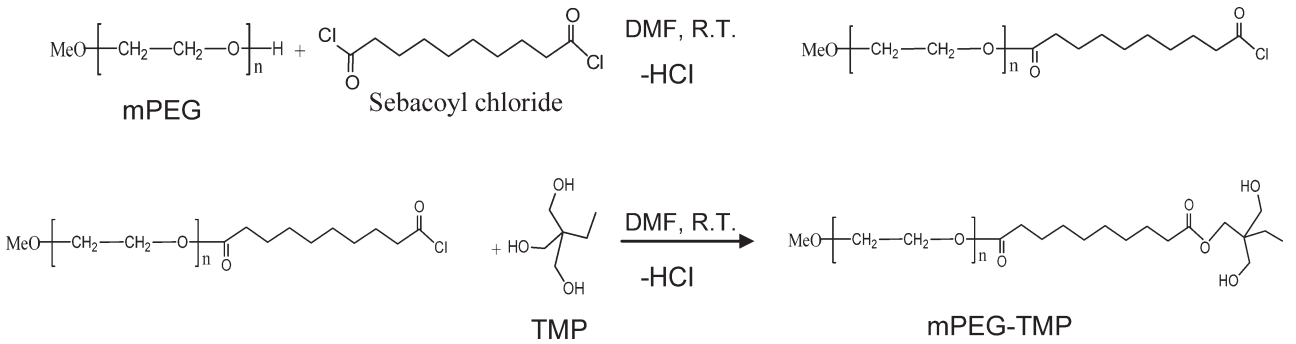
Polycaprolactone is a widely explored biodegradable polymer in drug delivery applications.[1–5] Similarly, biodegradable polyurethanes (PUs) prepared from polyols are also biocompatible and biodegradable having excellent flexibility. In our previous research,[6,7] we have shown that PUs containing polyethylene glycol (PEG) exhibited good biocompatibility and biodegradability.[8,9] Polyesterurethanes (PEUs) have also been studied because of their flexibility and superior biocompatibility.[10,11] Poly(ester urethane) copolymers were obtained by replacing short chain diol monomers (ethylene glycol, butanediol and hexanediol) by high molecular weight PEG. Lee et al.[12] synthesized some new types of PUs from poly(butyl succinate) (PBS) using PEG and 1,1′-methylenebis(dicyclohexyl-4,4′-diisocyanate) (^{12}HMDI) and found that by increasing the PEG content, the degradation rate of PU also increased because of the hydrolysis of polymers in aqueous media.[13,14]

As part of our continuing efforts[6,7] to develop novel types of biocompatible PUs, we now report on the synthesis of polycaprolactone diol-based PEUs grafted with methoxy polyethylene glycol (mPEG) to investigate their use in controlled release (CR) of repaglinide, an antidiabetic drug, a fast and short-acting meglitinide analog. The drug has low bioavailability (50%) and poor absorption in the upper intestinal tract[15,16] and hence, its consumption with the protective polymeric coating is necessary. In pursuit of this work, we report herein the synthesis of PEUs with PCL grafted onto mPEG. The polymers prepared were characterized by Fourier transfer infrared (FTIR) and gel permeation chromatography (GPC) techniques. Size of the microspheres was measured by laser light scattering technique. Drug-loaded microspheres were characterized by scanning electron microscope (SEM) to understand the surface morphology. Release kinetics of repaglinide from the microspheres were studied using the empirical equation.[17] The present study presents the development on novel is type of polymers to be used in the CR of repaglinide.

This paper is Center of Excellence in Polymer Science Communication # 195.

Correspondence to: T. M. Aminabhavi or B. S. Sherigara (aminabhavi@yahoo.com or bssherigara@rediffmail.com).

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



Scheme 1 Synthesis of methoxypolyethyleneglycol grafted trimethylol propane.

EXPERIMENTAL

Materials

Analytical grade *N,N′*-dimethyl formamide (DMF), poly(vinyl alcohol) (PVA) of M_w = 125,000 Da and dichloromethane were all purchased from s.d. fine chemicals, Mumbai, India. DMF was dried over 4 Å molecular sieves prior to use. Repaglinide was purchased from Loba Chemicals, Mumbai, India. 1,1′-Methylene-bis(dicyclohexyl-4,4′-diisocyanate) (^{12}HMDI), dibutyltindilaurate, methoxypolyethylene glycol (mPEG, M_w = 2000 Da), polycaprolactone diol (M_w = 750 Da), trimethylol propane (TMP), sebacoyl chloride and 1,4-butanediol were all purchased from Aldrich Chemical Company, Milwaukee, WI, USA.

Synthesis of methoxypolyethylene glycol *grafted* trimethylol propane

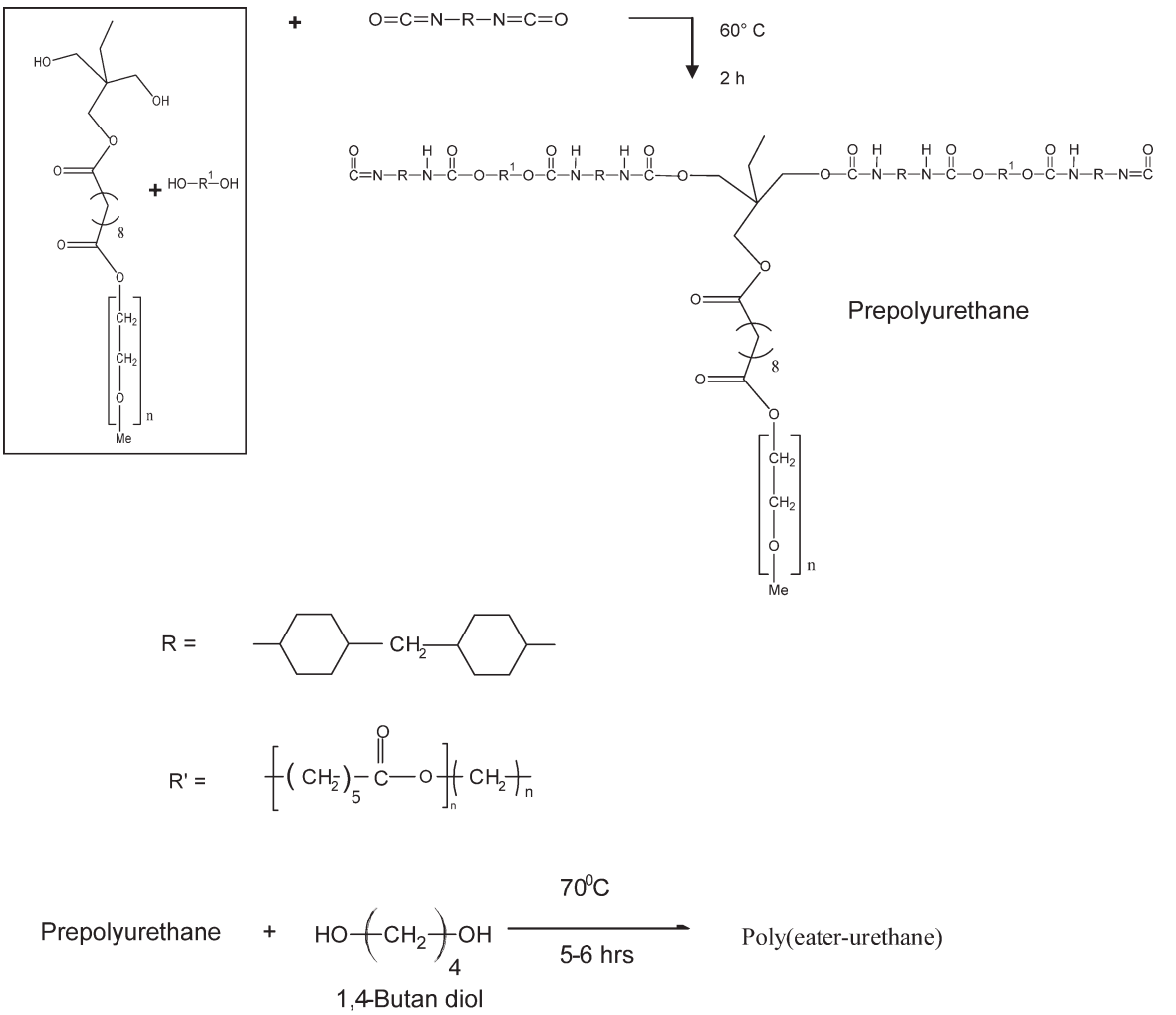
Sebacoyl chloride was taken in a dry round bottom flask fitted with a calcium chloride guard tube and addition funnel. To this, mPEG and triethyl amine dissolved in DMF in 1 : 1 ratio with respect to sebacoyl chloride were added drop-wise and, stirred overnight on a magnetic stirrer. The obtained —COCl terminated mPEG was filtered to remove triethylamine chloride salt. Trimethylol propane was taken into round bottom flask containing triethylamine. To this mixture, —COCl terminated mPEG was added drop-wise under magnetic stirring for overnight. The reaction mixture was filtered, DMF was removed under high vacuum and the product was stored in a desiccator until further use. The chemical reaction leading to the formation of mPEG-*g*-TMP is shown in Scheme 1.

Synthesis of poly(ester-urethane)

Polyurethanes were prepared by the method described earlier.[7] Methoxy polyethylene glycol-trimethylol propane (0.005 mol) and PCL (0.005 mol) were dissolved in DMF taken in a 100 mL round bottom flask fitted with addition funnel, nitrogen inlet and a guard tube. Then, dibutyltindilaurate (0.02%) was added and stirred on a magnetic stirrer for about 10–15 min under nitrogen atmosphere. A 0.02 mol of ^{12}HMDI was added drop-wise to the above reaction mixture, which was stirred for 30 min and heated at 60°C for 2 h to get the isocyanate-terminated PEU. The reaction mixture was cooled to ambient temperature and 1,4-butanediol (0.01 mol) was added to isocyanate-terminated PEU as a chain extender. The mixture was again heated at 70°C for 5–6 h. After completion of the reaction, the mixture was cooled to ambient temperature; the product was precipitated in distilled water, collected by filtration and dried in a vacuum oven at 60°C. Different PEUs were prepared by varying the ratio of PCL and mPEG-*g*-TMP. Reactions leading to the preparation of mPEG-*g*-TMP and PCL and the formation of PEUs are given in Schemes 1 and 2, respectively. Formulation codes and different ratios of the monomers used for PEU preparation are given in Table I.

Preparation of repaglinide-loaded microspheres

Microspheres of mPEG grafted PEU containing hydrophobic repaglinide drug were prepared by solvent evaporation technique. Poly(ester-urethane) (100 mg) was dissolved in dichloromethane (3 mL) followed by the addition of repaglinide to the polymer (1 : 0.1) at the appropriate weight ratio and stirred at ambient temperature. The polymer/drug solution was added drop-wise to 2.5% PVA solution under constant stirring using Eurostar high-speed stirrer (IKA Labortechnik, Germany) at 900 rpm rotor speed. The solution was further stirred for about 20–30 min to completely evaporate dichloromethane; the solution was diluted with distilled water and microspheres were isolated using a tabletop centrifuge (Jouan, MR 23i, France). The PEU microspheres were washed several times with fresh distilled water to remove the adhered particles such as stabilizers or unencapsulated drugs. The obtained microspheres were again redispersed into deionized water and



Scheme 2 Synthesis of PEU from mPEG-TMP and PCL.

lyophilized using freeze-dryer (Jouan, LP3, France) to obtain the completely dried microspheres.

Drug loading efficiency

Microspheres loaded with the drug were dissolved in DCM and the amount of repaglinide entrapped was determined by UV spectrophotometer (Secomam, Anthelie, France) at the λ_{max} value of 243 nm. These data were collected in triplicate, but the average values were considered in calculating % drug loading and encapsulation efficiency. These were calculated as follows:

$$\text{Actual drug loading}(\%) = \left(\frac{\text{Weight of drug in microspheres}}{\text{Weight of microspheres}}\right) \times 100 \quad (1)$$

$$\%\text{Encapsulation efficiency} = \left(\frac{\text{Actual drug loading}}{\text{Theoretical drug loading}}\right) \times 100 \quad (2)$$

Here, theoretical drug loading refers to the amount of drug taken for loading.

In vitro drug release

Weighed amounts of drug-loaded microspheres (10 mg) were suspended in 100 mL of phosphate buffer solution of pH 7.4. Dissolution medium was stirred at 100 rpm at 37°C using water bath attached with a shaker (Grant OLS200, Grant Instruments, Cambridge Ltd, UK). Aliquots of dissolution medium (3 mL) were withdrawn and filtered through 0.25 mm millipore filter at the predetermined time intervals. After appropriate dilution, the drug concentration was analyzed by UV spectrophotometer (Secomam,

TABLE I
Formulation Codes, Distribution of Molecular Weight and Polydispersity Index Used for Peu Preparation

Formulation codes	% mPEG-*g*-TMP	% PCL	$\overline{M}_w$	$\overline{M}_w/\overline{M}_n$
PEU-1	10	90	22,000	1.24
PEU-2	20	80	24,700	1.29
PEU-3	30	70	26,800	1.22
PEU-4	40	60	29,100	1.32
PEU-5	50	50	34,000	1.35

TABLE II
% Drug Loading, Drug Loading Efficiency, Particle Size and Exponent Value, *n* for 10% Repaglinide-Loaded PEU Microspheres

Formulation codes	% Drug loading	% Encapsulation efficiency	Diameter (μm) (SD)	Power Law	
				n	r^2
PEU-1	9.6	96	7 ± 1	0.65	0.96
PEU-2	9.2	92	8 ± 2	0.58	0.96
PEU-3	8.8	88	12 ± 2	0.51	0.98
PEU-4	8.1	81	15 ± 3	0.50	0.98
PEU-5	7.1	71	21 ± 4	0.50	0.97

SD, Standard deviation; r^2, correlation coefficient calculated at 95% confidence limit.

Anthelie, France) at the fixed λ_{max} value of 243 nm. Dissolution medium was maintained at constant volume by replacing the samples with a fresh dissolution medium.

Gel permeation chromatography

Molecular weights of the synthesized PEUs (without drug) were determined by Gel permeation chromatography (GPC) (Viscotek, Houston, TX, USA) using the differential refractive index detector (Viscotek, VE 3580) by employing two columns (Viscotek gel, GMHH *R*-H). The flow rate of the mobile phase viz., THF was set at 1 mL/min; polystyrene standards were used for calibration runs. Subsequently, the molecular weight of PEUs was reported as the polystyrene equivalent molecular weight. The results of molecular weight and polydispersity index are given in Table I.

Fourier transform infrared spectra

Fourier transform infrared spectra (FTIR) spectra of the polymers were determined using Nicolet 5700 spectrophotometer (Milwaukee, WI, USA) in the spectral range of 4000 to 400 cm^{-1}. Samples were crushed with KBr to get pellets under the hydraulic pressure of 600 kg/cm^2.

Nuclear magnetic resonance spectroscopy

Synthesized monomers and polymers were characterized by nuclear magnetic resonance spectroscopy (H^1-NMR and C^{13}-NMR) in deuterated chloroform using Bruker AV-300 Spectrometer (USIC, Karnatak University, Dharwad).

Scanning electron microscopy

Scanning electron microscopy (SEM) images of the microspheres were recorded using Jeol JSM 6400 scanning electron microscope (Japan) at the required magnification. A thin film of 10 nm gold coating was done before subjecting the samples to SEM.

Particle size analyzer

Particle size was measured by laser light scattering technique (Mastersizer 2000, Malvern, UK). Sizes of the completely dried microspheres of different formulations were measured using the dry sample adopter. The volume-mean diameter (*Vd*) was recorded and these results are included in Table II.

RESULTS AND DISCUSSION

Gel permeation chromatography

GPC data suggest that molecular weights of the polymers have increased with increasing content of mPEG-*g*-TMP with respect to PCL. This could be due to high molecular weight of mPEG (M_w = 2000) that was used to graft onto TMP monomer. The

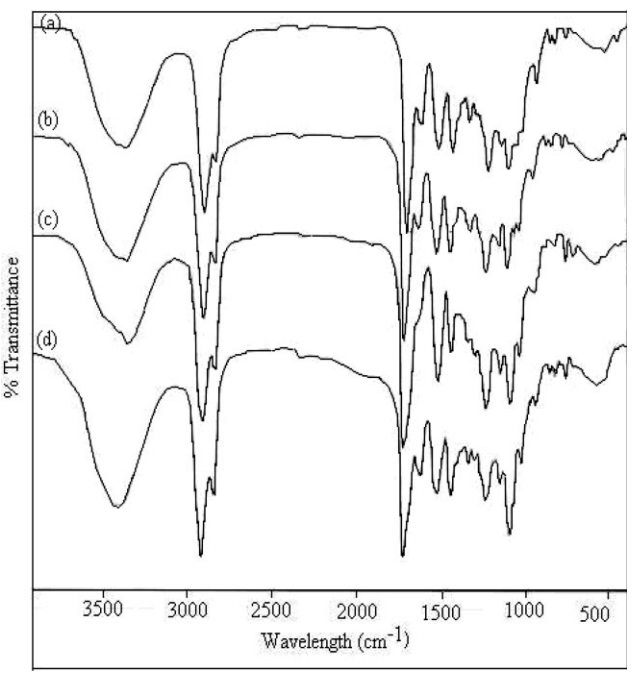


Figure 1 FTIR spectrum of PEU prepared from different formulations of mPEG-TMP and PCL (a) 10% mPEG-*g*-TMP, (b) 30% mPEG-*g*-TMP, (c) 40% mPEG-*g*-TMP and (d) 50% mPEG-*g*-TMP.

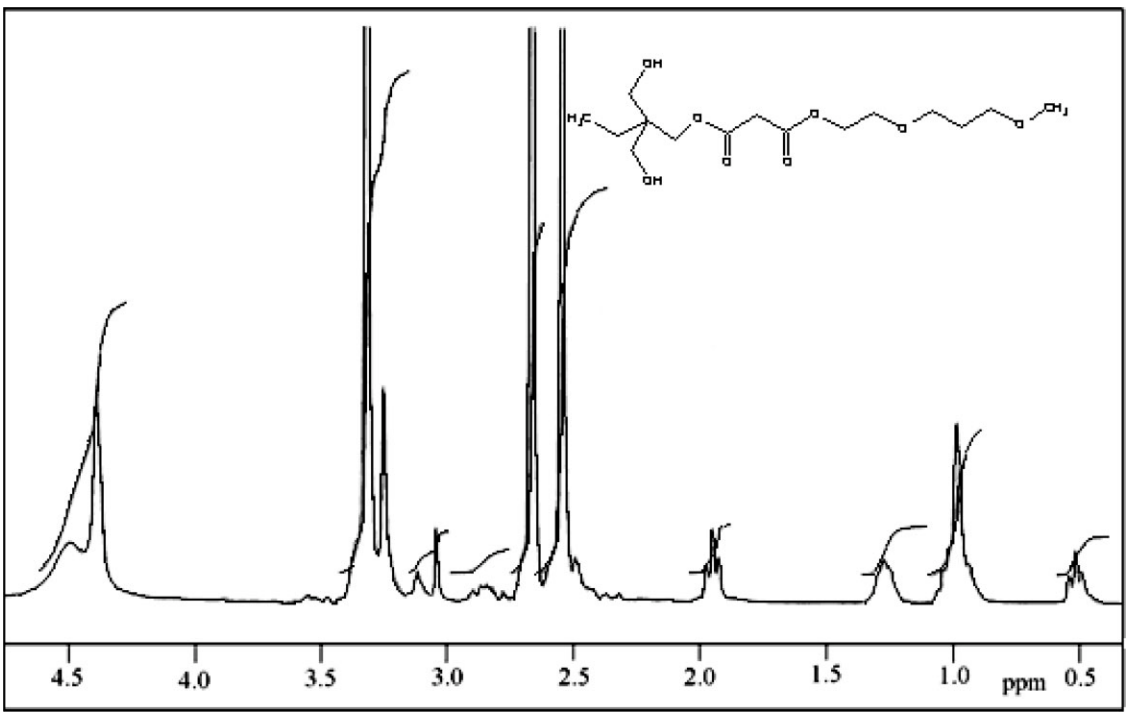


Figure 2 ^{1}H-NMR spectra of TMP-*graft*-mPEG.

results of molecular weight and polydispersity index (PDI) are given in Table I.

Fourier transform infrared spectra

FTIR curves of the different formulations of PEUs are displayed in Figure 1. Characteristic peak of the ester linkage is obtained around 1740 cm^{-1}, while the band around 3400 cm^{-1} is due to the merging of —NH and —OH groups. The absence of a peak due to isocyanate group seen around 2260 cm^{-1} indicates the successful reaction between alcohol and isocyanate, resulting in the formation of urethane linkage. The characteristic peak of carbonyl group of urethane linkage (—N=C=O) appears at 1708 cm^{-1}, while the peak due to —C—N stretching vibrations is located at 1530 cm^{-1}. The band around 2800 cm^{-1} indicates —C—H stretching viberation, while an increase in the intensity at 1110 cm^{-1} is observed from PU-1 to PU-5 indicate the high content of mPEG-*g*-TMP in PEUs.

Nuclear magnetic resonance spectroscopy (H^1-NMR and C^{13}-NMR)

H^1-NMR spectrum of the copolymer is shown in Figure 2. Ester peak due to the reaction between sebacoyl chloride, mPEG and TMP resonate at δ = 3.3–3.5 ppm, which confirms the successful grafting. The CH_3 proton of TMP appears at δ = 0.9 ppm. The methylene proton signals of sebacoyl chloride units resonate at δ = 2.72–1.8 ppm. The ether methylene protons in the PEG signals are assigned at δ = 4.4 ppm. The —OH peak of TMP appear as a broad peak at δ = 4.5 ppm.

C^{13}-NMR spectrum of the copolymer is shown in Figure 3. The appearance of a peak at δ = 175.96 ppm confirms the ester formation. Peak in the range of δ = 23.5–38.14 ppm are due to aliphatic methylene carbons present in mPEG-*g*-TMP. The —OCH_2 carbon of mPEG resonate at δ = 71.2, while the —OH—CH_2 carbon of TMP resonates at δ = 64.49 ppm, which confirms the presence of TMP and mPEG in mPEG-*g*-TMP.

Scanning electron micrograph

A typical Scanning electron micrograph (SEM) picture of the drug-loaded sample viz., PEU-3 shown in

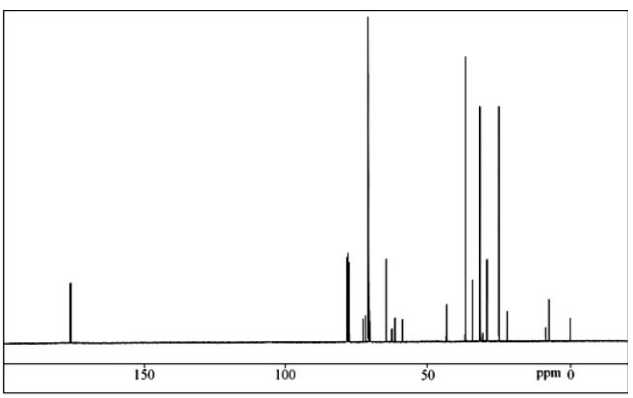


Figure 3 C^{13}-NMR spectra of TMP-*graft*-mPEG.

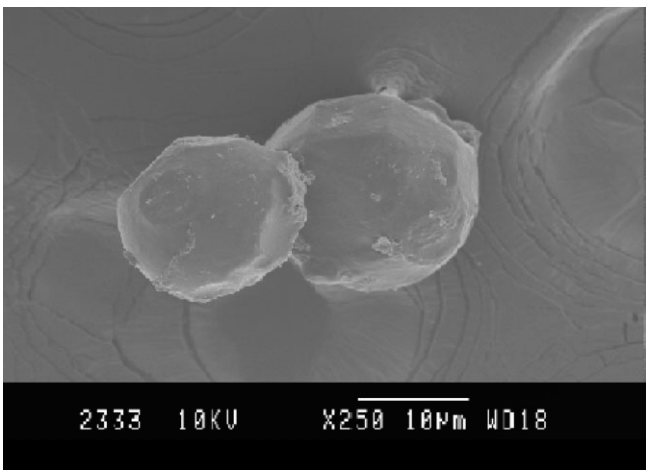


Figure 4 SEM picture of repaglinide loaded PEU-3 microspheres.

Figure 4 suggests that microspheres are spherical with some what wrinkled surfaces.

Particle size analysis

Particle size analysis showed an increasing trend with increasing amount of mPEG-*g*-TMP monomer in PEU-1, PEU-2, PEU-3, PEU-4, and PEU-5. Size of the microspheres was increased from 7 to 25 μm because of a increase in mPEG-*g*-TMP segment of the polymers that are more hydrophilic because of increased in mPEG-*g*-TMP content. The increase in mPEG-*g*-TMP segments with respect to PCL in the PEUs also resulted in an increased molecular weight of PEUs. Such an increase in size of the microspheres may be due to the bulky nature of mPEG attached to TMP. However, the microspheres were prepared at a constant stirrer speed of 900 rpm using 200 mg of polymer in about 6 mL of DCM.

Drug loading efficiency

Results of % drug loading and % encapsulation efficiency for different formulations are presented in Table II. A fixed amount of drug (10 wt %) was used for initial loading into PEUs. The UV results suggest that % repaglinide loading decreased because of the increasing amount of mPEG-*g*-TMP in PEUs. The loadings of repaglinide in PEU-1, PEU-2, PEU-3, PEU-4, and PEU-5 matrices are, respectively, 9.6, 9.2, 8.8, 8.1, and 7.1%, but the % encapsulation efficiencies decreased from 96 to 71 with increasing ratio of mPEG-*g*-TMP with respect to PCL. Significant reductions in % drug loading and % encapsulation efficiency data are attributed to the hydrophilic nature of mPEG and the hydrophobic nature of repaglinide present in PEUs.

In vitro drug release

Release characteristics of mPEG-*g*-TMP and PCL derived PEUs were evaluated to investigate the CR of repaglinide. Plots of % release patterns of repaglinide-loaded PEU microspheres are displayed in Figure 5. No burst effects were observed in all the formulations, because only 7, 10, 14, 18, and 22% of repaglinide drug was released from PEU-1, PEU-2, PEU-3, PEU-4 and PEU-5, respectively, during the first 2 hours. However, the release studies were extended up to 120 h. The amounts of drug released during this time were 78, 86, 91, 95, and 98%, respectively, indicating the usefulness of the matrices for a slow release of the drug.

Drug release kinetics

The empirical equation, $M_t/M_\infty = kt^n$ used earlier by Ritger and Peppas[17] was used to analyze the drug release characteristics from both swellable and nonswellable systems.[18] Fickian diffusion ($n = 0.5$) and Case II transport ($n = 1$) are often observed when the drug is released from the polymeric matrices. Any system that releases the drug by both diffusion and swelling mechanisms offers the n values in the range $0.5 < n < 1$. Such values of n have been calculated from the slope of the plots of $\ln(M_t/M_\infty)$versus $\ln t$ using the least squares analysis. The values of n for different PEU microspheres loaded with repaglinide are given in Table II. The estimated values of n in the formulations vary from0.5 to 0.65, indicating the Fickian type release trends. These trends are similar to those reported for other systems in the reported literature.[6,7,19,20]

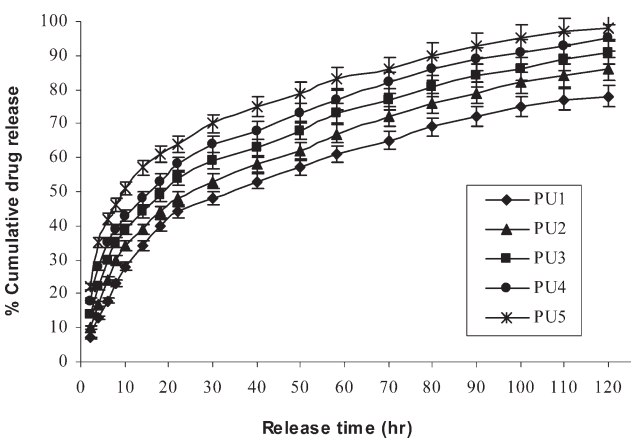


Figure 5 Drug release profiles of repaglinide from PEU microspheres prepared from (a) 10% mPEG-*g*-TMP (◆), (b) 20% mPEG-*g*-TMP (▲), (c) 30% mPEG-*g*-TMP (■), (d) 40% mPEG-*g*-TMP (●) and (e) 50% mPEG-*g*-TMP (x) at 37°C.

CONCLUSIONS

This study reports the synthesis of novel types of mPEG grafted PEUs by tailoring different ratios of the monomers. Microspheres were developed and repaglinide was loaded successfully. The release kinetics showed that the rate of drug release was controlled by varying the ratio of mPEG-*g*-TMP with that of PCL of the matrix. PEU matrices of this study have unique advantages in increasing the slow release rate of repaglinide up to about 120 h. However, the biodegradable nature of the matrices, as imparted due to the presence of PCL, has an advantage in further utilizing these matrices for *in vivo* studies. It is shown that the hydrophilic and lipophilic balance between the matrices can be controlled by varying the ratios of two different diols to obtain suitable PEU matrices for the release of repaglinide.

Mr. S. Vijay Kumar thanks the Kuvempu University, Shimoga for providing a fellowship. Professor T. M. Aminabhavi and Dr. N. B. Shelke thank the University Grants Commission (UGC), New Delhi, India for funding to establish Center of Excellence in Polymer Science (CEPS) at Karnatak University, Dharwad, during the period 2002–2007.

References

1. Bogdanov, B.; Toncheva, V.; Schacht, E.; Finelli, L.; Sarti, B.; Scandola, M. Polymer 1999, 40, 3171.
2. Li, F.; Hou, J.; Zhang, X.; Xu, M.; Luo, X.; Ma, D.; Kim, B. K. J Appl Polym Sci 1996, 62, 631.
3. Skarja, G. A.; Woodhouse, K. A. J Appl Polym Sci 2000, 75, 1522.
4. Vasnev, V. A. Polym Sci Ser B 1997, 30, 474.
5. Dahlmann, J.; Rafler, G.; Rafler, K.; Mehlis, B. Br Polym J 1990, 23, 235.
6. Shelke, N. B.; Sairam, M.; Halligudi, S. B.; Aminabhavi, T. M. J Appl Polym Sci 2007, 103, 779.
7. Shelke, N. B.; Aminabhavi, T. M. J Appl Polym Sci 2007, 105, 2155.
8. Kawai, F. Crit Rev Biotechnol 1987, 6, 273.
9. Kawai, F.; Enokibara, S. J Ferment Bioeng 1996, 82, 575.
10. Langer, R. S.; Folkman J. M. In Biomedical Polymers: Polymeric Materials and Pharmaceutics for Biomedical Use. Goldberg, E. P., Nakajima, A., Eds.; Academic Press: New York, 1980; p 113.
11. Lelah, M. D.; Lambercht, L. L.; Young, B. R.; Cooper, S. L. J Biomed Mater Res 1983, 17, 1.
12. Lee, S.; Lee, Y. S.; Nahm, K. S.; Hahn, Y. B.; Ko, S. B. Bull Korean Chem Soc 2000, 21, 1145.
13. DiBenedetto, L.; J; Haung, S. J.; Cameron, J. A. Polym Mater Sci Eng 1987, 57, 404.
14. Haung, S. J.; Roby, M. S.; Macri, C. A.; Cameron, J. A. In Biodegradable Polymers and Plastics; Vert, M., Feijen, J., Albertsson, A., Scott, G., Chiellini, E., Eds.; Royal Chem Society: Cambridge, England, 1992; p 147.
15. Jain, S. K.; Awasthi, A. M.; Jain, N. K.; Agrawal, G. P. J Control Release 2005, 107, 300.
16. Jain, S. K.; Agrawal, G. P.; Jain, N. K. J Control Release 2006, 113, 111.
17. Ritger, P. L.; Peppas, N. A. J Control Release 1987, 5, 23.
18. Colombo, P.; Bettini, R.; Catellani, P. L.; Santi, P.; Peppas, N. A. Eur J Pharm Sci 1999, 9, 33.
19. Han, W. W. T.; Stevens, W. F. Drug Dev Ind Pharm 2004, 30, 397.
20. Gudasi, K. B.; Vadavi, R. S.; Shelke, N. B.; Sairam, M.; Aminabhavi, T. M. React Functional Polym 2006, 66, 1149.