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Modification of Biodegradable Poly(Malate) and Poly(Lactic-*co*-Glycolic Acid) Microparticles with Low Molecular Polyethylene Glycol

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Poly(lactic-*co*-glycolic acid) microparticles (PLGA-MP) and cross-linked poly(malate) microparticles (PMAL-MP) were prepared by solvent/extraction and polycondensation, respectively. Microparticles were modified with polyethylene glycol (PEG 400) which lowered the drug loading of PMAL-MP, but did not influence the loading of PLGA-MP. The drug release from PMAL–PEG-MP occurred by initial degradation to oligomeric fractions, followed by further hydrolysis of oligomers to free drug (dipyridamole). The complete release was achieved for 22 days, whereas the release from non-modified PMAL-MP took 38 days. The drug release from PLGA–PEG-MP was characterized with an initial burst effect, followed by slower release during 26 days. Thus, the modification with PEG could be used to achieve appropriate release of the selected antithrombotic drug.

Keywords biodegradable microparticles; polyethylene glycol; poly(malate); poly(lactic-*co*-glycolic acid); controlled release

INTRODUCTION

Biodegradable drug delivery systems have been of particular interest because of the possibility either to sustain or to regulate drug release process. In addition, inclusion of labile drug molecules into polymeric particles could improve their stability and further, better efficiency of the therapy could be expected. The copolymers of poly(lactic-*co*-glycolic acid) (PLGA) are widely applied as carriers for microparticles and nanoparticles (Gref et al., 1994; Jiang, Gupta, Deshpande, & Schwendeman, 2005; Okada & Toguchi, 1995). The copolymers of malic acid are less investigated although they are also biodegradable and biocompatible (Rossignol, Boustta, & Vert, 1999). Recently, a new macromolecular nonimmunogenic carrier containing beta-poly(L-malic acid) as a segment was synthesized for target delivery of antisense oligonucleotides (Lee et al., 2006). In addition, the availability of free functional groups allows chemical bonding of drug molecules to the macromolecular carrier (Cammas et al., 1999). Previous studies have shown the possibility to achieve sustained release of dipyridamole from cross-linked microspheres based on D,L-malic acid (Yoncheva, Miloshev, Belcheva, & Lambov, 2001). However, the release rate was very low, which will possibly cause a problem for maintaining the therapeutic plasma concentrations constant. Dipyridamole is an attractive drug because of its anti-aggregating effect and low toxicity, although its light sensitivity should also be taken into account. The introduction of dipyradamole into biodegradable microparticles could improve its stability as well as spread the possibility to use its anti-thrombotic activity for treatment of patients with vascular diseases. It has been reported that dipyridamole-loaded PLGA-microparticles (PLGA-MP) could be considered as a parenteral system for sustained perivascular delivery of the antiproliferative drug (Zhu et al., 2006). Regarding oral administration, dipyridamole-loaded cellulose acetate microspheres were developed as a floating controlled release system, which allowed the microparticles to float over the gastric media more than 12 h (Soppimath, Kulkarni, & Aminabravi, 2001).

In this study, aiming to affect the dipyridamole release rate, experiments on the introduction of hydrophilic agents, such as polyethylene glycols (PEGs), into the cross-linked poly(malate) microparticles (PMAL-MP) have been carried out. PEGs were selected not only as hydrophilic agents, but also because of its possibility to form a surface coating layer around the particles. It has been reported that the presence of the hydrophilic PEG-chains on the nanoparticle surface significantly

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reduced their interaction with the blood proteins, thus, resulting in a prolongation of nanoparticle circulation time after intravenous administration (Gref et al., 1994; Stolnik et al., 1994). Hence, the PEG-attached chains could sterically stabilize the nanoparticles against opsonization and subsequent phagocytosis because of their complex physicochemical properties, particularly high chain flexibility and specific structural conformation. On the other hand, surface-extended PEG-chains could establish specific bioadhesive interactions with mucosal tissues because of their ability to interdiffuse across mucus network (Huang, Leobandung, Foss, & Peppas, 2000). Indeed, it has been demonstrated that the presence of PEG coating layer around poly(methyl vinyl ether-*co*-maleic anhydride) particles enabled their intracellular transport through intestinal mucosa (Yoncheva, Gomez, Campanero, Gamazo, & Irache, 2005). Taking these data into account, the cross-linked poly(malate) particles were modified either with high molecular poly(ethylene oxide) (PEO) or with low molecular polyethylene glycol (PEG 400) aiming to obtain different release rates. In parallel, microparticles based on PLGA copolymer were also developed and modified with PEG 400.

MATERIALS AND METHODS

Materials

D,L-Malic acid, *N*,*N*′-dicyclohexylcarbodiimide (DCC), tetrahydrofuran (THF), diethyl ether, and methanol were purchased from Merck (Darmstadt, Germany). Poly(D,L-lactide-*co*-glycolide) (PLGA) (Resomer RG 503, lactic:glycolic ratio 52:48, Mw 40,000) was obtained from Boehringer Ingelheim (Ingelheim, Germany). PEO with molecular weight of 200,000 Da was purchased from Union Carbide Corp. (Bound Brook, USA). Dipyridamole (2,2′,2″,2‴-[(4,8–dipiperidinopyrimido [5,4–*d*] pyrimidine-2,6–diyl) dinitrilo] tetraethanol) was obtained from Sopharma Ltd (Sofia, Bulgaria). All the other chemicals were of analytical grade.

Methods

Preparation of Cross-Linked Poly(malate) Microparticles

The cross-linked PMAL-MP were prepared using polycondensation method in the presence of DCC and *p*-toluenesulfonic acid (*p*-TSA) as catalyst (Panayotov, Belcheva, & Tsvetanov, 1987). Thus, 1.0 mmol dipyridamole (0.505 g), 1.25 mmol pyridine (0.17 mL), and 11.0 mmol DCC (2.28 g) were dissolved in THF. An organic solution of 5.0 mmol D,L-malic acid (0.67 g) and 0.4 mmol *p*-TSA (0.08 g) in THF was added under stirring (900 rpm) to the organic phase containing drug and DCC. The reaction was carried out at a temperature of 40–45°C and continued for 24 h. Then, the mixture was filtered and the yellow precipitate of the cross-linked PMAL-MP was subsequently purified by washing with hot ethanol. Finally, the resulting microparticles were rinsed with diethyl ether (10 mL) and vacuum dried. The modified PMAL-MP were obtained by applying the same procedure but with the addition of PEO 200,000 (0.0067 or 0.0268 g) or PEG 400 (0.335 g) to the organic solution of the drug.

Preparation of PLGA-Microparticles

The weighed amount of PLGA (0.9 g) and dipyridamole (0.1 g) was dissolved in 15 mL of mixed phase containing chloroform:acetone (1:1, vol/vol). The organic solution was subsequently added to 150 mL of aqueous phase containing PVA (1%, wt/vol) under stirring at 800 rpm and the emulsification was carried out during 30 min. The resulting suspension was poured into 500 mL distilled water under stirring at 600 rpm. After 1 h, the solidified microparticles were collected by centrifugation (10,000 rpm, 10 min), washed with distilled water and dried under vacuum at room temperature for 24 h. The modified PLGA–PEG-MPs were prepared following the same procedure, but PEG 400 was added to the organic phase at a concentration of 10 mg/mL.

Determination of Surface Morphology

The shape and surface characteristics of microparticles were examined by scanning electron microscopy (SEM) using a JSM 5300 scanning microscope (JEOL 5300, Japan). Samples were coated with gold to a thickness of 200–500 Å prior to SEM examination.

Drug Loading

A weighed quantity of microparticles was hydrolyzed in 100 mL of 1 N NaOH medium. After suitable dilution, the concentration of dipyridamole was measured by high-performance liquid chromatography (HPLC). Liquid chromatography apparatus equipped with a UV-detector Waters 991 and a column μBondapak C_{18} was used. Determinations were made at a flow rate of 0.4 mL/min using methanol:water (30:70 vol/vol) as a mobile phase and UV detection adjusted at 284 nm.

In Vitro Drug Release

In vitro drug release was conducted in a shaker bath (75 rpm) at 37°C using phosphate buffer (pH = 7.4) as a medium. The amount of dipyridamole released was measured by HPLC method.

Thin-layer chromatography of samples during drug release was performed on Merck DC-Alufolien, Kieselgel, 60 F_{254} (0.20 mm) plates and the mixture of butanol:water:acetic acid (68:22:10 vol/vol) was used as a mobile phase.

Gel Permeation Chromatography

The molecular weights of oligomeric fractions obtained during drug release from PMAL–PEG-MP were determined using Waters gel-permeation chromatograph equipped with a differential refractometer R-410 and a UV-detector Waters 490. The chromatographic separation was carried out using Ultrahydrogel columns (with porosities 250 and 500 Å)

calibrated on the basis of PEG standards. The methanol:water mixture (15:85 vol/vol) was used as a mobile phase at a flow rate of 0.8 mL/min and at a temperature of 36°C.

Statistical Analysis

One-way analyses of variance (ANOVA) at α-level of 0.05 were performed to compare the cumulative release of the drug using Origin 7.0 SR0 software (OriginLab Corporation, Northampton, MA, USA).

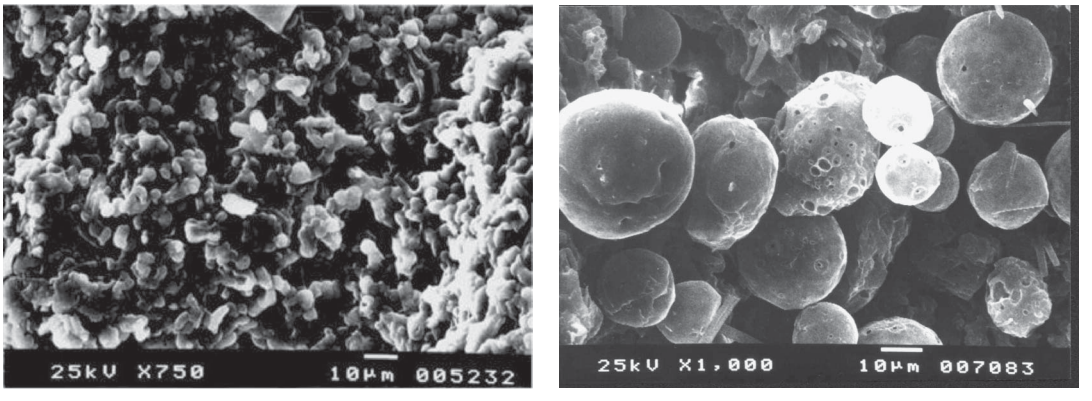
RESULTS AND DISCUSSION

Preparation and Characterization of Poly(malate) Microparticles

The availability of free functional groups in a drug molecule is the main prerequisite for the preparation of cross-linked PMAL-MP using polycondensation. As a result, ester bonds between hydroxyl groups of dipyridamole and carboxyl groups of D,L-malic acid could be formed (Yoncheva et al., 2001). In this study, aiming to affect the release rate as well as to achieve surface coating of the microparticles, experiments on the introduction of hydrophilic agents have been carried out. The participation of PEO or PEG into cross-linking was expected taking the availability of hydroxyl functional groups in their molecule into account. The concentration of PEO added to the polycondensation mixture was varied between 1 and 4% with respect to malic acid. It was found that the higher concentrations of PEO led to the formation of microparticle agglomerates. In addition, the amount of dipyridamole loaded onto PMAL–PEO-MP was extremely low (around 1%) at all examined PEO concentrations. The participation of PEG 400 into the cross-linking reaction seemed to produce microparticles with better characteristics. However, the tendency of agglomeration of the cross-linked microparticles still appeared (Figure 1A). The degree of drug loading of PMAL–PEG-MPs was higher than that for the particles modified with PEO, but lower than the loading of PMAL-MP (Table 1). Thus, it seemed that the addition of PEG 400 reduced the dipyridamole loading. The reason for the lower drug loading of PMAL–PEG-MP could be due to the competition between hydroxyl groups of PEG and dipyridamole during cross-linking. The size of the resulting PMAL–PEG-MP was similar to that of nonmodified PMAL-MP ranging from 1 to 15 μm (Figure 1A).

Preparation and Characterization of PLGA-Microparticles

The microparticles were primarily obtained from the classic solvent/evaporation method using methylene chloride as a solvent. However, the resulting microparticles tended to associate in irregular agglomerates. The possible reason for this observation could be due to the fact that dipyridamole was not completely dissolved into the inner emulsion phase during microencapsulation. Previous investigations showed that when the model drug isopropylantipyrin was completely dissolved into the organic phase of the emulsion, the microspheres were characterized with narrow size distribution and regular shape (Yoncheva & Lambov, 2000). According to these considerations, another technique was developed aiming to prepare PLGA- and PLGA–PEG-modified microparticles. Particularly, the mixed organic phase containing chloroform and acetone was used aiming to improve dipyridamole dissolution. Both types of microparticles were spherical and similar in size (5–50 μm), but some cracks and pores appeared on their surface (Figure 1B). This phenomenon could be explained with the faster extraction of the acetone from the polymeric droplets to the outer aqueous phase, which is in accordance with another study (Kawashima, Yamamoto, Takeuchi, Hino, & Niwa, 1998). Then, after the rapid acetone extraction, the slow evaporation of remaining chloroform took place. The degree of drug loading for PLGA-MP and PLGA–PEG-MP was similar (Table 1) suggesting that the presence of PEG in the organic



(A) (B)

FIGURE 1. Scanning electron microscopy of (A) cross-linked PMAL–PEG-MP prepared by polycondensation method and (B) PLGA-PEG-MP prepared by solvent/extraction method.

TABLE 1
Drug Loading and Encapsulation Efficiency Obtained From the Cross-Linked PMAL-MPs and PLGA-MPs

Sample	Drug Loading (%)	EE (%)[a]
PLGA-MP	4.15 ± 0.81	41.50 ± 8.12
PLGA–PEG-MP	3.71 ± 0.73	44.38 ± 8.73
PMAL-MP	32.40 ± 2.15	66.69 ± 4.43
PMAL–PEG-MP	12.72 ± 2.71	46.47 ± 9.90

Mean ± *SD* (*n* = 3).
[a]Encapsulation efficiency (%) = (actual drug loading/theoretical drug loading) × 100.

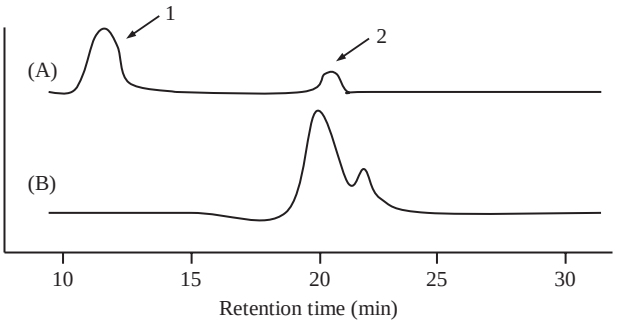


FIGURE 3. GPC chromatograms of samples taken on the second day (A) and on the sixth day (B) of in vitro release study from PMAL–PEG-MP.

phase did not influence the dipyridamole incorporation into the polymeric droplets.

In Vitro Drug Release from Poly(malate) Microparticles

The addition of PEO, regardless of its concentration, did not significantly affect the profile of dipyridamole release from PMAL–PEO-MP. On the contrary, the results achieved for PMAL-MP modified with PEG 400 showed that the release profile differed significantly when compared with that of PMAL-MP (Figure 2). The initial degradation of PMAL-MP resulted not in a free dipyridamole but in oligomeric products, followed by further hydrolysis to a free drug (Yoncheva et al., 2001). Here, the same phenomenon was observed during the release study of PMAL–PEG-MP. As shown in Figure 2, the free drug was delivered on the sixth day of the release process. These data were confirmed by thin-layer chromatography and GPC of the samples taken from the release medium at different time intervals. The GPC chromatograms of the sample withdrawn on the second release day revealed only the availability of high molecular fractions with an average weight of 18,900 Da (Figure 3A, peak 1) and oligomeric fractions of about 1,000 Da (Figure 3A, peak 2). The analysis of the GPC chromatograms of the sample taken on the sixth release day, which was plotted after triple detection by differential refractometer (Figure 3B) and UV detection at 208 and 284 nm, (Figure 4) showed that the peak at the 20th minute corresponded to two fractions. The first fraction was with molecular weight of about 500 Da (Figure 4A), and the second was with molecular weight of about 384–420 Da (Figure 4B). They could be assigned to free dipyridamole and PEG 400, respectively. The appearance of the lag-time phase in the case of PMAL–PEG-MP could be explained because PEG was bounded into the cross-linked structure and the bounds were not accessible for cleavage. However, after the lag-time phase, the release rate was enhanced almost twice compared with the nonmodified particles. The complete release of free dipyridamole from PMAL–PEG-MP was achieved for 22 days, while the release process from PMAL-MP took 38 days (Figure 2). The association of PEG promoted faster delivery of either oligomeric fractions or free dipyridamole compared with the nonmodified PMAL-MP (Table 2). Thus, after the initial cleavage of the cross-linked structure to oligomers, the properties of the oligomers were more hydrophilic because of the presence of PEG.

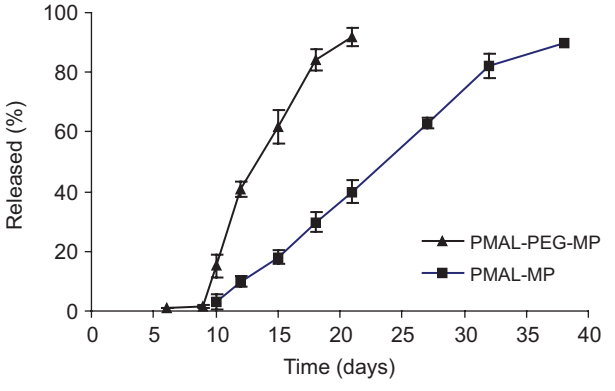


FIGURE 2. In vitro release profiles of dipyridamole depending on the composition of the cross-linked microparticles, PMAL-MP or PMAL–PEG-MP, respectively. Data represented mean ± *SD* (*n* = 3).

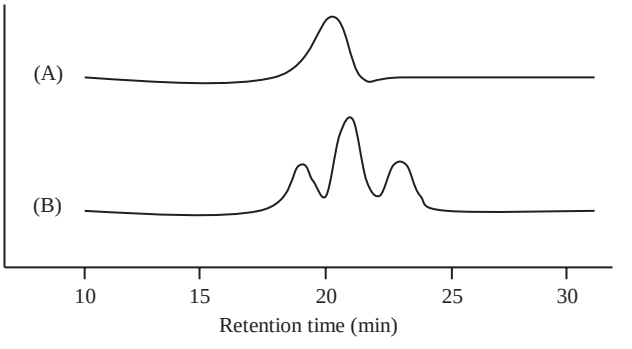


FIGURE 4. GPC chromatograms of the sample taken on the sixth day of in vitro release study from PMAL–PEG-MP observed by UV detection at 284 nm (A) and 208 nm (B).

TABLE 2
MRT-Values for Oligomers and Free Dipyridamole Calculated on the Basis of the Data Obtained From Release Study in a Phosphate Buffer (pH = 7.4)

Samples	MRT_{olig} (days)	$MRT_{Dipyridamole}$ (days)
PMAL-MP	13.14	22.10
PMAL–PEG-MP	6.98	13.26

In Vitro Drug Release from PLGA-Microparticles

The in vitro release profiles of PLGA-MP and PLGA-PEG-MP are shown in Figure 5. As shown, dipyridamole was released in a biphasic manner, which appeared to be typical for particulate systems where the drug is physically loaded. The initial burst effect for PLGA-MP reached approximately 49% during the first 2 h followed by slower continuous release thereafter. The intensity of the burst effect depended on many factors, such as the type and molecular weight of polymer carrier, the degree of drug loading, and drug properties. The higher drug loading of microparticles containing hydrophobic drug was reported to lead to higher level of burst release (Birnbaum, Kosmala, Henthorn, & Brannon-Peppas, 2000). However, the hydrophobic nature of dipyridamole was not the only responsible reason for the high initial delivery. The initial release probably increased because of the appearance of many pores, suggesting the more specific area available for water penetration. In addition, water penetration would be facilitated because of the more hydrophilic properties of the modified particles. Indeed, the data showed a slightly increased burst release for PLGA–PEG-MP (52%). This observation was in accordance with another study, where large burst effect appeared during in vitro release from microspheres based on PLA/PEG copolymer (Huang, Chung, & Tzeng, 1999).

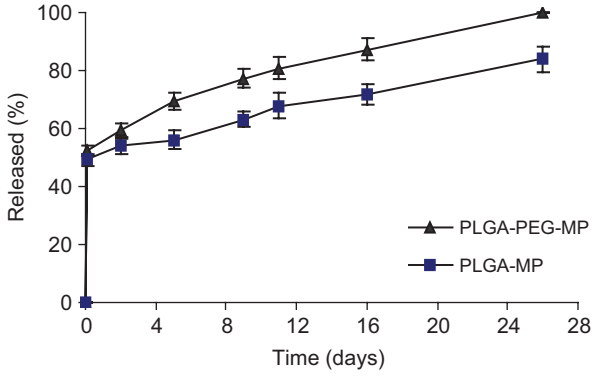


FIGURE 5. In vitro release profiles of dipyridamole depending on the microparticle formulation, PLGA-MP and PLGA–PEG-MP. Data represented mean ± *SD* (*n* = 3).

During the second phase of the release process, faster release was observed for PLGA–PEG-modified microparticles when compared with PLGA-MP. Thus, the complete release from PLGA–PEG-MP occurred for 26 days, whereas at the same time 84% of dipyridamole was released from PLGA-MP. This fact could be attributed to the enhanced degradation of the polymeric matrix as a consequence of the high affinity of PEG to the release medium. Taking into account that during the second phase the release was controlled by PLGA erosion, the presence of PEG probably increased the porosity of the system, which additionally promoted degradation.

CONCLUSION

Cross-linked PMAL-MP and PLGA-microparticles were modified using a low molecular PEG 400. The drug release from the cross-linked PMAL–PEG-MP occurred by initial degradation to oligomeric fractions, followed by further hydrolysis of the oligomers to free dipyridamole. In fact, PEG accelerated both phases, although its effect was more pronounced on the second one. Drug delivery from PLGA–PEG-MP was characterized with a significant burst effect, which was followed by slower release thereafter. In conclusion, both types of microparticles should be optimized aiming to obtain appropriate systems for controlled release of antithrombotic drug, such as dipyridamole.

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