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Effect of plasticization on heparin release from biodegradable matrices

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Abstract

Heparin-loaded polymer films of poly-L-lactide (PLLA) and poly-L-lactide-co-glycolide (PLLGA) as well as poly-DL-lactide-co-glycolide (PLGA) were produced. A plasticizer, PEG, was added to the polymers. It was found that the release profile in general consisted of a burst effect, a diffusion-controlled phase and a degradation-controlled phase. The plasticizer accelerated the onset of degradation in all cases, but its effect on the release profile differed significantly depending on the polymer. The plasticizer depressed the burst effect for PLLA, and accelerated the kinetics of the diffusion-controlled phase. For the PLLGA 80/20, however, the plasticizer had no significant effect on the release profile or kinetics. We explain these observations in terms of hydrophilicity and crystallinity effects.

Keywords: Local delivery; Biodegradable films; Stent; Heparin

1. Introduction

Biodegradable polymeric coronary stents have been given increasing attention in the recent years owing to some crucial limitations of the metallic stents. One of the most significant limitations is that these stents are permanent and the fact that stents are needed only temporarily, for up to 6 months (McBride et al., 1988). Hence a second intervention would be necessary to remove the redundant stent to prevent long-term effects of a foreign object in the body. Ideally, a stent should degrade after serving its purpose and this has led to the switch of scrutiny from metallic to biodegradable polymeric stents.

Apart from biodegradability, a polymeric stent has another significant advantage in that drugs can be released locally from the body of the stent in a controlled fashion. Some exciting new results have been reported on drug-coated metallic stents, which show reduced restenosis rates at the 6-month timepoint (Morice et al., 2002). To improve the performance, further, we have been developing biodegradable stents with capacity for sustained concurrent release of two or more drugs.

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Since a coronary stent is a blood-contacting device, inhibition of thrombus formation on the surface is of paramount importance. Heparin is a strong anticoagulant and has been often applied to prevent thrombosis. Researchers have been working extensively on surface modification using heparin to increase the blood compatibility of the polymer (Allmer et al., 1990; Bamford and Al-Lamee, 1996; Kang et al., 1998; Xie and Yang, 2002; Yang and Lin, 2002; Zhu et al., 2002). Unfortunately, this approach is probably more applicable to non-degradable systems with stable surfaces.

Localized heparin release has also been researched for prevention of cardiovascular thrombosis and vascular repair effects but most of the studies involved coatings or microspheres (Hinrichs et al., 1997; Vasudev et al., 1997; Edelman et al., 2000; Kreitz et al., 2004). These do not solve the problem of low drug loadings and the lack of prolonged period of release.

The purpose of this study was to assess the heparin release of PLLGA 80/20, PLLA and PLGA 53/47 matrices for a coronary stent application. Addition of plasticizer into these matrices to manipulate the release profiles was evaluated as well (for PLLA and PLLGA 80/20, as these polymers have relatively high T_gs and hence an effect due to plasticizer is expected). These materials have been evaluated for mechanical integrity after fabrication into stent prototypes, and the properties were found to be satisfactory (Tan et al., 2002, Venkatraman et al., 2003). With heparin loaded directly into the polymer matrices, the drug loading could be increased and in this study, a loading of about 10 wt.% was used.

2. Materials and procedures

The polymers were bought from PURAC Far East, Singapore. Table 1 shows the properties of the polymers.

Table 1
Properties of polymer matrices

Polymer type	T_m (°C)	T_g (°C)	Intrinsic viscosity (dl/g)	M_w (g/mol)
PLLA	174.43	67.3	8.4	3.3×10^6
PLLGA 80/20	153.51	53.6	4.8	9.3×10^5
PLGA 53/47		35.21	0.47	5.0×10^4

Heparin, in the form of a sodium salt, from the porcine intestinal mucosa was supplied by FLUKA and Polyethylene glycol (PEG) 4000 was from MERCK-Schuchardt.

Polymer granules were first dissolved in dichloromethane (DCM). Heparin powder was dried at 60 °C for 20 min and subsequently pounded to reduce the particle size. It was then added into the polymer solutions at a concentration of 200 g per dry polymer film of dimensions 14 mm × 10 mm × 0.1 mm. The mixture was then stirred using high-speed homogenizer for 15 min and then a magnetic stirrer overnight.

An automatic film applicator, AG-2150, manufactured by BYK-Gardner was used to cast the polymer films. After drying the films, three samples of 14 mm × 10 mm × 0.1 mm dimensions were cut from each polymer mixture. Each sample was then suspended in phosphate buffer solution and put into an incubator set at 37 °C, the human body temperature. Heparin release was measured at fixed intervals over 56 days.

Heparin release was measured by a colorimetric toluidine blue (Smith et al., 1980) method modified for this experiment. In this method a dye consisting of a 0.0035% toluidine blue solution was prepared in 0.01N HCL containing 0.2% NaCl. This dye will form a complex with heparin and by measuring the residual dye after complexation, the concentration of heparin could be deduced. The actual amount of release could then be determined using a calibration curve. The residual dye was measured using UV-2501PC Spectrometer.

A modulated differential scanning calorimeter (MDSC) from TA Instruments was used to determine the thermal properties of the samples. For melting enthalpy determination, the area under the melting peak was used to indicate the melting enthalpy. The changes in molecular weight were measured using the gel permeation chromatography (GPC) series 1100 from Agilent.

3. Results and discussion

3.1. Heparin release from different polymer matrices

PLLA and PLLGA 80/20 are semi-crystalline polymers whereas PLGA53/47 is an amorphous polymer.

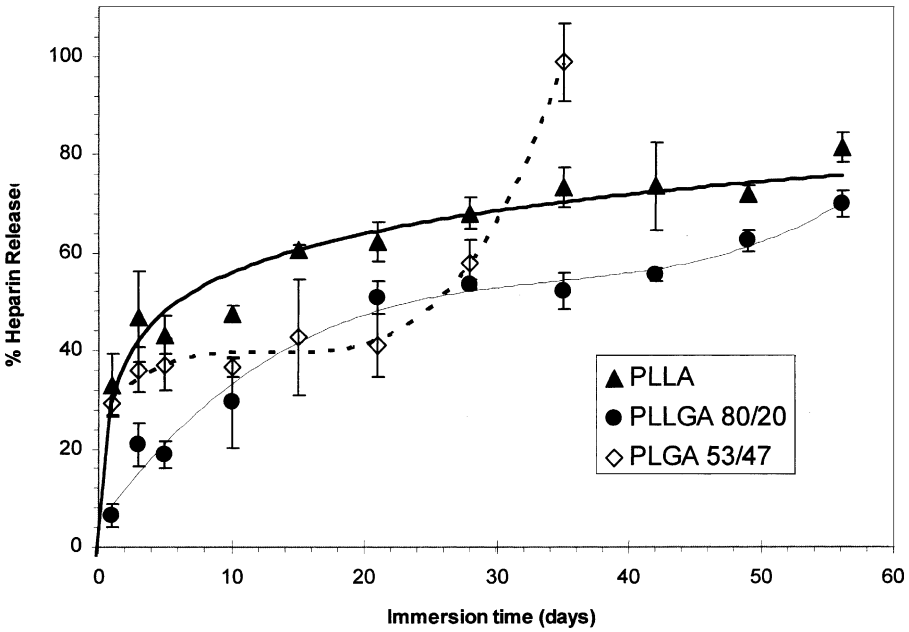

Fig. 1. The drug release profiles of PLLA, PLGA 80/20 and PLGA 53/47 over 56 days of immersion at 37 °C.

Fig. 1 shows the release profile of heparin from the three polymer matrices over about 2 months of observation. PLLA and PLLGA 80/20 showed biphasic release patterns whereas the release was triphasic for PLGA 53/47. There was an initial burst for the first 3 days of immersion for all the polymers, which accounted for about 40%, 20% and 30% of accumulated release for PLLA, PLLGA 80/20 and PLGA53/47 respectively. After the burst, the profile shows a typical diffusion controlled release for both PLLA and PLLGA 80/20. For PLGA 53/47, the initial burst was followed by a lag phase of about 18 days and then an accelerated release was observed from the 21st day till completion of release by the 35th day. By the 56th day, about 80% and 70% release of the heparin was observed for PLLA and PLLGA 80/20, respectively.

The burst effect is likely due to the release of (undissolved) heparin particles near the surface of the polymer film. This correlation between undissolved drug on the surface and the existence of an initial burst effect has been shown previously in our work (Alexis et al., 2004). The low solubility of the hydrophilic heparin in hydrophobic polymer matrices may aggravate this burst effect as a large fraction of the loaded drug was not dissolved in the matrix. PLLA is the most hydrophobic polymer among the three and this probably explains why the burst effect is the most severe for PLLA.

For PLGA 53/47, the sudden increase of the drug release at about day 21 is due to advanced stages of degradation of the polymer matrix. The matrix is observed to disintegrate after 21 days causing the large burst in the release.

After the initial burst and from day 3 onwards, the release resembled that of diffusion-controlled kinetics for both PLLA and PLLGA 80/20. The release rate constants of PLLA and PLLGA 80/20 were calculated from the slope of plots of amount released against the square root of time ($t^{1/2}$) as shown in Fig. 2. The rate constant for PLLGA 80/20 (8.5%/day$^{1/2}$) is higher than that of PLLA 8.4 (6.5%/day$^{1/2}$). This is due to the presence of the glycolide group in PLLGA 80/20, which caused the material to be less hydrophobic, less crystalline, absorb more water, and therefore become more plasticized (with water) than the PLLA. The higher degradation rate for the PLLGA 80/20 may also contribute to higher water absorption, through the generation of more –COOH end groups. Fig. 3 shows the changes in the molecular weight of the two polymers over 56 days of immersion. PLLA was observed to

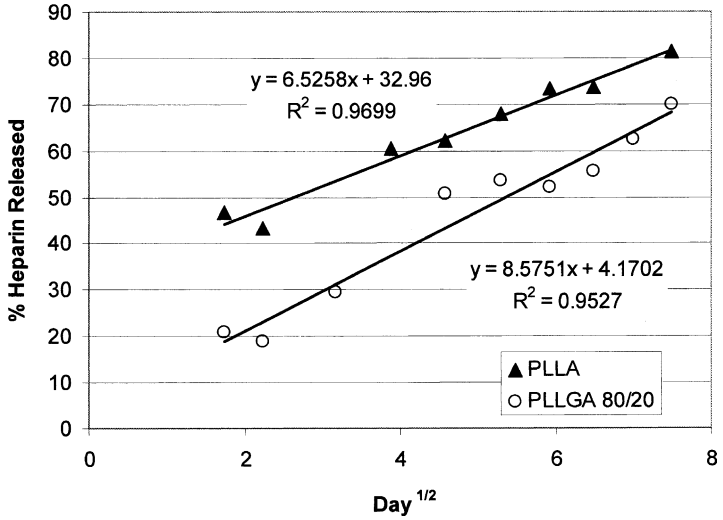

Fig. 2. The release rate constants of PLLA and PLGA 80/20.

be stable throughout the time frame studied while the molecular weight of PLLGA 80/20 decreased linearly with time.

3.2. Plasticization effect on heparin release

The effect of plasticization on drug release was also investigated with the aim of manipulating the drug release profiles. After several optimization trials, 5 wt.% of polyethlyene glycol 4000 was added to pure PLLA 8.4 and PLLGA 8020. The glass transition temperatures (T_g) of both the polymers were significantly modified after addition of PEG. The T_g reduced from 67.3 to 59.4 °C and 53.6 to 45.1 °C for PLLA and PLL GA 80/20, respectively.

Fig. 4 shows the effect of plasticization on PLLA. An interesting point worth noting is that the burst effect is greatly suppressed in the plasticized matrix. The re-

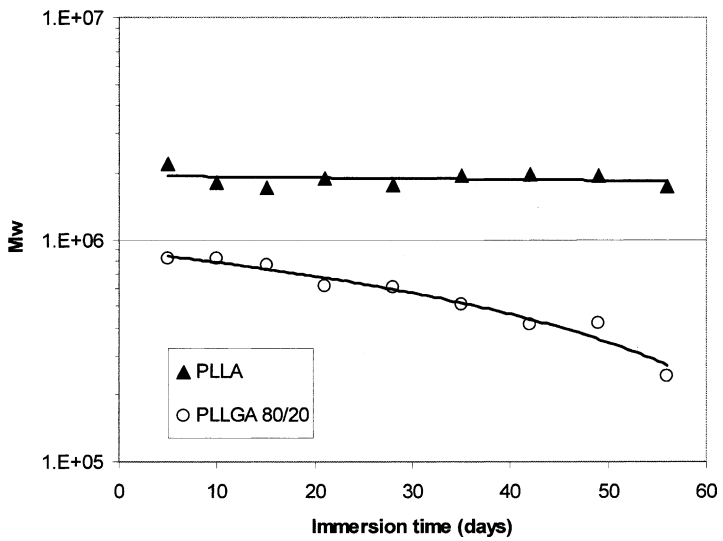

Fig. 3. Molecular weight changes of PLLA and PLLGA 80/20 over 56 days of immersion at 37 °C.

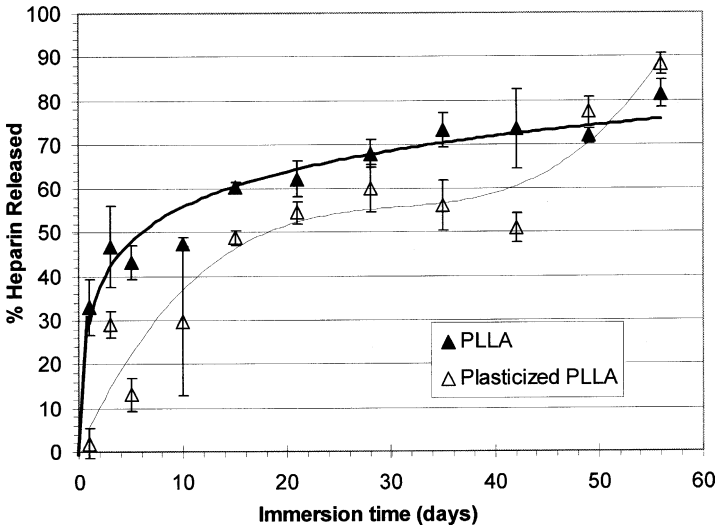

Fig. 4. The effect of plasticization on the drug release of PLLA.

lease of heparin as a function of time was almost linear for the first 15 days. The release slowed down for the next 27 days, then accelerated again, presumably due to degradation onset. By the 56th day, the total release reached almost 90%, 10% more than the unplasticized PLLA.

However, the effect of plasticization on PLLGA 80/20, which is depicted in Fig. 5 shows no significant effect on the release profile. This is explained further below.

The suppression of the burst effect in the plasticized PLLA could be due to the hydrophilic nature of both PEG and heparin causing the heparin to have a higher solubility in the PEG-plasticized PLLA, which is now more hydrophilic. On the other hand, the plasticizer increased the free volume promoting freer chain motion as indicated by the drop in the T_g, which is favorable for water diffusion into the polymer and a consequent faster release of drugs into the medium. Hence a higher rate of release is expected with addition of a plasti-

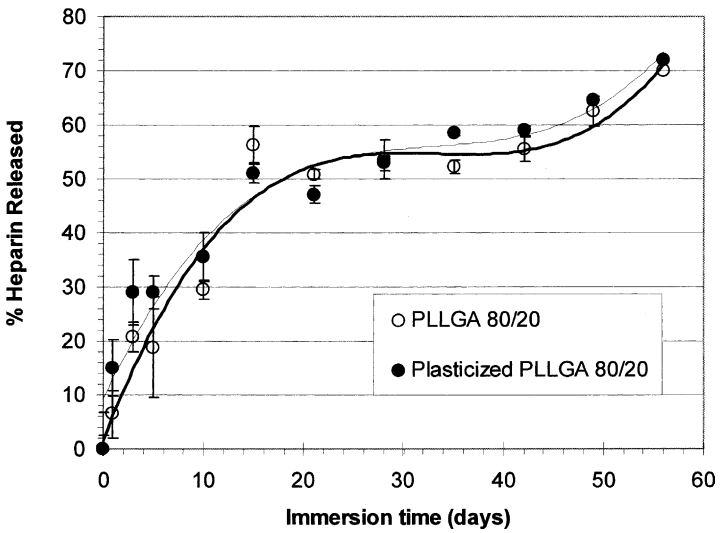

Fig. 5. The effect of plasticization on the drug release of PLLGA 80/20.

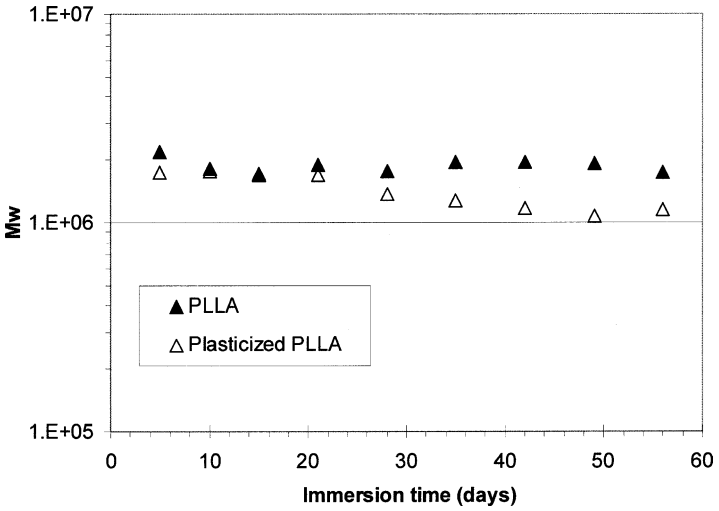

Fig. 6. The effect of plasticization on the molecular weight changes of PLLA.

cizer. For the PLLA this was confirmed by the results in Fig. 4, which shows a steeper slope in the plasticized PLLA release profile. The inflection at 42 days of immersion for the plasticized PLLA suggests the onset of degradation-controlled release. In Fig. 6, it can be seen that the molecular weight of the plasticized PLLA starts to deviate from that of PLLA after day 30 and this probably accounts for the emerging role of degradation in the drug release mechanism.

For PLLGA 80/20, plasticization did not alter the drug release and the drug release profile significantly. Unlike the plasticized PLLA, the burst effect was still substantial for the plasticized PLLGA 80/20. This is probably due to the less hydrophobic nature of PLLGA 80/20 as compared to PLLA and hence the difference in hydrophilicity due to added PEG may be insignificant. However, similar to PLLA, the rate of release was expected to be higher in the plasticized matrix, which clearly was not observed. Studies into the molecular weight changes with immersion revealed that starting from day 10 onwards, the plasticized matrix indeed had a lower molecular weight, which confirmed the more rapid degradation after plasticization (Fig. 7). Nonetheless, this reduction of molecular weight was accompanied by an increase in the melting enthalpy as shown in Fig. 7. The melting enthalpy is directly proportional to the degree of crystallinity. This increase in crystallinity, which retards drug release (which occurs predominantly through the available amorphous phase) explains why the drug release of the plasticized matrix was not enhanced even when there was a notable reduction in the molecular weight of the matrix.

The increase in melting enthalpy could be due to three possible mechanisms. The first mechanism is that during the degradation, the chain scission occurs first in the amorphous region of the polymer and then proceeds to the crystalline region of the polymer (Shalaby, 1994). Hence there is a continued loss of the polymer mass but preferentially of the amorphous component which leads to a gradual increase in crystallinity. Another possible mechanism is the rearrangement of short chains to form crystallites as degradation proceeds. When the chain length decreases during degradation, the chain molecules have greater mobility and can re-organize to form new crystallites (Pitt and Gu, 1987). A third possibility, based on our earlier work, is hydrolysis occurs preferentially at the glycolide linkages. This generates residual polymer that is richer in lactide units, which may then recrystallize. We have observed this recrystallization phenomenon in degrading PLLGA 80/20 (Alexis et al., unpublished results); in this instance, the lowering of the Tg by the presence of plasticizer may ensable recrystallization at 37 °C. At the moment, it is not possible to defintively identify a mechanism, as all can be operative at 37 °C; however, as extensive weight loss is not observed, the first mechanism is not expected to contribute. We believe the third mechanism to be dominant.

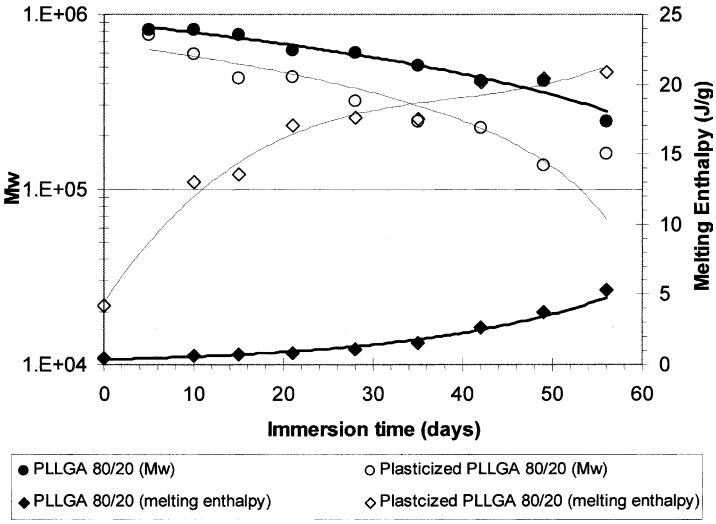

Fig. 7. The effect of plasticization on the molecular weight and melting enthalpy changes of PLLGA 80/20.

4. Conclusions

Different polymer matrices released drugs at different rates, and the mechanisms of release depended on the microstructure and glycolic acid content as presented in this work. For pure PLLA, the rate of release was the slowest followed by PLLGA 80/20 and finally PLGA 53/47. However, the burst effect is most severe in PLLA due to the most hydrophobic nature of the polymer among the three. This burst effect was effectively suppressed by plasticizer addition. For PLLGA 80/20, plasticization by PEG did not have a significant effect. These results reinforce the observation that in designing systems for controlled release, mechanistic details are important in the selection of plasticizers for release modification.

With these preliminary results, the approach of combining these profiles that are vastly different in a single system becomes feasible.

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