Controlled Release of Argatroban from PLA Film—Effect of Hydroxylesters as Additives on Enhancement of Drug Release

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ABSTRACT: The aim of this study was to develop a drug eluting stent that prevents vein restenosis. For this, we selected argatroban as the study drug and poly(lactic acid) (PLA) as the matrix. To enhance the release of argatroban from PLA film, the addition of hydroxylesters (additives) was investigated. The additives investigated were diethyl tartrate (DET), diethyl malate (DEM), and triethyl citrate (TEC). Marked enhancement of drug release was observed in DET-added film, while TEC- or DEM-added film showed little enhancement. To clarify the effect of DET, the release profile based on the contents of the drug and DET in the film and the effect of alkyl chain length of tartrate were studied. Tartrates used were dimethyl, di-*i*-propyl, and di-*n*-butyl esters (DMT, DiPT, and DnBT, respectively), and the enhancement order was DMT $\cong$ DET $\gg$ DiPT $\cong$ DBT $\cong$ PLA alone. The reasons for enhancement were discussed from the viewpoint of drug release behavior, degradation of PLA, water uptake within the film, and SEM observations. It was concluded that enhancement of drug release was due to large amounts of water uptake within the film which resulted in the formation of open pores at its surface. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 3353–3360, 2008

Key words: drug delivery system; additive; electron microscopy; poly(lactic acid); argatroban

INTRODUCTION

In the past thirty years, much research on the sustained-release of drugs has been carried out, and several systems are clinically available. During this research, many kinds of polymers have been investigated as a matrix for drugs. The most promising polymers are poly(lactic acid) (PLA) and copoly(lactic acid-glycolic acid), as these polymers have good safety, biocompatibility, and degradability.[1] Many factors have been observed to affect the release of a drug from a polymeric matrix. For example, when PLA is used as a matrix, factors that affect drug release are molecular weight, crystallinity, glass transition temperature, and the end group of PLA, in addition to physicochemical properties of the drug and the preparation method.[2–6] However, as the release of drugs from a PLA matrix is generally very low, many methods to improve drug release have been proposed, including the addition of low molecular weight substances to the PLA matrix. Nakano and coworkers reported that the addition of long chain fatty acid esters to the matrix enhanced the release of lipophilic drugs.[7–9] The reason for this acceleration in drug release was due to the phase separation between the PLA matrix and the ester phase. Another method to improve drug release is the addition of low-molecular-weight esters such as citric ester, which induces a decrease in the glass transition temperature of PLA.[10,11] Thus, additives increase the mobility of the polymer matrix, resulting in the enhancement of drug diffusion in the matrix.[11] In addition, there are reports showing that drug release is increased by forming a porous structure in the matrix, which results in a large surface area.[12] On the basis of these facts, it can be concluded that the addition of low-molecular-weight substances is effective in enhancing drug release when PLA is adopted as the matrix.

On the other hand, a recent trend in research has shown that the afore-mentioned defect of the PLA matrix can be overcome by using microparticles or microspheres as they have a large surface area and can be used as injectables.[13] However, when incorporated in a medical device used in implantation, drug release cannot be achieved by using microparticles or microspheres. It is possible to obtain such a devise using coating technology, for example the drug eluting coronary stent (DES). To develop DES, many polymeric coating materials or matrices have been proposed.[14]

We aim to develop a DES which prevents vein restenosis. To achieve this we selected argatroban as a

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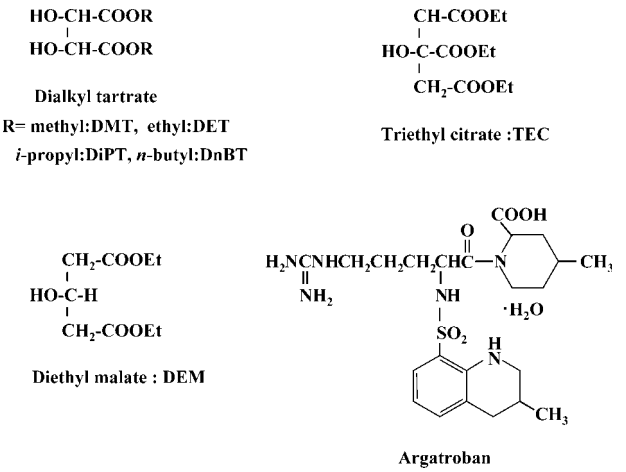

Figure 1 Chemical structures and abbreviations of additives and the drug used.

drug and PLA as a matrix. Argatroban is known as a synthetic direct thrombin inhibitor, and has a more potent inhibitory effect on fibrin- or clot-incorporated thrombin than other thrombin inhibitors such as heparin and hirudin. In addition, there is evidence that argatroban reduces restenosis due to abnormal proliferation of smooth muscle cell.[15,16] PLA is used as the matrix in this study due to its good biocompatibility, degradability, and safety as mentioned earlier.[1] However, it was observed from earlier research that the matrix composed of PLA alone does not release drugs efficiently. Thus, we investigated the effect of low-molecular-weight additives on the release of argatroban, and found that the addition of certain hydroxylesters significantly enhanced release of this drug. In this work, the effects of these esters on the release of argatroban from the PLA matrix were studied.

MATERIALS AND METHODS

Materials

Hydroxylesters used as additives were L-(+)-dimethyl tartrate (DMT), L-(+)-diethyl tartrate (DET), L-(+)-di-*i*-propyl tartrate (DiPT), L-(+)-di-*n*-butyl tartrate (DnBT), DL-diethyl malate (DEM), and triethyl citrate (TEC) (Tokyo Chemical Industry, Tokyo, Japan). Argatroban, an antithrombin agent, was kindly supplied by Mitsubishi Pharma (Tokyo, Japan). The chemical structures of these additives and argatroban are shown in Figure 1, and physicochemical properties of the additives are listed in Table I. Poly-DL-(lactic acid) (PLA) used as the matrix was purchased from biodegradable polymers (Florida, USA), (Lactel: DL-PLA, inherent viscosity 0.55–0.75). Trifluoroethanol was used as a solvent for PLA and argatroban (Wako Pure Chemicals, Tokyo, Japan).

Preparation of PLA film with drug

PLA film with drug and additive was prepared by the following procedure: adequate amounts of drug, additive and PLA were dissolved in trifluoroethanol and the solution was transferred onto a SUS 316L dish (diameter 16 mm). After the solvent was evaporated at room temperature for 24 h then 37°C for 24 h, the contents of the dish were dried *in vacuo* for 24 h at room temperature and the sample (weight 35–45 mg), was obtained. PLA film without drug and PLA film alone were also prepared according to this procedure. The composition of the films are listed in Table II, where the weight ratio of PLA, additive, and drug are listed together with wt %. The composition is presented using the weight ratios (parts) of drug and additive.

X-ray diffractometry of PLA film with drug

Powder X-ray scattering pattern of the PLA film was determined at room temperature with an X-ray diffractometer (RINT 2000, Rigaku, Tokyo, Japan). The X-ray source was Cu-Kα, and its voltage and current were 40 kV and 40 mA, respectively. The goniometer was scanned at 2°/min from $2\theta = 2°$–40°. The PLA films contained 30 parts of DET and 30 parts of argatroban.

Release of drug

Samples were soaked in 50 mL of phosphate buffered saline (PBS), pH 7.4, in a glass vial, and the

TABLE I
Physicochemical Properties of Hydroxylesters

Additive	Abbreviation	Molecular weight	Solubility in water[a]
L-(+)-Dimethyl tartrate	DMT	178.14	miscible
L-(+)-Diethyl tartrate	DET	206.19	miscible
L-(+)-Di-*i*-propyl tartrate	DiPT	234.25	partially soluble
L-(+)-Di-*n*-butyl tartrate	DnBT	262.30	nonsoluble
DL-Diethyl malate	DEM	190.19	small amount was not miscible
Triethyl citrate	TEC	276.28	partially soluble

[a] at room temperature.

Test of solubility: additive (200 μL) and water (800 μL) were mixed at room temperature and miscibility was judged by the naked eye.

TABLE II
Composition of PLA Films by Weight Ratio and Weight %

	Weight ratio (part)			wt%	
No.	PLA	Additive	Argatroban	Additive	Argatroban
1	90	0	0	0	18.2
2	90	10	20	8.3	16.6
3	90	20	20	15.4	15.4
4	90	30	20	21.4	14.3
5	90	30	10	23.1	7.7
6	90	30	30	20.0	20.0

vial was shaken in a 37°C water bath at a frequency of 15 strokes/min. The release of argatroban from the film was determined by periodically measuring the absorbance of the PBS solution at 330 nm that was λ_{max} of argatroban (UV–Vis spectrophotometer: U3410 Hitachi, Tokyo, Japan).

Degradation of PLA and PLA with DET

The PLA alone and PLA with DET films were soaked in PBS at 37°C for a predetermined time, then washed with distilled water, and dried *in vacuo* at 50°C for 4 h. The weight-average-molecular weight of PLA was determined by gel permeation chromatography (Pump: LC6AD, Shimazu, Tokyo, Japan, Detector: IR-2031, JASCO, Tokyo, Japan, Column: Shodex FL804, Showa Denko, Tokyo, Japan, Standard: polystyrene, and Eluting solvent: THF). The measurement was carried out under the following conditions: column temperature = 40°C, and eluting rate = 1.0 mL/min.

Weight loss of PLA by degradation in PBS at 37°C was determined by gravimetry according to the following equation:

$$\text{Weight loss (wt\%)} = [(W_i - W_d)/W_i] \times 100 \quad (1)$$

where W_i and W_d are the weights of the initial sample (before soak) and the dried sample after soaking in PBS for a predetermined time, respectively.

Weight change of PLA film

Weight changes of DET- and TEC-added PLA films containing 30 parts of additive and 20 parts of drug were determined by the following method: the films were soaked in PBS at 37°C for a predetermined time, then carefully wiped with filtration paper and weighed. The percentage weight change was determined according to the following equation:

$$\text{Weight change (wt\%)} = [(W_w - W_i)/W_i] \times 100 \quad (2)$$

where W_i and W_w are the weights of the initial dry sample and wet sample measured at time t, respectively.

SEM observation of PLA film

After the DET- and TEC-added films with drug had been soaked in PBS for 21 days, they were washed with distilled water, dried at room temperature *in vacuo*, and sputter-coated with gold. SEM observations were carried out using a SEM (Microscope TM-1000, Hitachi, Tokyo, Japan).

RESULTS

Drug status in PLA film

X-ray diffraction analysis of the film with drug did not show any crystalline peaks due to the drug or PLA in the range from 2θ = 2°–40° even when 30 parts of argatroban was loaded (Fig. 2).

Effects of the type of hydroxylesters on drug release from PLA film

The effects of the additives (DET, DEM, and TEC) on drug release from PLA film containing 30 parts of additive and 20 parts of argatroban are shown in Figure 3, where the absorbance change of the PBS solution is plotted against time. This figure shows that only DET enhanced the release of argatroban. The drug release from the DEM- or TEC-added film was close to that of the film without additive, and a

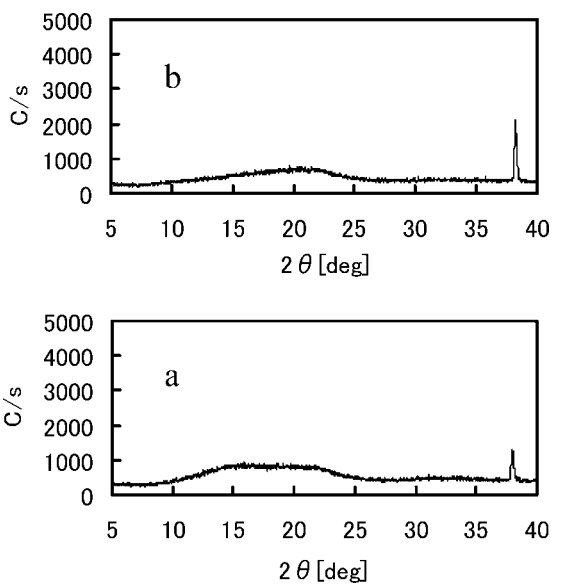

Figure 2 X-ray diffraction of PLA alone and drug-loaded PLA with DET (a) PLA alone, (b) PLA containing 30 parts of Argatroban and 30 parts of DET.

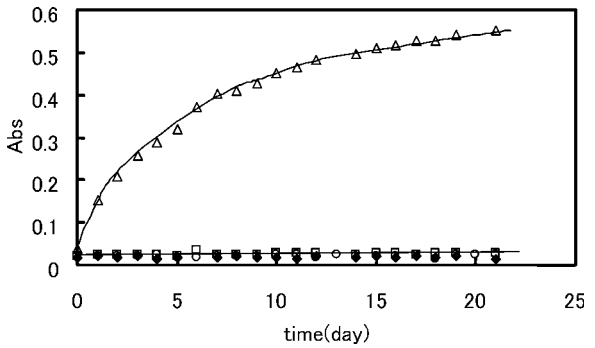

Figure 3 Effect of the type of additive on release of drug from PLA film ○ = TEC, ◆ = DEM, △ = DET, □ = PLA alone, Argatroban content = 20 parts, Additive content = 30 parts, Data are given as the average of $n = 3$.

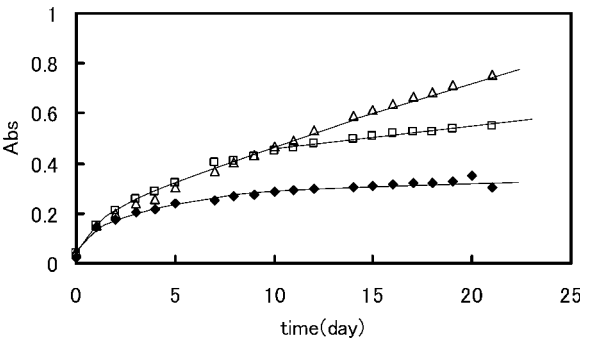

Figure 5 Dependence of drug release on drug content in PLA film Argatroban content: ◆ = 10 parts, □ = 20 parts, △ = 30 parts, DET content = 30 parts, Data are given as the average of $n = 3$.

time-dependent increase in the absorbance was not observed.

Factors affecting release of drug from PLA matrix

Dependence of drug release on DET content is illustrated in Figure 4, where the content of DET is varied from 0 to 30 parts. The release rate increased with an increase in the content of DET.

The relationship between argatroban content and the release rate is shown in Figure 5, where the weight ratio of DET in the film was constant, 30 parts, to the PLA weight. The cumulative amount of drug increased from Abs = 0.2 to 0.82 on the 21st day with an increase in drug from 10 to 30 parts. When argatroban content was lower than 20 parts, the release rate tended to decrease in a time-dependent manner.

The effect of alkyl chain length of the tartrates (dimethyl, diethyl, di-*i*-propyl, and di-*n*-butyl esters (DMT, DET, DiPT, and DnBT, respectively)) on drug release is shown in Figure 6. DMT and DET significantly enhanced drug release, with both esters having almost the same efficiency. The cumulative release was about 50 times larger than that of the PLA film alone at 21 days. On the other hand, DiPT and DnBT did not significantly enhance drug release, and release was very close to that of the PLA matrix alone.

Degradation behavior of PLA

The degradation behavior of PLA in phosphate buffer was investigated from the viewpoint of molecular weight (M_w) and weight loss of PLA by using PLA alone and PLA with DET. The decrease in M_w is shown in Figure 7, where the difference of the initial M_ws is caused by the difference of PLA lots. In the case of PLA alone, M_w was decreased to 58,800 within the time investigated (21 days), while M_w of PLA with DET decreased gradually to 69,700 within 21 days.

Weight losses of PLA alone and PLA with DET films are shown in Figure 8. After a rapid weight

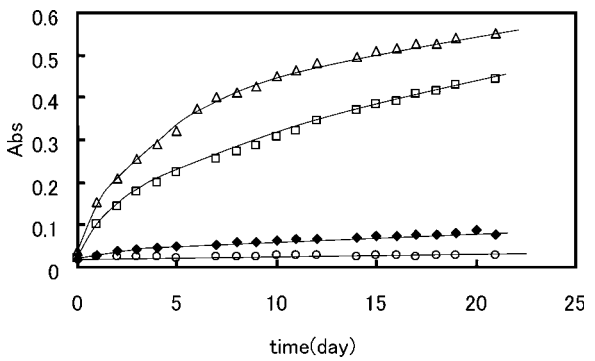

Figure 4 Dependence of drug release on DET content in PLA film DET content: ○ = 0 part, ◆ = 10 parts, □ = 20 parts, △ = 30 parts, Argatroban content = 20 parts, Data are given as the average of $n = 3$.

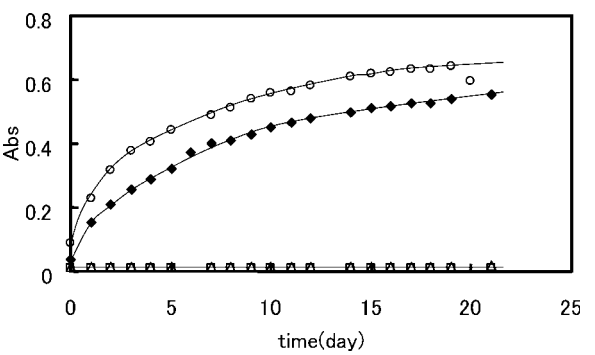

Figure 6 Effect of the alkyl chain length of tartrate on drug release Argatroban content = 20 parts, Additive content = 30 parts, ○ = DMT, ◆ = DET, □ = DiPT, △ = DnBT. Data are given as the average of $n = 3$.

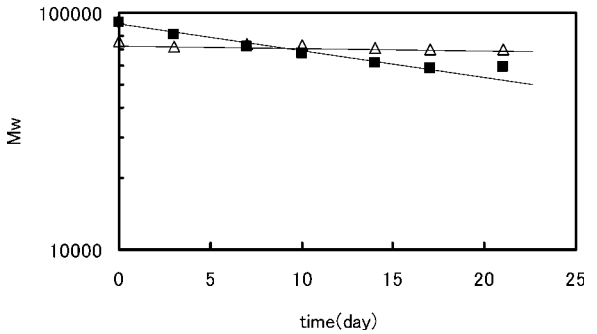

Figure 7 Change of molecular weight of PLA in PBS ■ = PLA alone, △ = PLA with 30 parts of DET, pH = 7.4, Temperature = 37°C.

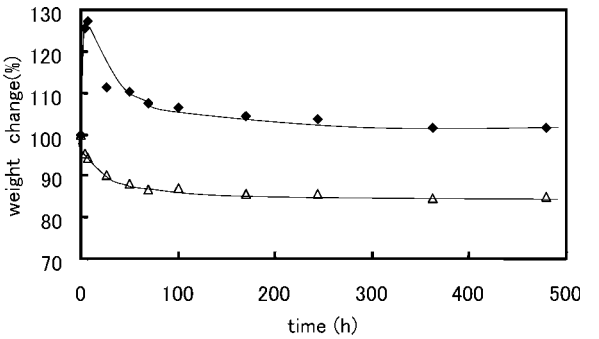

Figure 9 Effect of the additive on weight change of PLA film in PBS pH = 7.4, temperature = 37°C Argatroban content = 20 parts. Additive content = 30 parts, Additive: △ = TEC, ◆ = DET.

decrease at the initial stage, the weight losses of PLA alone and PLA with DET decreased gradually to 7 and 22 wt %, respectively.

Weight change of PLA films in PBS

The weight change of PLA films with drug and additive are shown in Figure 9, where DET or TEC added to PLA is represented. The weight change was caused by elution of additive, release of drug, and water uptake. The weight of DET-added PLA film increased dramatically to 130% in 24 h, then decreased and reached a plateau (∼ 100%). The weight of TEC-added PLA film decreased slightly to 85 wt % of the initial weight according to time.

DISCUSSION

Drug status in PLA film

The finding that X-ray diffraction analysis of PLA film containing both argatroban and DET did not show any crystalline peaks (Fig. 2) indicates that the drug was not dispersed as a crystalline particle in the matrix.

Effect of hydroxylesters on drug release from PLA film

The effect of the additive species (DET, DEM, and TEC) on drug release was compared by using films containing 30 parts of the additive and 20 parts of argatroban. The results in Figure 3 show that only DET enhanced drug release and that the released amount from DET-added film was 50 times greater than that from DEM- and TEC- added films at day 21. In the film containing DEM or TEC, enhancement of drug release was hardly detectable during the observation time except for an initial small burst of drug release, and the release profile was very close to that of PLA film without additive. To understand the role of DET, we focused our attention on DET and other tartrates as additives which might enhance the release of the drug; this is discussed in the next section.

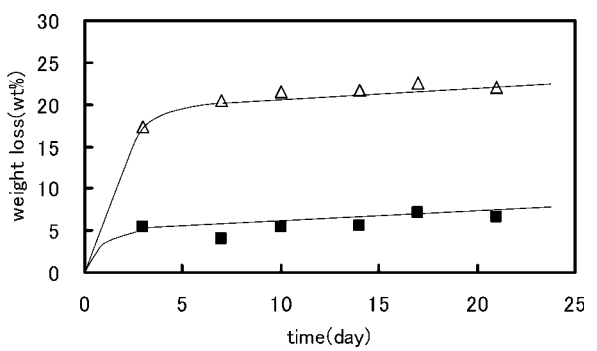

Figure 8 Weight loss of PLA films in PBS ■ = PLA alone, △ = PLA with 30 parts of DET, pH = 7.4, temperature = 37°C.

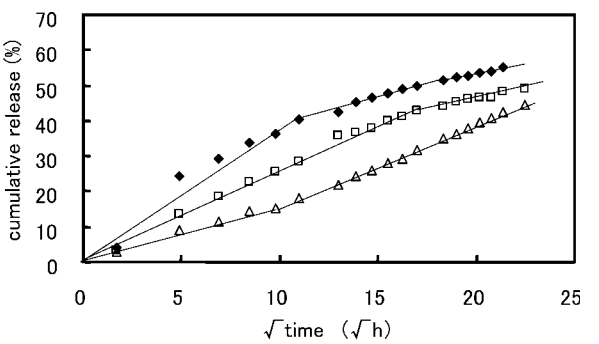

Figure 10 Relationship between the cumulative release of drug and the square root of time. Argatroban content: ◆ = 10 parts, □ = 20 parts, △ = 30 parts, Additive content = 30 parts of DET, Data are given as the average of $n = 3$.

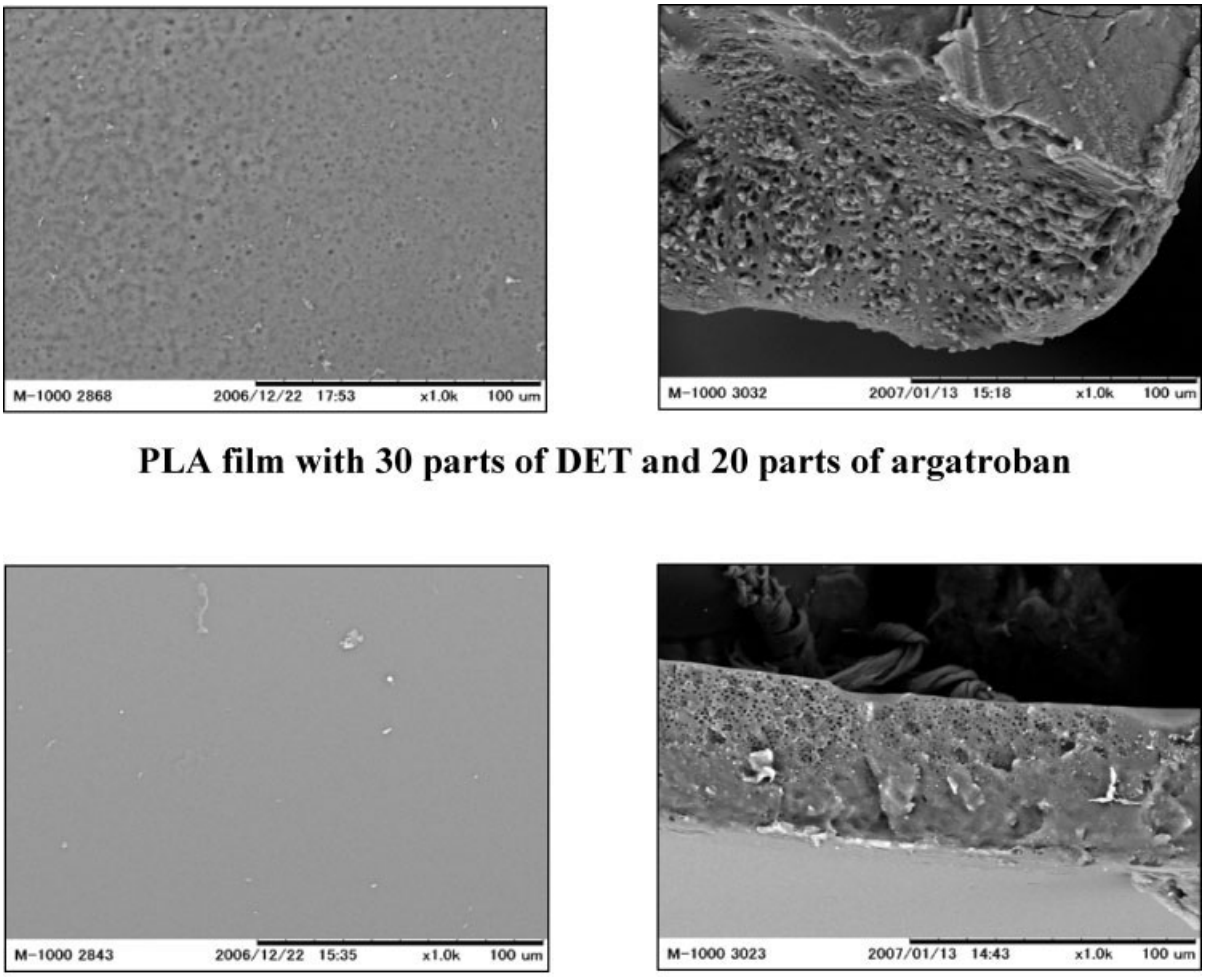

Figure 11 Scanning electron micrograph of PLA films. After immersion in PBS at 37°C for 21 days. left: surface, right: cross section.

Factors affecting drug release in tartrate-added films

The effect of DET content on the release of the drug was investigated by varying DET content from 0 to 30 parts. The results shown in Figure 4 clearly indicate that drug release is enhanced when DET content is increased, especially, when more than 20 parts of DET is added to the PLA matrix, drug release is most effective.

The effect of argatroban content (10, 20, and 30 parts) on the release itself is discussed taking the example of the content of DET in the film as 30 parts. The results shown in Figure 5 suggest that drug release was more effective when the content of argatroban was more than 20 parts. When argatroban content was less than 20 parts, the release rate tended to decrease according to time. This decrease in drug release was not caused by depletion of drug in the film, as Figure 10 representing cumulative release versus time$^{1/2}$, shows that more than 50% of the drug remained in the matrix at day 21. Figure 10 also shows that the film containing 30 parts of drug, released the drug continuously at a high rate for 21 days.

The effects of alkyl chain length of the tartrate on drug release was studied by using DMT, DET, DiPT, and DnBT. The findings in Figure 6 that the significant enhancement of drug release by DMT and DET compared with DiPT and DnBT indicate that the alkyl chain length markedly affects the release profile. One of the reasons for the effect of the alkyl chain length may be the solubility of the additives in water. As shown in Table I, DMT and DET are miscible in water, while DiPT and DnBT are not.

Mechanism of drug release enhancement

The reason for the enhancement of drug release by DET is discussed. It is understood that the release of drugs from a polymer matrix is generally due to the decomposition of the matrix and/or drug diffusion.[17,18]

The effect of the matrix decomposition is described on the basis of molecular weight change and weight loss. In the cases of PLA alone and PLA with DET, drastic degradation was not observed as shown in Figure 7. The gradual decrease in molecular weight of PLA alone was higher than that of DET-added PLA. This suggests that DET prevents the decomposition of the PLA molecule. As for the weight losses of PLA alone and PLA with DET films shown in Figure 8, the initial loss may be attributed to the release of low-molecular-weight PLA. When the loss of PLA with DET film was compared with that of PLA alone, the former was larger than the latter. This was attributed to the elution of DET from the PLA matrix in addition to the release of low-molecular-weight PLA. On the basis of these results, it is concluded that DET does not promote the degradation of PLA and thus the enhanced release of the drug is not due to degradation of the matrix.

It is well-known that in controlled release by the diffusion mechanism, a plot of released drug (M_t/M_o) against the square root of time ($t^{1/2}$) is linear [eq. (3)] and its slope (k) is proportional to the diffusion coefficient.[19–21]

$$M_t/M_o = k \times t^{1/2} \quad (3)$$

where M_t and M_o are the amounts of the released drug at time t and the initial loaded drug, respectively. The result of plotting M_t/M_o versus $t^{1/2}$ shown in Figure 10 indicates that the profiles for the films with 10, 20, and 30 parts of drug cannot be represented by a straight line. That is, the slope of the line is changed. Therefore, it is concluded that the release enhancement by DET is not controlled by simple pore diffusion but by a more complex mechanism.

The reason for the enhancement of drug release was next studied from the viewpoint of weight change (water uptake) in PBS and morphology of the PLA matrix by using DET-added and TEC-added films. The weight change of the films in PBS may be caused by elution of the additive, the release of drug and water uptake. As demonstrated in Figure 9, the profiles of weight change were very different. The weight of DET-added PLA film increased dramatically within 24 h up to 130% of the dry weight then decreased and finally reached a plateau (100%). This marked increase in weight at the initial stage may be explained by water uptake within the matrix, and the following decrease would be due to the release of low-molecular-weight PLA, DET, and drug from the matrix. On the other hand, the weight of TEC-added film decreased according to time and reached a plateau (85%) within a few days. This decrease was mainly due to the elution of TEC and low-molecular-weight PLA. Thus, this marked difference in water uptake between DET-added and TEC-added films can be attributed to the solubility of the additives in water. As DET is water-soluble, the matrix can absorb water, while the matrix with the poorly-soluble TEC does not absorb water.

The morphology of DET-added and TEC-added films were observed under SEM, and the micrographs of their surface and cross section (Fig. 11) showed the following: the film containing DET had open pores at the surface and a porous structure in cross section, while the film with TEC has no open pores at the surface although it had porous structure. Consequently, the open pores at the surface were associated with enhanced drug release. That is, through the open pore the drug will diffuse to PBS.

We are currently investigating the reasons for enhancement of drug release by DET in terms of the elution of additive, water uptake, and SEM observations. These results will be reported elsewhere.

CONCLUSIONS

The addition of DMT or DET to PLA film significantly enhanced the release of argatroban when compared with the other additives investigated, TEC, DEM, DiPT, and DnBT. On the basis of the findings from water uptake studies and SEM observations of DET-added and TEC-added films, we concluded that the release enhancement of argatroban observed in DET-added film was attributable to the formation of open pores at the film surface caused by large amounts of water uptake within the film.

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