Controlled Release of Insulin from Plasma-Irradiated Sandwich Device Using Poly-DL-lactic Acid

Ichiro YAMAKAWA,* Sumio WATANABE,* Yoko MATSUNO* and Masayuki KUZUYA*

Tsukuba Research Laboratories, Eisai Co., Ltd.,* 1-3, Tokodai 5-chome, Tsukuba-shi, Ibaraki 300-26, Japan and Laboratory of Pharmaceutical Physical Chemistry, Gifu Pharmaceutical University,* 5-1, Mitahora-Higashi 5-chome, Gifu-shi, Gifu 502, Japan. Received July 1, 1992

The release behavior of insulin from a plasma-irradiated sandwich (PIS) device using poly-DL-lactic acid (PLA) was studied. The controlled release device can be obtained by oxygen plasma irradiation (radiofrequency discharge operating at 13.56 MHz) on the outer layer of the sandwich device which was fabricated from an insulin–PLA matrix tablet as a core material and a mixture of plasma-degradable polyoxymethylene (POM) and biodegradable PLA as a wall material. The release test indicated that insulin was released through the micropores formed by the vaporization of POM, and that the release behavior of insulin was affected largely by the molecular weight of PLA used as the outer layer rather than the plasma operational condition. The release of insulin can be controlled by the use of PLA with an average molecular weight of 11000 as the outer layer of the PIS device. Insulin from the PIS device maintained normal blood glucose levels for 10 d in diabetic rats as an implantable dosage form. The duration of insulin effectiveness was relatively short considering the degradation rate of PLA, indicating that the degradation characteristics of biodegradable PLA were not well reflected in the PIS device.

Keywords oxygen plasma irradiation; sandwich device; poly-DL-lactic acid; insulin; sustained release; implant

Introduction
Recently, cold plasma chemistry utilizing glow discharge under reduced pressure has received much attention as a method for manufacturing new and useful materials and improving the function of polymer surface. It has been reported as a pharmaceutical application of plasma chemistry that the sustained release of drugs were obtained by the use of plasma polymerizing, in which the direct exposure of drug substances to plasma gas may lead to the degradation of drugs. It is well known that a perfect, pin-hole free, thin film is produced by plasma polymerizing, and it was considered that a drug released through the polymerized film can be affected by a cracking and/or an imperfect part of the film which can not be predicted.

Kuzuya et al. developed a new sandwich device to avoid the direct exposure to plasma gas and obtain a zero-order release rate. A new sandwich type controlled release device can be obtained by oxygen plasma irradiation on the outer layer of the double-compressed tablets which were fabricated from a drug as a core material and a mixture of plasma-degradable polyoxymethylene (POM) and plasma-crosslinkable polystyrene (PST) as a wall material. The microporous structure of the outer layer was formed by the vaporization of POM with oxygen plasma irradiation. It was also found that drug release rates can readily be controlled by the selection of a variety of factors for tablet fabrications as well as plasma operational conditions. Furthermore, Kuzuya et al. reported that the new sandwich type controlled release device can be obtained by oxygen plasma irradiation on the outer layer consisting of biodegradable poly-DL-lactic acid (PLA) and POM under a low supply of power of 6 W.

Biodegradable polymer such as PLA have a long history of use in sutures, bone plates and prosthetic devices. The use of PLA as implantable pellets and injectable microspheres of various drugs which do not require removal when their delivery role is finished, has been studied during the past decade. Especially, sustained release dosage forms containing peptide drugs using PLA have been recently reported, and they often show initial rapid release phases of drugs which may cause side effects. In the previous report, a plasma irradiated sandwich (PIS) device containing theophylline did not show an initial rapid release phase, but a lag time phase as a reservoir type controlled release system. It was the purpose of this study to investigate whether the plasma-irradiated PLA device can be applied to an implantable dosage form or not.

In this paper, PIS devices containing insulin were prepared according to the plasma operational condition described previously, and their sustained release of insulin in a buffer solution was reported. Change in blood glucose levels after the subcutaneous implantation of the PIS device in diabetic rats was also examined.

Experimental
Materials Poly-DL-lactic acid (PLA) was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. The molecular weights of PLA used in the present study are represented as weight averages, and are designated as PLA5000, PLA11000 and PLA20400 for the PLA of molecular weights 5000, 11000, and 20400, respectively. PLA powders were triturated in an agate mortar with a pestle, and the fractions under a 200-mesh sieve were used.
Polyoxymethylene (POM) was synthesized according to the literature, and the fractions under a 60-mesh sieve were used.
Insulin crystals (bovine, 24.7 IU/mg) were purchased from Sigma Chemical Company, St. Louis, MO, U.S.A. All other chemicals used were of reagent grade.
Preparation of the Sandwich Device Insulin, or a mixture of insulin (250 U) and PLA powder, was compressed into a flat-faced tablet, 10 mm in diameter, at a pressure of 200 kg/cm² (core tablet). Subsequently, a mixture of PLA and POM as a wall material and a core tablet were compressed into a flat-faced tablet, 13 mm in diameter, at a pressure of 200 kg/cm² after reducing pressure for 5 min to form a sandwich structure, as shown in Fig. 1.

Plasma Irradiation Apparatus for plasma irradiation in this study was described previously. Briefly, samples were put on a glass-plate in a coil chamber to ensure homogeneous exposure to oxygen plasma gas by use of radio-frequency discharge operating at 13.56 MHz as shown in Fig. 2. Flow volume and pressure of oxygen gas for plasma-irradiation were controlled by the change of evaporating speed.

© 1993 Pharmaceutical Society of Japan
PLAs used in this study tended to melt by the plasma-irradiating heat because their softening points were relatively low. Therefore, operational conditions of plasma-irradiation were as follows: supplied power, 6 W; pressure, 0.5 Torr; flow rate of oxygen gas, 50 mL/min; plasma duration, 1–3 h.

**Release Test of Insulin from Plasma-Irradiated Sandwich Device** A release test in a buffer solution was performed as previously described. The sample was put into a flask with 100 mL of phosphate buffer containing 0.001% methyalkalwave at pH 7.4 and incubated at 37 ± 0.5 °C (50 U/min).

The released insulin was periodically determined by high performance liquid chromatography. Chromatography conditions were as follows: column, a Nucleosil CN (5 μm) column (150 mm×4.6 mm i.d.); a mobile phase, a mixture of acetonitrile and 1.2% perchloric acid aqueous solution in 35:65 by volume; flow rate, 1.0 mL/min; column temperature, 35 °C; detection wavelength, 220 nm.

**Change in Blood Glucose Levels in Diabetic Rats** Sprague-Dawley male rats weighing from 200 to 250 g were made diabetic with streptozotocin. Each animal received an intravenous injection of 50 mg/kg of streptozotocin dissolved in 0.005 M citric acid at pH 4.5.

Implantation was performed by making a 1.5-cm incision in the lower back area of the rat with a pair of scissors and creating a pocket in the subcutaneous tissue. The implant site was then closed with surgical sutures.

Blood samples were collected by needle puncture of the tail vein two or three times a week. Blood glucose was determined by the β-D-glucose oxidase method.

**Observation of Surface Morphology** The surface characteristics of the plasma-irradiated device were examined by the use of a scanning electron microscope (model ALPHA 30A TOPCON Co., Ltd., Tokyo, Japan). After the release test the wet samples were lyophilized before coating Au onto them.

**Biodegradation of PLA** PLA plates were prepared by a hot press method (PLA 100 mg, 8 mm in diameter). PLA plates were implanted subcutaneously into the lower back area of Sprague-Dawley male rats weighing from 200 to 250 g. Animals were sacrificed at various times and the plate was removed. After drying under reduced pressure for 24 h, the plate was weighed exactly and the molecular weights of the residual polymer were measured by a gel permeation chromatography (GPC) using a polystyrene standard. GPC conditions were as follows: column, Shodex® PAK GF-802 and GF-802.5 column (300×8 mm i.d.×2) (Showa Denko Co., Ltd., Tokyo, Japan); a mobile phase, tetrahydrofuran; flow rate, 1.0 mL/min; column temperature, 40 °C; detection, refractive index (Shodex® RI SE-61, Showa Denko Co., Ltd., Tokyo, Japan). Weight loss and change in molecular weight of the PLA plate were expressed as the percentages remaining (the percentages of initial value).

**Absorption of Water in PLA Plate** PLA plates were prepared by a hot press method (PLA 100 mg, 8 mm in diameter), and were incubated in phosphate buffer, pH 7.4 at 37 ± 0.5 °C. The outsides of the plates were wiped with tissue paper before the weighing.

**Results and Discussion**

**Observation of Surfaces of Outer Layers of the PIS Device** The surface of the plasma-irradiated outer layer (a mixture of PLA11000 and POM in 3:1 by weight as an outer layer) is shown in Fig. 3. Micropores caused by the vaporization of POM were seen on the surface of the outer layer, which was found to be smoother compared with that before plasma operation. The softening points of PLA used were relatively low (50–55 °C, measured by Wako), therefore, it is apparent that melt of PLA near the surface of the outer layer by the plasma-irradiating heat became
likely in spite of a low supply of power of 6 W in the apparatus for plasma irradiation.

**Release Test of Insulin from the PIS Device** The PIS devices containing insulin were prepared from insulin tablet (insulin 30 mg= ca. 750 IU, 10 mm in diameter) as a core material and a mixture of PLA and POM in 3:1 by weight as a well material (mixture 100 mg, 13 mm in diameter). Figure 4 shows release profiles of insulin in a buffer solution at pH 7.4 from the PIS device. Although the release of insulin from the device of plasma-duration for 3 h was almost finished within 4 h, insulin was released continuously with pseudo zero order release rate for 8 h from the device of plasma-duration for 2 h. However, insulin was scarcely released before the plasma operation. These results suggested that the release rates of insulin were controlled by oxygen plasma irradiation on the outer layer consisting of PLA and POM, as was the case with the PIS device containing theophylline. If the PIS device as a reservoir system combines a monolithic matrix core system, sustained release for longer periods can be achieved from the combined device.

**Choice of Molecular Weight of PLA Used as a Core Tablet** Figure 5 shows the release profiles of insulin from core tablets prepared with PLA as a matrix material in core tablets. Insulin tablets (insulin alone 20 mg, ca. 500 IU) were dissolved completely in phosphate buffer at pH 7.4 within 15 min. In contrast, insulin was released continuously for about 6 h from the matrix tablets consisting of PLAS00 and insulin in 1:1 by weight, whereas insulin was released completely within 2 h from the matrix tablet prepared with PLA11000 or PLA20400. The difference in the degradation of PLA depending on its molecular weight may not be related to the release behaviors of insulin shown in Fig. 5, because the degradation rates of PLA in a buffer solution were extremely smaller than the release rates of insulin from the core tablets. PLAS00 tended to soften so readily at room temperature that PLAS00 powder can be compressed into a tablet more densely than PLA11000 and PLA20400 powder, and thus the penetration of water into the PLAS00-insulin matrix tablet may eventually become slow. PLA5000 was chosen as the matrix material for a core tablet on the basis of the result of release test as shown in Fig. 5.

**Choice of Molecular Weight of PLA Used as an Outer Layer Material** Figure 6 shows the change in weight of the PIS device consisting of PLA and POM as the outer layer with plasma-duration. It has been reported that the degradation of PLA was very small under the condition of plasma irradiation, while POM vaporized quantitatively with an oxygen plasma duration time under the extremely low supply of power of 6 W. Weight loss of the PIS device caused by the vaporization of POM with plasma-duration was not affected by the molecular weight of PLA being present as a mixture in the outer layer. It can be considered, therefore, that an increase of micropores was not related to the molecular weights of PLA used as the outer layer.

Figure 7 shows the release profiles of insulin from the PIS device prepared with PLAS00 as the outer layer. All of the samples showed a lag time of 2—4 h and a steep increase of release rate at 6 h, and thus insulin was released completely within 24 h at all conditions of plasma-duration. Furthermore, a certain amount of insulin was released from blank samples before plasma operation, which may be attributed mainly to the swelling property of PLAS00 in
Fig. 7. Effect of Plasma-Duration on Release of Insulin from the PIS Device Prepared with PLA5000 as the Outer Layer
○, plasma-duration for 1 h; △, plasma-duration for 2 h; □, plasma-duration for 3 h; ●, before plasma operation.

Fig. 8. Effect of Plasma-Duration on Release of Insulin from the PIS Device Prepared with PLA11000 as the Outer Layer
○, plasma-duration for 1 h; △, plasma-duration for 2 h; □, plasma-duration for 3 h; ●, before plasma operation.

Fig. 9. Effect of Plasma-Duration on Release of Insulin from the PIS Device Prepared with PLA20400 as the Outer Layer
○, plasma-duration for 1 h; △, plasma-duration for 2 h; □, plasma-duration for 3 h; ●, before plasma operation.

Fig. 10. Increase of Weight of PLA in Phosphate Buffer, pH 7.4 at 37°C
Each point represents the mean of three experiments. ○, PLA5000; △, PLA11000; □, PLA20400; ●, POM.

a buffer solution as shown in Fig. 10. Many cracks on the surface of the outer layer prepared with PLA5000 were found after release tests as shown in Fig. 11. It may be considered that the microporous structure of the outer layer prepared with swellable PLA5000 was extremely fragile against the condition of the release test, and consequently insulin was released completely from the PIS device within 24 h.

Figure 8 shows the release profiles of insulin from the PIS device prepared with PLA11000 as the outer layer. Insulin was not released from blank samples. It was clearly shown that release rates of insulin were controlled depending on the plasma-duration when PLA11000 was used as the outer layer. Insulin was continuously released with pseudo zero order rate for 34 h after a lag time of 4 h from the device of plasma-duration for 3 h, and the release rate of insulin from it decreased after 34 h. Almost the same release rate of insulin was observed from the device of plasma-duration for 2 h, however, it decreased after 24 h in the release test. Less than 20% of contained insulin was released from the device of plasma-duration for 1 h.

Figure 9 shows the release profiles of insulin from the PIS device prepared with PLA20400 as the outer layer. Insulin was not released from blank samples. Less than 20% of containing insulin was released at all conditions of plasma duration in the release test.

The weight average/number average molecular weight ratios of PLAs measured by GPC were 1.60, 1.66 and 1.78 for PLA5000, PLA11000 and PLA20400, respectively, indicating that the molecular weight distribution of PLA used in this study was fairly sharp. Therefore, the difference in characteristics of PLA with different molecular weights may be reflected considerably in the release behaviors of insulin as shown in Figs. 7—9. Figure 10 shows the increase of weight of PLA in a buffer solution as an indication of swelling property. The absorbed amount of water in PLA5000 was extremely large in the phosphate buffer at pH 7.4, whereas a little amount of water was absorbed in PLA11000, PLA20400 and POM scarcely absorbed water (less than 2%) within 24 h. Figure 11 shows scanning electron photomicrographs of the surfaces of the PIS devices after release tests. Many cracks on the surface of the PIS device prepared with PLA5000 and POM as the outer layer may be attributed to a large swelling of PLA5000. In contrast, porous surfaces of the PIS device prepared with PLA11000 or PLA20400 seemed to be intact. Although
the formation of micropores (weight loss) in the PLA11000 outer layer with plasma-duration time was similar to those in the PLA20400 outer layer as shown in Fig. 6. The difference in release profiles of insulin was observed between PLA11000 and PLA20400 as shown in Figs. 8 and 9. It is possible that a little difference in the absorption of water between PLA11000 and PLA20400 would affect the penetration of water to the outer layer and cause the difference in the release behavior of insulin from the PIS device.

Change in Blood Glucose Levels after the Subcutaneous Implantation of the PIS Device

Figure 12 shows a change in blood glucose levels of diabetic rats receiving the PIS devices prepared with PLA11000 as the outer layer. Normal blood glucose levels were maintained for 10 d in the PIS device with a plasma-duration of 3 h. The release rate of insulin in the steady state from the device of plasma-duration of 3 h was about 5 IU/h which was calculated from the data from 4 to 34 h.

It has been reported that a non-degradable ethylene vinyl acetate pellet showing the insulin release rate of 2 IU/d lowered blood glucose levels for 26 d in rats, while biodegradable PLA implants showing the insulin release rate of 5–6 IU/h lowered blood glucose levels for 14–19 d. Although containing insulin of the PIS device was different from that of the previous PLA implant, its cumulative released amounts of insulin in a buffer solution for 72 h (plateau) agreed well with that in the previous PLA implant. The release behavior of insulin from the PIS device in a buffer solution showed a lag time phase followed by a steady state release phase at almost the same release rate as the previous PLA implants. In contrast, the duration of insulin effectiveness of the PIS device was shorter than that of the previous PLA implants although the diabetic state of rats used in this study was light compared to the diabetic state in the previous study. When the PIS device was recovered from the administrated site at 17 d post implantation, a fibrous capsule was found surrounding the administrated site. Visual examination of the sites, however, showed no apparent inflammation. The decline of insulin effectiveness may not be attributed to the catastrophic release of insulin from the PIS device based on the result that it apparently remained intact in the fibrous capsule at 17 d post implantation.

Figure 13 shows the biodegradation of PLA11000 and PLA5000 in the subcutaneous tissue of rats. A weight loss of PLA11000 and PLA5000 was relatively small within 10 d post implantation indicating that the biodegradation of PLA was not related to the release rate of insulin from the
PIS device in the subcutaneous tissue of rats. However, it may be considered that the physical properties of PLA, such as glass transition temperature \( T_g \), were changed by the initial rapid decrease of an average molecular weight of PLA in the subcutaneous tissue as shown in Fig. 11. Therefore, the microporous structure of PLA in the outer layer of the PIS device can be changed in the subcutaneous tissue post implantation, and the change in the microporous structure may eventually affect the release behavior of insulin.

In conclusion, the PIS device consisting of PLA and POM as the outer layer can be applied to an implantable dosage form in the subcutaneous tissue. The release profile of insulin in a buffer solution was affected largely by the molecular weight of PLA used as the outer layer. The duration of insulin effectiveness in diabetic rats was relatively short considering the degradation rate of PLA used as the outer layer, indicating that the degradation characteristics of biodegradable PLA have not been well reflected in the PIS device as yet. It was considered that the drug release from biodegradable polymer formulation proceeded by two mechanisms, namely first a diffusion-dependent release from the polymer matrix and subsequently, a polymer erosion-dependent release.\(^{11}\) If the erosion of PLA is available in the release behavior of insulin from the present PIS device, a longer duration of insulin effectiveness will be obtained. Intensive research on the relationship between the molecular weight of PLA used as the outer layer and the drug release in vivo from the PIS device will be further needed.

References and Notes
1) A part of this work was presented at the 7th Annual Meeting of the Japan Society of Drug Delivery System, Tokyo, July 1991.