Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Controlled Release of Lastet, an Anticancer Drug, From Poly(3-Hydroxybutyrate) Microspheres Containing Acylglycerols

Hideki Abe[a]; Yoshiharu Doi[a]; Yuji Yamamoto[b]
[a] Reseach Laboratory of Resources Utilization, Tokyo Institute of Technology, Midori-ku, Yokohama, Japan [b] Medical School, Yokohama City University, Minami-ku, Yokohama, Japan

To cite this Article Abe, Hideki , Doi, Yoshiharu and Yamamoto, Yuji(1992) 'Controlled Release of Lastet, an Anticancer Drug, From Poly(3-Hydroxybutyrate) Microspheres Containing Acylglycerols', Journal of Macromolecular Science, Part A, 29: 11, 229 — 235
To link to this Article: DOI: 10.1080/10101329208054587
URL: http://dx.doi.org/10.1080/10101329208054587

MACROMOLECULAR REPORTS, A29(SUPPL. 3), 229-235 (1992)

CONTROLLED RELEASE OF LASTET, AN ANTICANCER DRUG, FROM POLY(3-HYDROXYBUTYRATE) MICROSPHERES CONTAINING ACYLGLYCEROLS

Hideki Abe and Yoshiharu Doi*
Reseach Laboratory of Resources Utilization, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 227, Japan
Yuji Yamamoto
Medical School, Yokohama City University, Urafunecho, Minami-ku, Yokohama 232, Japan

ABSTRACT

Poly(3-hydroxybutyrate) (PHB) microspheres containing 10wt% of lastet, an anticancer drug, were prepared by a solvent-evaporation process. The release rate of lastet from PHB microspheres in vitro (in 0.01M phosphate buffer) was significantly increased by incorporating glycerol tristearate as an additive. The significant difference in the surface characteristics was found between two types of PHB microspheres with and without glycerol tristearate by the scanning electron micrographs. In the surface of PHB microspheres containing 25wt% of glycerol tristearate, large holes of about 5μm were detected together with small holes of about 1μm diameter. It has been proposed that lastet is released through a number of internal channels in PHB microspheres.

INTRODUCTION

A wide variety of bacteria synthesize optically active poly(3-hydroxybutyrate) (PHB) and accumulate it within cells as reserve material[1]. The isolated PHB is a biodegradable thermoplastic. The biodegradability of PHB has been evaluated by hydrolytic[2-5] and enzymatic[6-10] degradation processes. In addition, PHB has been found to exhibit good biocompatibility with no cytotoxic response[3,10,13]. Because of these properties, PHB has attracted much attention for biomedical applications such as drug delivery systems, surgical sutures, and implant materials.

Korsatko et al.[11-13] prepared PHB matrix tablets containing 7-hydroxyethyltheophyllin and studied the release rate of the drug from tablets in vivo (in mice). The degradation of PHB in animal tissue and the liberation of the drug over a period of 20 weeks were reported. Nakano et al.[14,15] prepared PHB microspheres containing aclarubicin and studied the release rate of the drug

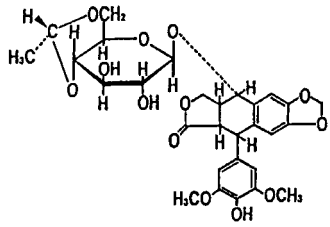

FIGURE 1. Structure of lastet, an anticancer drug.

from the microspheres in vitro (in phosphate buffer). The release rate of the drug was found to be significantly increased by incorporating ethyl or butyl esters of fatty acids into the PHB microspheres.

In this paper, we have prepared the PHB microspheres containing acylglycerols and an anticancer drug, lastet (Figure 1), and studied the morphologies, the hydrolytic degradation and the drug release of the PHB microspheres in vitro (in 0.01M phosphate buffer). The relation between the morphologies of microspheres and the release rate of drug is discussed.

EXPERIMENTAL

Poly(3-hydroxybutyrate) microspheres were prepared by a solvent-evaporation process. Prescribed amounts of PHB (150mg), lastet (40mg), and an acylglycerol (45mg) were dissolved in 25ml of methylene chloride. This solution was poured rapidly into 100ml of distilled water containing 0.5wt% of poly(vinyl alcohol) as a surfactant. The mixture was stirred at the rate of 1200 rpm with mechanical stirrer to form an emulsion. Stirring was then continued at room temperature for 120 min until methylene chloride had evaporated. PHB microspheres were collected by filtration, washed with distilled water, and dried in vacuo.

The diameters of PHB microspheres were measured by an optical microscope (Nikon OPTIPHOTO-2, Nikon MICROFLEX AFX-DX). The surface characteristics of PHB microspheres before and after drug release in vitro were observed with a scanning electron microscope (JEOL JMST 220) after gold coating of PHB microspheres using an ion coater.

PHB microspheres of 4mg were dissolved in 10ml of chloroform, and the concentration of lastet was determined from the UV absorbance at 284nm by using HITACHI U-2000 Spectrophotometer.

The release profiles of lastet from PHB microspheres were studied at 37°C in a 0.01M phosphate buffer (pH 7.4). About 10mg of PHB microspheres were immersed into a small bottle containing 30ml of phosphate buffer. This small bottle was shaken in a thermostat at 37±0.1°C. Small portions of solution were periodically removed

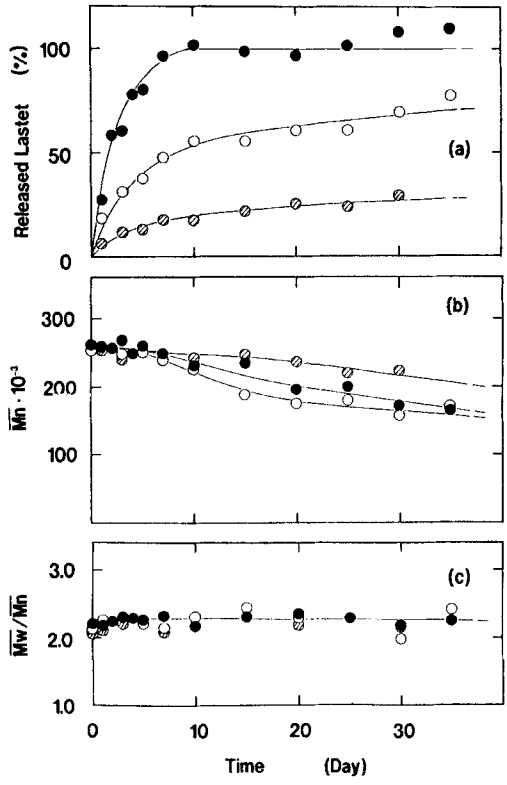

FIGURE 2. In vitro result of lastet release in 0.01M phosphate buffer (pH 7.4) at 37°C from PHB microspheres containing 10wt% of lastet and 25wt% of glycerol monostearate (○), 25wt% of glycerol tristearate (●), and without acylglycerol (⊘).
(a): Release profiles of lastet from PHB microspheres.
(b): Number-average molecular weights ($\overline{Mn}$) of PHB in microspheres.
(c): Polydispersities ($\overline{Mw}/\overline{Mn}$) of PHB in microspheres.

and separated PHB microspheres and buffer by filtration. The release of the drug into the phosphate buffer was determined by spectrophotometric assay of buffer at 284nm. The concentration of drug was calculated from a calibration curve of lastet in the phosphate buffer. PHB microspheres were washed with distilled water, dried in vacuo, and used for analysis.

All molecular weights of PHB samples were obtained at 40°C by using a Shimadzu 6A GPC system with a Shodex 80M column. Chloroform

was used as eluant at a flow rate of 0.5 ml/min, and sample concentration of 1.0 mg/ml was used. Polystyrene standards with a low polydispersity were used to make a calibration curve. The amounts of acylglycerols in PHB microspheres were determined by liquid chlomatography (LC) with a Shodex KF802 column in chloroform at 40°C.

The differential scanning calorimetry (DSC) data of PHB samples with an acylglycerol were obtained by a Shimadzu DSC-50 equipped with a cooling accessory under a nitrogen flow of 30 ml/min. The samples of 5 mg were encapsulated in aluminum pans and heated at 10°C/min from -100 to 200°C. The melting temperature (Tm) and enthalpy of fusion (ΔHm) were determined from the DSC endotherms of the first scan, while the glass-transition temperature (Tg) and crystallization temperature (Tc) were determined from a second scan. Tg was taken as the midpoint of the heat capacity change.

RESULTS AND DISCUSSION

Three different PHB microspheres of average diameter 20μm containing 10wt% of lastet were prepared. Two PHB microspheres contained 25wt% of glycerol monostearate and 25wt% of glycerol tristearate as an additive, respectively. The studies of lastet release from the PHB microspheres were carried out in a 0.01M phosphate buffer (pH 7.4) at 37°C.

Figure 2(a) shows the profiles of drug release in vitro. The release rate of lastet from PHB microspheres without acylglycerol was very small and only 25% of the loaded drug was released for 30 days. The drug release from PHB microspheres was accelated, when an acylglycerol was mixed with PHB and lastet. In the case of PHB microspheres containing 25wt% of glycerol monostearate, 55% of the loaded drug was released for 10 days. In addition, all of the loaded drug were released for 10 days from the PHB microspheres containing 25wt% of glycerol tristearate.

Figure 2(b) shows time-dependent changes in the number-average molecular weights ($\overline{Mn}$) of PHB during the drug release. The $\overline{Mn}$ values of PHB in microspheres containing only lastet decreased gradually from 260,000 to 220,000 with time by hydrolysis for 30 days. On the other hand, the $\overline{Mn}$ values of PHB in microspheres containing glycerol monostearate or glycerol tristearate decreased from 260,000 to 180,000 for 30 days. Thus, the molecular weight loss of PHB was accelated by the addition of acylglycerol.

As shown in Figure 2(c), the polydispersities ($\overline{Mw}/\overline{Mn}$) of PHB were unchanged in the range of 2.2±0.2 during the chain scission by hydrolysis, and the molecular weight distributions of PHB were unimodal. This result suggests that a random chain scission of PHB by hydrolysis proceeds throughout the whole polymer matrix.

The weights of PHB in microspheres were unchanged during the hydrolysis for 30 days, indicating that no polymer erosion occurs at 37°C. The amounts of acylglycerol in PHB microspheres remained unchanged during the hydrolysis for 30 days. These results indicate that only lastet is released from PHB microspheres in phosphate buffer at 37°C.

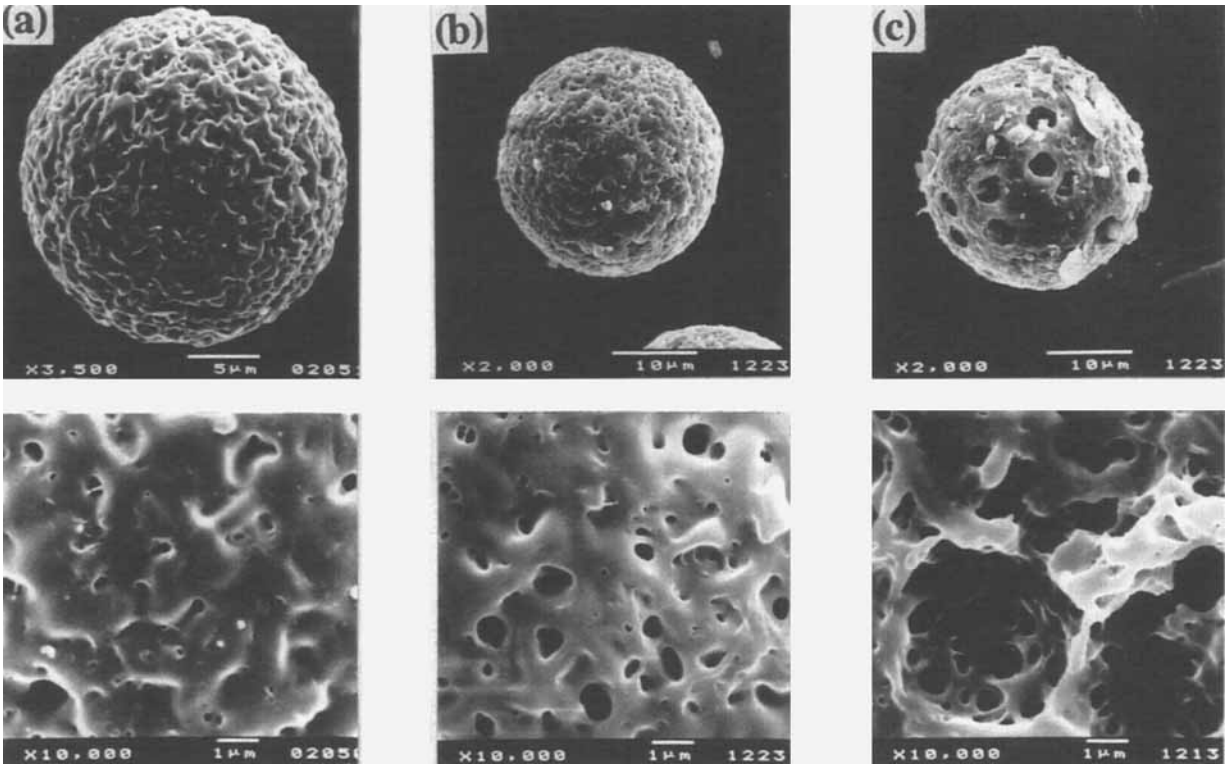

FIGURE 3. Scanning electron micrographs (SEMs) of PHB microspheres of average diameters 20μm, containing 10wt% of lastet (a), 10wt% of lastet and 25wt% of glycerol monostearate (b), and 10wt% of lastet and 25wt% of glycerol tristearate (c).

TABLE 1
DSC data of PHB samples containing glycerol monostearate (GMS) and glycerol tristearate (GTS)

Sample	T_g[a] (°C)	T_c[b] (°C)	T_m[c] (°C)	ΔH_m[d] (J/g)
PHB	4	48	177	111
PHB with GMS (10wt%)	-10	25	169	73
(25wt%)	-24	20	156	59
(35wt%)	-43	8	145	57
PHB with GTS (10wt%)	2	46	173	82
(25wt%)	3	47	175	72
(35wt%)	4	44	176	51

a) Glass-transition temperature
b) Crystallization temperature
c) Melting temperature
d) Enthalpy of fusion

Figure 3 shows the scanning electron micrographs (SEMs) of PHB microspheres before experiments of drug release. In the surface of PHB microspheres (a) containing only lastet, a number of small holes of about 1μm diameter are observed. These small holes may be formed upon evaporation of methylene chloride from PHB microspheres during the preparation. No significant difference in surface characteristics was found between PHB microspheres (b) with 25wt% of glycerol monostearate and those (a) without acylglycerol. In contrast, large holes of about 5μm diameter were detected together with small holes of about 1μm diameter in the surface of PHB microspheres (c) containing 25wt% of glycerol tristearate. These large holes were not observed in the PHB microspheres (a) and (b).

The differential scanning calorimetric (DSC) study was made to clarify the cause of different surface characteristics of PHB microspheres. Table 1 lists the DSC data of different PHB samples. The glass-transition temperature (Tg) of PHB was 4°C. The Tg values of PHB decreased from 4 to -43°C with increasing content of glycerol monostearate (GMS) in PHB from 0 to 35wt%, indicating that GMS is miscible with PHB, i.e., GMS and PHB molecules are mixed on molecular level in the amorphous PHB phase. In contrast, the Tg values of PHB samples containing glycerol tristearate (GTS) was 3±1°C, independently of the content of GTS in PHB, which indicates that GTS is immiscible with PHB. The DSC result suggests that the formation of large holes in the PHB microspheres containing GTS is caused by the immiscible blend of PHB with GTS.

The SEMs of PHB microspheres before and after drug release *in vitro* showed no appreciable changes in the size and surface of microspheres. Lastet may be released through a number of internal channels in PHB microspheres. In the case of PHB microspheres containing glycerol tristearate, the internal channels may be connected with large holes. Consequently, the release rate of lastet is enhanced and all of lastet in PHB microspheres are released.

REFERENCES

[1] Y.Doi, "Microbial Polyesters", VCH Publishers, New York (1990)
[2] S.J.Holland, A.M.Jolly, M.Yasin, B.J.Tighe, Biomaterials, 8, 289(1987)
[3] N.D.Miller, D.F.Williams, Biomaterials, 8, 129(1987)
[4] Y.Doi, Y.Kanesawa, Y.Kawaguchi, M.Kunioka, Makromol.Chem., Rapid Commun., 10, 227(1989)
[5] Y.Kanesawa, Y.Doi, Makromol.Chem.,Rapid Commun., 11, 679(1990)
[6] F.P.Delafield, M.Doudoroff, N.J.Palleroni, C.J.Lusty, R.Contoponlos, J.Bacteriol., 90, 1455(1965)
[7] H.Hippe, H.G.Schlegel, Arch.Microbiol., 56, 278(1967)
[8] T.Tanio, T.Fukui, Y.Shirakura, T.Saito, K.Tomita, T.Kaiho, S.Masamune, Eur.J.Biochem., 124, 71(1982)
[9] Y.Doi, Y.Kanesawa, M.Kunioka, T.Saito, Macromolecules, 23, 26(1990)
[10] T.Saito, K.Tomita, K.Juni, K.Ooba, Biomaterials, 12, 309(1991)
[11] W.Korsatko, B.Wabnegg, G.Braunegg, R.M.Lafferty, F.Strempfl, Pharm.Ind., 45, 525(1983)
[12] W.Korsatko, B.Wabnegg, H.M.Tillian, G.Braunegg, R.M.Lafferty, Pharm.Ind., 45, 1004(1983)
[13] W.Korsatko, B.Wabnegg, H.M.Tillian, G.Braunegg, R.Pfrgner, V.Walser, Pharm.Ind., 46, 952(1984)
[14] K.Juni, M.Nakano, M.Kubota, J.Controlled Release, 4, 25(1986)
[15] K.Juni, M.Nakano, M.Kubota, Chem.Pharm.Bull., 36, 333(1988)