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RESEARCH PAPER

Preparation and Characterization of Oil-in-Water Type Poly (D,L-Lactic Acid) Microspheres Containing Testosterone Enanthate

Satoru Tsubuku,[1,*] Shinya Sugawara,[1]
Masaharu Miyajima,[1] Masaru Yoshida,[2]
Masaharu Asano,[2] Kazuhiko Okabe,[3]
Daishiro Kobayashi,[3] and Hidetoshi Yamanaka[3]

[1]*Central Research Laboratories*
Zeria Pharmaceutical Co., Ltd.
Saitama, Japan, 360-0111
[2]*Department of Material Development*
Takasaki Radiation Chemistry Research Establishment
Japan Atomic Energy Research Institute
Gunma, Japan, 370-1292
[3]*Department of Urology*
School of Medicine
Gunma University
Gunma, Japan, 371-8511

ABSTRACT

Poly (D,L-lactic acid) (PLA) microspheres containing testosterone enanthate (ET) were prepared by using an oil-in-water (O/W) emulsion technique. The size distribution of the microspheres obtained could be explained by a log-normal distribution, and as a result, it was found that ET fully incorporates into microspheres even when the drug is loaded at up to 50%. On the other hand, the dissolution behavior of ET from microspheres was strongly dependent on particle size, suggesting that dissolution of the drug from microspheres can be easily controlled by controlling the preparative conditions.

* To whom correspondence should be addressed. Central Research Laboratories, Zeria Pharmaceutical Co., Ltd., 2512-1, Oshikiri, Kohnan-Machi, Osato-Gun, Saitama, Japan, 360-0111.

INTRODUCTION

Recently, many studies have been reported on the preparation of microspheres containing drugs, especially using biodegradable materials. Microspheres have been prepared for purposes such as stabilization, taste masking, and reduction of drug side effects (1,2). However, their most useful role is as drug-delivery vehicles for controlled drug release (2). For instance, patients receiving hormone treatment must take frequent doses of these drugs over long periods of time for treatment to be effective, because of the short half-life of hormone preparations. Several preparations for the controlled-release of implant-type microspheres have been developed to improve patient compliance and quality of life (3).

Various materials are used in the production of microspheres. In particular, poly (D,L-lactic Acid) (PLA) (4), copoly (D,L-lactic/glycolic acids) (5), and albumin (6) have often been applicable to the preparation of long-acting implant forms of drugs because of their high biodegradability and biosuitability (2,7,8).

Recently, we reported the clinical effect of an implantable form of biodegradable microsphere containing a drug used in the field of urology (9). We confirmed that testoster one was continuously released into the blood from these microspheres, with good efficacy in the prostate glands of castrated rats (10).

In order to greatly prolong the clinical drug effect in the present study, we attempted to prepare PLA microspheres containing testosterone enanthate (ET), a lipophilic ester of testosterone with a longer clinical effect than testosterone. We estimated the influence of preparative conditions on the physicochemical characteristics of PLA microspheres, such as their drug-incorporating efficacy, morphology, size distribution, thermal properties, and dissolution properties in vitro.

It is well known that the particle size and size distribution of microspheres affects their drug-dissolution property (11). It is important for microspheres to be of uniform size when they are to be administered by injection; moreover, uniform size is also an important factor in the maintenance of product quality and economic manufacture. For these reasons, the normality of particle size distribution and the correlation between particle size and dissolution behavior was evaluated in this study.

MATERIALS AND METHODS

Materials

Testosterone enanthate (ET; MW = 400.6) was purchased from Wako Pure Chemical Ind. (Osaka, Japan). An aqueous solution of D,L-lactic acid (LA) was purchased from Sigma Chemical Company (St. Louis, MO). Polyvinyl alcohol (PVA; degree of polymerization- 500; 86.5–89.0% hydrolyzed) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Polysorbate 80 was supplied by Nikko Chemicals Co. (Tokyo, Japan). All other chemicals were of reagent grade. Deionized distilled water was used throughout the study.

Preparation of PLA

PLA was synthesized by direct polycondensation at 200°C for 20 hr, without the use of catalyst, by bubbling nitrogen gas through a 90% aqueous solution of LA at a rate of 200 ml/min (12). The number average molecular weight (Mn), weight average molecular weight (Mw), and molecular-weight distribution (Mw/Mn) of the PLA obtained were determined with a Waters ALC-244 high-performance liquid chromatograph (Waters Associate, Milford, MA) at 25°C at a flow rate of 1 ml/min, using 10^2 Å, 10^3 Å, and 10^4 Å Waters ultrastragel columns in tetrahydrofuran as an eluent. These respective values were found to be Mn = 5100, Mw = 11,730 and Mw/Mn = 2.3.

Preparation of Microspheres

The formulation and schematic diagram of microsphere preparation are shown in Table 1 and Fig. 1, respectively. As shown in Fig. 1, ET and PLA were previously dissolved in the solvent, and the solution obtained was added to the PVA solution. This mixture was stirred with a mixer (Scroller, SCR-110, Iuchi, Osaka, Japan) at 25°C until the solvent vaporized. Microspheres were obtained by centrifugation and resuspended in fresh water. This procedure was repeated 5 times. The microspheres were then dried by lyophilization.

Determination of ET Concentrations in Microspheres

The amount of ET actually trapped in microspheres was assayed spectrophotometrically at 242 nm (UV-240 spectrophotometer, Shimadzu, Tokyo, Japan) in a 50% ethanol solution containing 5 mg of microspheres.

Observation of Microspheres

The morphologic characteristics of lyophilized microspheres were observed by scanning electron microscopy (SEM; JEOL JXA-733, Tokyo, Japan).

Table 1

Formulations and Preparative Conditions of Microspheres

No.	PLA20 (g)	Solvent Kind	Solvent ml	ET (g)	PVA (g)	Water (ml)	Stirring Rate (rpm)
1			20				
2	2	CH_2Cl_2	10	1.0	2.0	200	400
3			30				
4				0.2			
5	2	CH_2Cl_2	20	0.5	2.0	200	400
6				1.5			
7				2.0			
8							200
9	2	CH_2Cl_2	20	1.0	2.0	200	800
10							1000
11					0.0		
12					0.4		
13	2	CH_2Cl_2	20	1.0	1.0	200	400
14					6.0		
15					10.0		
16	2	$CHCl_3$	20	1.0	2.0	200	400

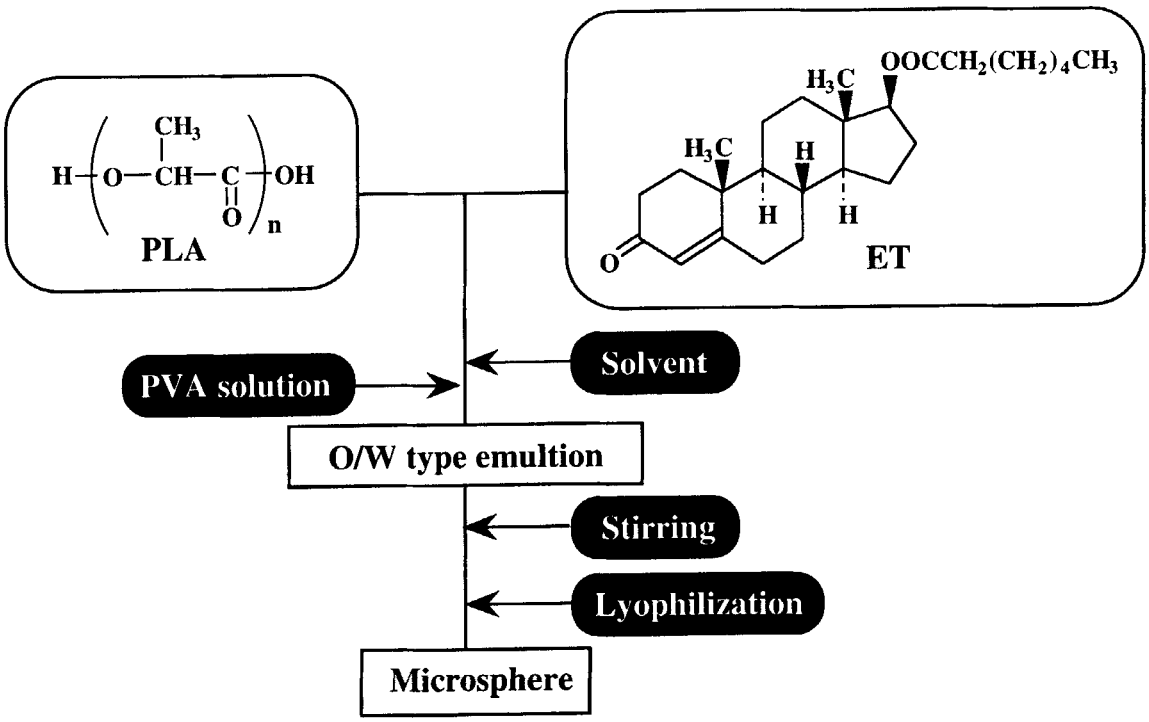

Figure 1. Schematic diagram for preparation of microspheres.

Particle-Size Analysis

Particle size was measured with a laser diffraction particle-size analyzer (LMS-24, Seishin Enterprise Co., Ltd, Tokyo, Japan). Before measurement, microspheres were dispersed in water. Microsphere size was assumed to be log-normally distributed. The log-normal distribution is described in equation (1) (13).

$$F(\ln d) = \frac{\sum n}{\ln \sigma \sqrt{2\pi}} \exp\left\{-\frac{(\ln d - \ln dg)^2}{2 \ln^2 \sigma_g}\right\} \quad (1)$$

Median diameter was determined as the microsphere diameter equivalent to 50% of the cumulative volume % of microspheres $[F(\ln d)]$, plotted as a function of the logarithm of microsphere diameter (d). Uniformity of microsphere diameter is expressed as σ_g. As calculated from equation (2), σ_g is the geometrical standard deviation of microsphere diameter.

$$\sigma_g = \frac{F(\ln d)_{84\%}}{F(\ln d)_{50\%}} \quad (2)$$

where $F(\ln d)_{84\%}$ is the diameter of a microsphere equivalent to 84% of $F(\ln d)$, and $F(\ln d)_{50\%}$ is the diameter of a microsphere equivalent to 50% of $F(\ln d)$, respectively.

Thermal Analysis of Microspheres

Thermal analyses of ET, PLA, and prepared microspheres were done with a differential scanning calorimeter (DSC-3100, Mac Science Co., Ltd, Tokyo, Japan) to evaluate the change in glass-transition temperature between the raw materials and prepared microspheres.

Drug-Release Study of Microspheres

A study of ET release from microspheres was done according to the second method for the Japanese Pharmacopoeia dissolution test. Microsphere samples (containing 120 mg of ET) were added to 900 ml of phosphate buffer (pH 7.2) containing Polysorbate 80 (0.1%), and were rotated with a paddle (100 rpm) at 37°C. The procedure was done in shade because ET is decomposed by light. Portions of the solution of 5-ml were withdrawn periodically, and the same volume of fresh medium was added to the test medium. The ET concentration in the dissolution medium was assayed spectrophotometrically at 242 nm. The release rates of ET were determined in duplicate.

Measurement of Microsphere Surface Area

Microsphere surface area was determined by the adsorption/desorption method, using nitrogen as the carrier gas (SA3100, Coulter, Tokyo, Japan).

RESULTS AND DISCUSSION

Incorporating Efficacy of ET

The efficacy of drug incorporation into microspheres has been reported to be greatly affected by such preparative conditions as the stirring rate, concentration of emulsifing agent, and kind or amount of solvent (14), as well as the solubility of the drug in the solvent used when microspheres are prepared using the oil-in-water (o/w) solvent evaporation method. The incorporating efficacy with hydrophilic drugs was reported to be much lower than that with lipophilic drugs (15).

As shown in Table 2, ET concentrations above 90% were loaded into all of the microspheres prepared in this study. No difference in incorporating efficacy was found with dichloromethane versus chloroform as solvents. This was due to high lipophilicity and solubility of ET in the solvent used (16). Therefore, a high incorporating efficacy was obtained under every condition used in the study.

Morphology of Microspheres

The morphology of microspheres is well known to be affected by the physicochemical characteristics of individual drugs, their amounts, and the preparative conditions (14). Kishida et el. reported that an irregular microsphere shape or porous surface depend strongly on drug concentrations (17). Also, in our previous study, microspheres prepared with dichloromethane and chloroform as solvents had different shapes and surface characteristics (10). As shown in Figs. 2 and 3, the microspheres obtained in this study had regular and spherical shapes and smooth surfaces, except when 10 g of PVA was used. In this last case, the microspheres aggregated during lyophilization.

Size of Microspheres

Size distribution parameters for the microspheres are shown in Table 2. Good linearities were obtained from log-normal size distribution plots, indicating that particles conformed to log-normal distribution under all preparative conditions. As described later, the median diam-

Table 2

Results of Incorporating Efficacy and Size Distribution Analysis

No.	Incorporating Efficacy			Size-Distribution Analysis		
	Theoretical (%)	Analytical (%)	Trap (%)	D_{50}(μm)	σ_g	R^2
1	33.3	30.0	90.1	82.6	1.44	0.959
2	33.3	32.4	97.3	73.6	1.52	0.941
3	33.3	32.0	95.9	111.4	1.36	0.992
4	9.1	8.2	90.6	72.5	1.40	0.986
5	20.0	19.0	95.0	66.7	1.67	0.976
6	42.9	41.5	96.9	68.4	1.63	0.981
7	50.0	48.6	97.2	84.7	1.46	0.981
8	33.3	30.9	92.7	180.8	1.46	0.925
9	33.3	31.3	93.8	37.6	1.55	0.989
10	33.3	30.7	92.1	30.9	1.60	0.967
11	33.3	31.4	94.2	323.4	1.61	0.980
12	33.3	31.8	95.4	65.2	1.45	0.985
13	33.3	32.3	96.9	68.3	1.34	0.999
14	33.3	31.4	94.2	39.5	1.50	0.961
15	33.3	30.9	92.6	—	—	—
16	33.3	30.5	91.6	63.6	1.39	0.996

Numbers refer to the formulation and preparative condition given in Table 1.

eter of microspheres varied with the preparative conditions. On the other hand, the σ_g value did not vary with different preparative conditions, indicating that the width of the size distribution was almost the same for all conditions.

Effect of Stirring Rate

Scanning electron micrographs of microspheres prepared at 200, 400, and 1000 rpm are shown in Figs. 2(a), 2(b), and 2(c), respectively. Particle size decreased as stirring rate increased. This tendency was also seen in microsphere size distributions, as shown in Fig. 4. With regard to median diameter (D_{50}), there was little difference between that for 800 rpm and 1000 rpm, but the diameter at 200 rpm was about 5 times as large as at 1000 rpm. This result was attributed to the high shearing force caused by mixer propeller rotation in the O/W emulsion. These findings agreed with those in other studies, showing a smaller microsphere size with a higher stirring rate (18).

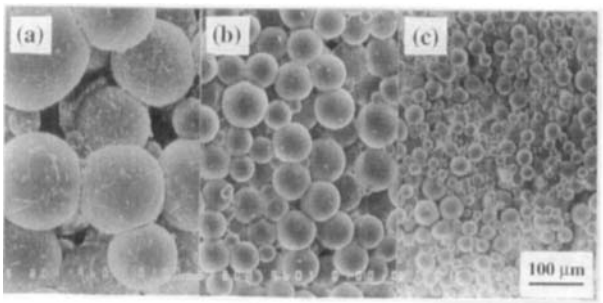

Figure 2. SEM photographs of microspheres prepared as a function of stirring rate. (a) 200 rpm (No.8 in Table 1). (b) 400 rpm (No.1 in Table 1). (c) 1000 rpm (No.10 in Table 1).

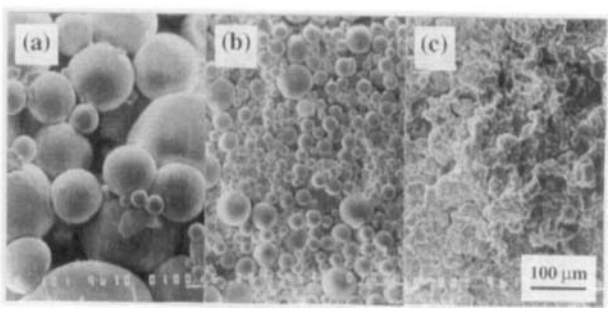

Figure 3. SEM photographs of microspheres prepared as a function of PVA concentration. (a) 0 g (No.11 in Table 1). (b) 6 g (No.14 in Table 1). (c) 10 g (No.15 in Table 1).

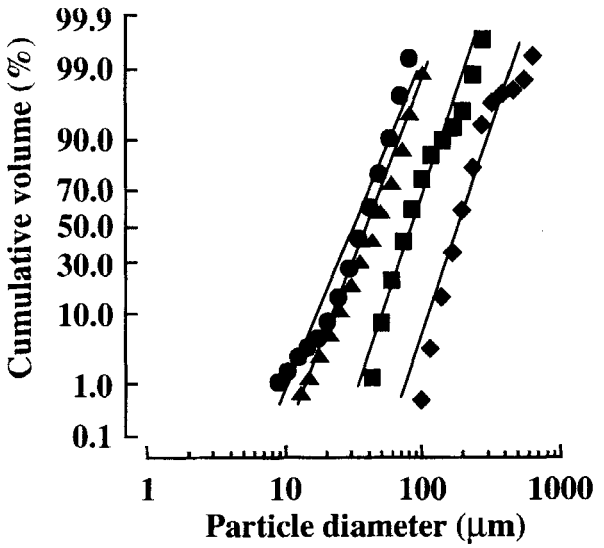

Figure 4. Effect of stirring rate on the size distribution of microspheres. (◆) 200 rpm; (■) 400 rpm; (▲) 800 rpm; (●) 1000 rpm.

Effect of PVA Content

Scanning electron micrographs of microspheres prepared with 0 g, 6 g, 10 g, and 2 g of PVA are shown in Figs. 3 (a), 3 (b), and 3 (c) and Fig. 2 (b), respectively. Log-normal plots are shown in Figs. 4 and 5. The median microsphere diameter decreased with increasing PVA content. When 10 g of PVA was added, microspheres formed aggregates during the lyophilization. On the other hand, microspheres prepared without PVA were able to have large median diameters. These results indicated that microsphere size was strongly influenced by the content of PVA added. As some previous investigators reported (18,19), this was considered to be caused by the smaller microsphere size with a decreasing surface-tension in the O/W emulsion as a result of the increased content of emulsifing agent such as PVA.

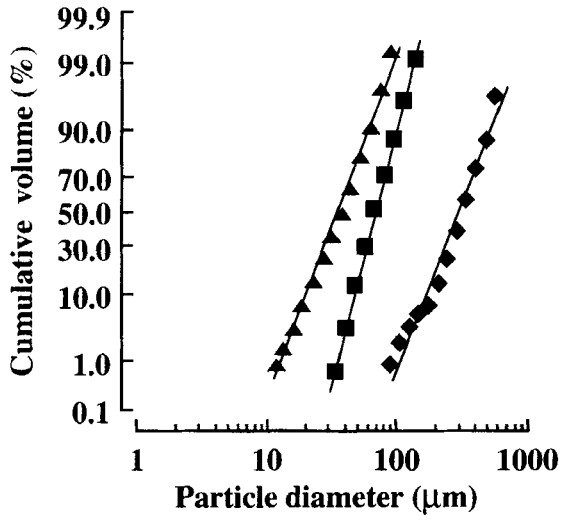

Figure 5. Effect of PVA concentration on the size distribution of microspheres (◆) 0%;(■) 0.5%;(▲) 3.0%.

Effect of Drug Concentration and Solvent

ET concentrations of up to 50%, and the kind of solvent used, had little influence on median microsphere diameter. It has been found in some studies that the effect of ET concentration and solvent on microsphere size distribution was caused by interfacial tension between the water phase and the oil phase of O/W emulsions (20,21). Our results confirmed that the slight differences in median diameter found with our preparative conditions were attributable to this difference in interfacial tension.

On the other hand, the amount of solvent had a fair degree of influence on median microsphere diameter, in that microspheres prepared with 10 ml of solvent had a larger median diameter than those prepared with 20 ml or 30 ml. This result accorded with the results of previous studies (21,22), in that median diameter increased as solvent amount decreased. Those studies reported that the increases of median microsphere diameter were caused by the increases of solvent viscosity resulting in the formation of larger droplets.

Thermal Analysis of Microspheres

The results of thermal analyses for ET, PVA, and microspheres are shown in Fig. 6. Both ET and a mixture of ET and PLA exhibited endothermic peaks at around 37°C due to the melting of ET. However, microspheres prepared with or without PVA, and those prepared with dichloromethane or chloroform as the solvent, showed no peaks originating from ET. Furthermore, no peak of this type was found in microspheres with ET concentrations ranging from 9% to 50% and stirring rates ranging from 200 rpm to 1000 rpm. Despite the greater than 90% incorporating efficacy for ET, no ET-related thermal peak was observed. This suggested that ET in microspheres exists in an amorphous state. In a previous study

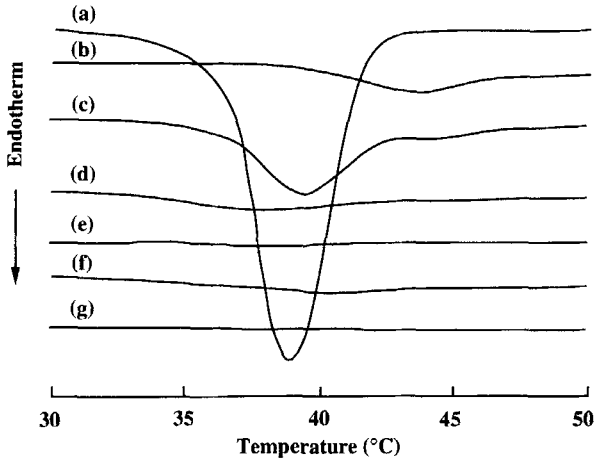

Figure 6. DSC curves. (a) Bulk ET; (b) bulk PLA; (c) bulk mixture of ET and PLA (1:2) (d) No.7 in Table 1; (e) No.10 in Table 1; (f) No.1 in Table 1; (g) No.16 in Table 1.

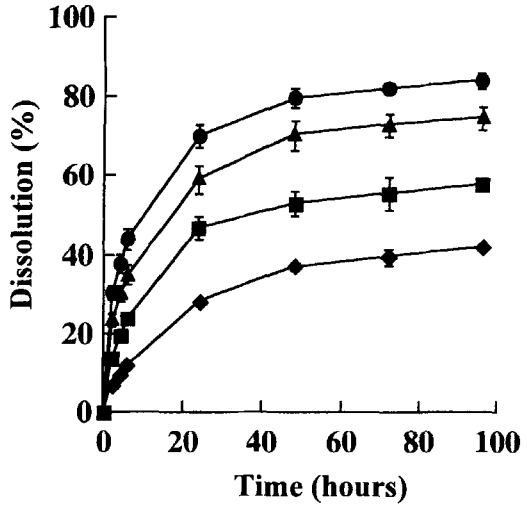

Figure 7. Effect of stirring rate on the dissolution of ET. (◆) 200 rpm; (■) 400 rpm; (▲) 800 rpm; (●) 1000 rpm.

(10), we found that testosterone-loaded microspheres exhibited no peak when chloroform was used as the solvent, but exhibited a clear peak when dichloromethane was used. We confirmed in this manner that testosterone crystallinity differs greatly with the kind of solvent used. We also confirmed that this difference influenced the clinical effect on rats given microspheres in vivo. The difference between ET and testosterone in thermal analysis was attributable to drug solubility in the solvent used, as described previously.

Dissolution Study of Microspheres In Vitro

Dissolution profiles of ET from microspheres prepared with different stirring rates are shown in Fig. 7. ET dissolution rates from microspheres prepared at low stirring rates were lower than from those prepared at higher stirring rates. As described above, several stirring rates yielded different particle sizes of microspheres. On the other hand, good correlation was found between microsphere median diameter and surface area per gram of microspheres, as shown in Fig. 8. It was confirmed that different microsphere surface areas in the dissolution medium caused differences in ET dissolution rates. In this way, microsphere particle size, instead of surface area, can be simply considered a parameter governing microsphere dissolution. In order to estimate this relationship for microspheres with the same ET concentration, dissolution percentage at 24 hr was plotted against median microsphere diameter (Fig. 9). Dissolution percentage at 24 hr showed a tendency to decrease exponentially as median particle diameter increased. The same tendency was also found in the dissolution percentage at 72 hr in plots of median particle diameter (data not shown). We therefore concluded that median microsphere diameter was highly correlated with the dissolution property of ET from microspheres.

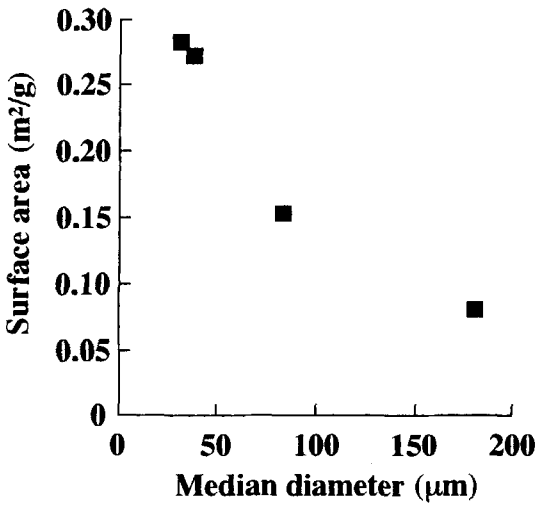

Figure 8. Relationship between the median diameter and surface of microspheres as a function of stirring rate.

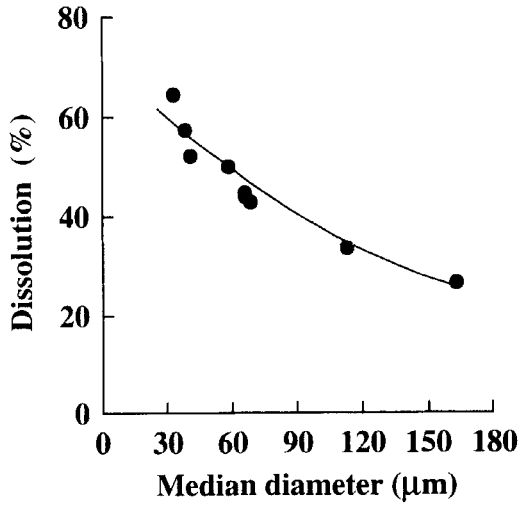

Figure 9. Relationship between the median diameter and dissolution of ET after 24 hr.

CONCLUSION

The physicochemical characteristics of microspheres containing ET and prepared under several conditions were investigated in this study. With regard to drug-incorporating efficacy, ET contents of greater than 90% were loaded into all microspheres prepared in the study. Depending on the mixer stirring rate, microspheres with different median diameters were prepared. The size distribution of these microspheres appeared to closely follow a log-normal distribution. As for geometric standard deviation, the width of the size distribution of the microspheres was highly uniform regardless of the preparative conditions. However, even though the microspheres could form without PVA, it was impossible to control particle size appropriately under this latter condition. On the other hand, microspheres aggregated during lyophilization with excess PVA. From these results we concluded that an appropriate PVA content existed during microsphere preparation. No difference was indicated in any characteristic of microspheres prepared using dichloromethane or chloroform, in contrast to the results with loading of testosterone. In the dissolution study, good correlation was found between the median particle diameter of microspheres and ET dissolution percentage, indicating that ET-loaded PLA microspheres can be easily prepared by the O/W emulsion technique (i.e., a solvent-evaporation process).

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