

Preparation of Poly(lactic acid) Microspheres Containing Anti-cancer Drugs

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Biodegradable poly(L-lactic acid) and poly(D, L-lactic acid) microspheres incorporating anti-cancer drugs, adriamycin and cisplatin, were prepared by the solvent evaporation method. Effects of several variables on the drug release were studied and it was found that the drug distribution in the matrix was the most important, which in turn depended on the solubility of the drug in the matrix polymer. Release rates of the drug could be altered to some extents by changing the drug loading, the molecular weight and chemical composition of the matrix polymer, and the drug/polymer mixing method. Especially, the mechanical mixing could greatly reduce the drug burst seen in an early stage of the release.

KEY WORDS: Poly(lactic acid)/ Anti-cancer drugs/ Microsphere/ Solvent evaporation method/ Drug delivery system/

INTRODUCTION

In connection with achievement of maximum bioavailability of conventional drugs with minimum side-effects, new drug delivery systems (DDS) have attracted much attention.¹⁾ Controlled or sustained release of drugs is one of these concepts. To develop the systems, polymeric materials probably are essential as matrix which slowly releases the drug incorporated in its interior.

We have been studying DDS using the polylactides which include poly(lactic acid), poly(glycolic acid), and their copolymers. These polymers belong to the most well-known biodegradable polymers which are first hydrolyzed without enzymes and then metabolized in the body.²⁾ They have been used as surgical sutures and have been proved to be nontoxic.³⁾ Moreover, their degradation rate can be regulated by changing their molecular weight, chemical composition, and crystallinity. Therefore, polylactides seem to be very promising as matrix for DDS. Indeed, a large number of studies which utilize these polymers have been intensively done in the DDS field in recent years,⁴⁻⁸⁾ but their basic data and informations for drug release are insufficient from the viewpoint of polymer science.

The present work was carried out in an attempt to prepare poly(lactic acid) microspheres containing anti-cancer drugs with different physical properties.

EXPERIMENTAL

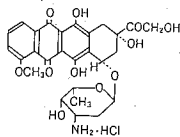
Materials

Doxorubicin hydrochloride (adriamycin, ADR) and cis-dichlorodiamine platinum

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(II)(cis-platin, CDDP) were donated by Kyowa Hakko Co., Tokyo, Japan, and Nihon Kayaku Co., Tokyo, Japan, respectively, and used without purification. Their chemical structures, solubility in water and methylene chloride, and molecular weight are represented in Table 1.

Table 1. The Physical properties of drugs used.

	Adriamycin (ADR)	Cisplatin (CDDP)
		
solubility in water	good	1 mg/ml
solubility in CH ₂ Cl ₂	2-3 µg/ml	negligibly small
molecular weight	579.99	300.05

Poly(lactic acid)s were synthesized by polycondensation of L-lactic acid or D,L-lactic acid at 180°C under a reduced pressure without any catalyst. Poly(lactic acid)s of different molecular weights were obtained by changing the polymerization time. For removal of the monomers, the oligomeric products were dissolved in methylene chloride or methanol, followed by precipitation with methanol or water, respectively. The weight average molecular weights of the oligomers were determined by gel permeation chromatography (GPC) using standard polystyrenes. Other chemicals were of guaranteed grade and used without further purification.

Microsphere Preparation

The microspheres containing ADR or CDDP were prepared by the solvent evaporation method.⁹⁾ The schematic presentation is given in Figure 1. Briefly, one gram of poly(lactic acid) was dissolved in 10 ml of methylene chloride in the case of (O/W) method and in 10 ml of acetonitrile in the case of (O/O) method. A given amount of drugs was added to these solutions and then suspended homogeneously by sonication. The suspensions were added dropwise under agitation to water containing 2 wt % poly(vinyl alcohol)(PVA), or to silicone oil containing 0.1 wt % soy bean lecithin. PVA and lecithin were used as emulsifier. The temperature of emulsions was raised to 30 or 45°C to allow evaporation of methylene chloride or acetonitrile, respectively. Following evaporation of the solvents, the microspheres were collected by centrifugation, washed three times with cold distilled water or petroleum ether, and then lyophilized.

Characterization of Microspheres

To determine the ADR content of the microspheres, a weighed amount of microspheres was dissolved in 1 ml of methylene chloride and the solution obtained was

Preparation of Poly(lactic acid) Microspheres

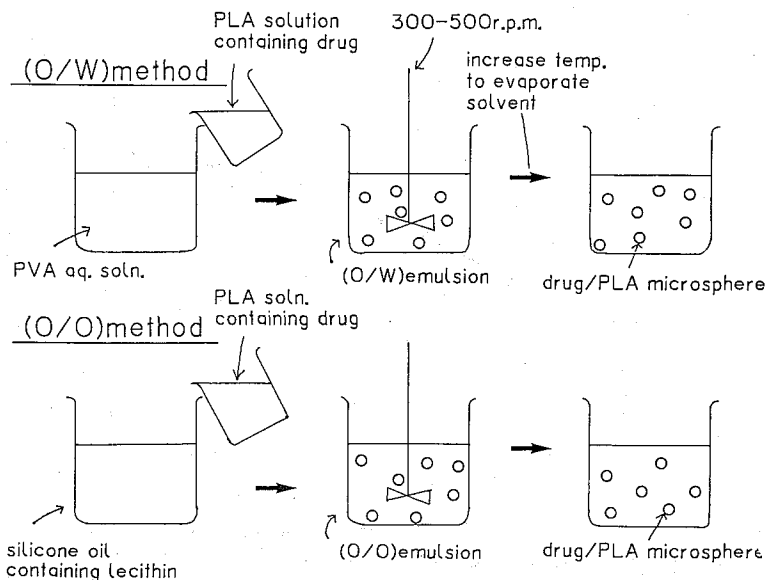


Figure 1. Scheme of solvent evaporation method.

diluted to 100 ml with methanol. A fluorescence spectrophotometer (Hitachi, Model 650 10-S, Tokyo, Japan) was used to determine the ADR concentration at the excitation wavelength of 470 nm and the emission wavelength of 580 nm. The microsphere containing CDDP was dissolved in 1 ml of chloroform and the organic solution was extracted with the same volume of distilled water. The CDDP concentration of the extract was determined by HPLC assay.¹⁰⁾ The size of microspheres was determined by observation with scanning electron microscopy (SEM, Hitachi Model S-450, Tokyo, Japan). SEM was also used to follow the hydrolytic degradation of microspheres.

In Vitro Release Test

A weighed amount of microspheres was suspended in a given amount of Tris buffer solution at pH 7.4 and the resulting suspension was stored in a vial. For the *in vitro* release study, the vial was immersed in a shaker bath kept at 37°C and the release medium was periodically removed, the same amount of the fresh medium being added to the vial. The amount of ADR and CDDP released into the medium was measured using the fluorescence spectrophotometer and HPLC, respectively, as described above. Bioactivity of ADR released from the microspheres was evaluated by bioassay using *Bacillus subtilis* ATCC 6633.¹¹⁾

RESULTS AND DISCUSSION

Effects of Preparation Conditions on Drug Release

Figure 2 shows the *in vitro* release profiles of ADR from the poly(L-lactic acid) microspheres with different drug contents. The molecular weights of PLA were all

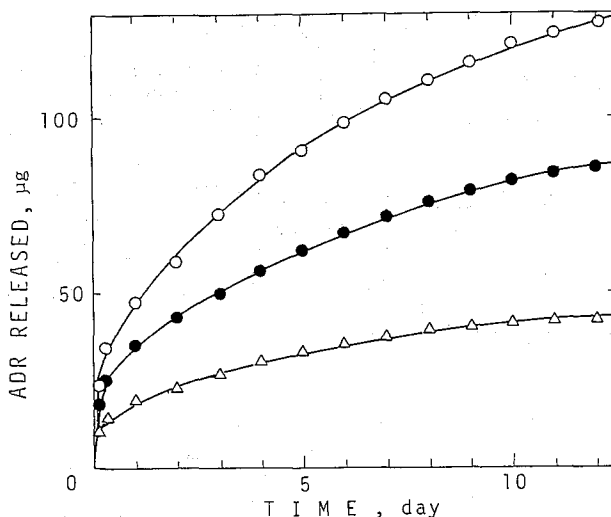


Figure 2. Release profiles of ADR from ADR/L-lactic acid oligomer microspheres ($\bar{M}_w=6,100$). (○) 24.6 μg -ADR/mg-sphere, (●) 14.4 μg -ADR/mg-sphere, (△) 5.3 μg -ADR/mg-sphere.

6,100. Amounts of ADR released from 10 mg of each microsphere are shown as cumulative microgram. Retention of bioactivities of ADR released was confirmed by bioassay. Initial loadings of ADR were varied by changing the ADR amount used for the preparation of the microspheres. Weight percentage of ADR trapped in the microspheres was as high as 50 to 70%.

ADR is partially soluble in methylene chloride from which poly(lactic acid) solution was prepared and hence some amounts of ADR can be entrapped in the microspheres, leading to the sustained release of ADR from the microspheres. In the case of CDDP, however, it is very difficult to prepare the CDDP microsphere using the O/W solvent evaporation method because of the poor solubility of CDDP in organic solvents. The CDDP drug crystals tend to diffuse out from the organic phase to the water phase, resulting in quite low trapping of CDDP in the microsphere, mostly less than 10%. In order to achieve high trapping of CDDP, the O/O method was applied to the preparation of CDDP microspheres. The O/O method essentially does not allow the transfer of CDDP to the outer phase of silicone oil. Indeed, almost 100% of CDDP could be entrapped in the microsphere by this method, although 80% of CDDP of the initial loading burst out from this microsphere in one hour in the beginning of the release test. Probably, this is due to heterogeneous distribution of CDDP in the polymer matrix, as the CDDP drug crystals dispersed in the matrix seem to form continuously connecting channels which must enable penetration of the outer aqueous medium into the microsphere, resulting in quite fast leakage of the drug from the microsphere. Spenlehauer et al. also evaluated the effect of preparation condition of the O/W solvent evaporation method on release profiles of poly(D, L-lactide) microspheres containing CDDP.¹²⁾ They reported that the drug trapping was markedly enhanced by saturation of the outer aqueous phase with CDDP.

Preparation of Poly(lactic acid) Microspheres

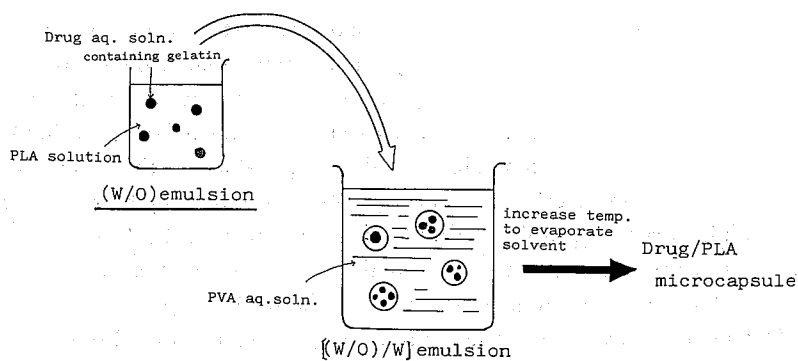


Figure 3. Scheme of (W/O)/W emulsion type solvent evaporation method.

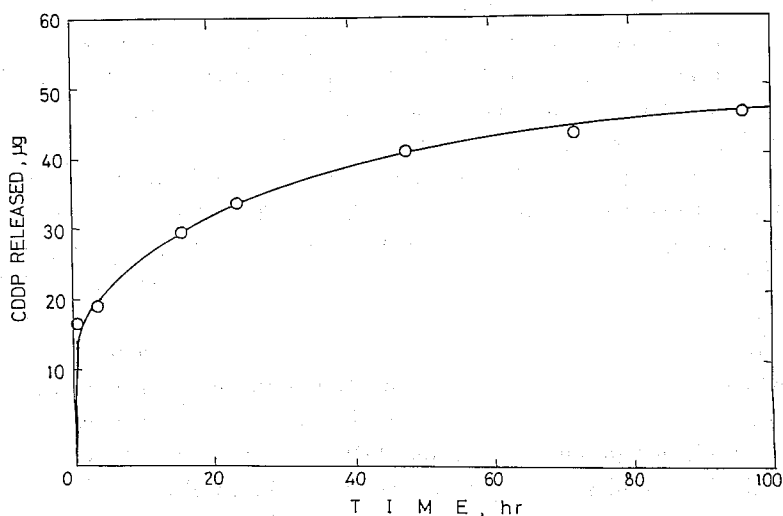


Figure 4. Release profile of CDDP from CDDP/L-lactic acid oligomer microsphere ($\bar{M}_w=13,000$ $2.0 \mu\text{g-CDDP/mg-sphere}$).

To improve the CDDP entrapping and the release profile, we applied the (W/O)/W type solvent evaporation method as shown in Figure 3. The CDDP microspheres involving the aqueous microphase of a diameter from 1 to $10 \mu\text{m}$, was prepared by this method. The diameter of the final microspheres was 10 to $100 \mu\text{m}$. The release profile of CDDP from this microsphere is shown in Figure 4. The internal aqueous microphase containing gelatin and the wall material made of poly(lactic acid) successfully retained the drug, but the quite low solubility of CDDP in water phase limited the initial drug loading in the microspheres even when the internal aqueous microphase was saturated with CDDP higher than 1(w/w)% CDDP by this (W/O)/W method. A new method is necessary to prepare the CDDP microspheres which have higher loadings and longer release periods.

The O/W type solvent evaporation method was first described by Beck et al. for preparation of poly(lactic acid) microspheres containing progesterone, a steroid hormone.⁹⁾ This method will be appropriate for the preparation of the microspheres incorporating highly lipophilic drugs such as steroids and aclacinomycin.¹³⁾ Slightly lipophilic drugs such as adriamycin are also applicable to this method as above described, but the trapping efficiency and the release profile may be not so good as for highly lipophilic drugs. Highly hydrophilic drugs such as CDDP and many other drugs are not appropriate for the O/W method. It appears that an O/O method, a (W/O)/W method, or more improved ones are should be improved for this group of drugs. Our results show that physical properties, especially solubility of the drugs are the most important factor for the preparation of poly(lactic acid) microspheres containing these drugs.

Effect of Matrix Polymer on Drug Release

As shown in Figure 2, the initial loading of ADR microspheres affects the release profile, but the initial content of ADR in the microspheres is not proportional to the release amount. On the other hand, the degradation rate of the poly(lactic acid) is likely to influence the release rate of drugs.

Therefore, we studied the effect of molecular weight of the poly(lactic acid)s on the drug release. Table 2 represents the molecular weights of various poly(lactic acid)s used for the microsphere preparation, together with the loadings and trapping efficiencies of ADR in the microspheres prepared at the ADR/polymer ratio of 0.02 (w/w). The amount of ADR loaded in the resulting microspheres varied with the molecular weight of the polymer, probably because the microsphere was prepared with the O/W method which allowed partial dissolution of ADR into the outer aqueous phase. To evaluate the effect of molecular weight of the polymer on the release profiles, the microspheres which had almost the same ADR contents were selected out of several samples. Release profiles of ADR from the microspheres of poly(L-lactic acid)s with different molecular weights are shown in Figure 5. The release rate is reduced as the molecular weight increases, indicating that the release rate is controllable by changing the degradation rate of the matrix polymer. The results obtained with the poly(D, L-lactic acid) microspheres are shown in Figure 6. Generally speaking, the polymer from optically active L-lactic acid is crystalline, whereas the polymer from racemic D, L-lactic acid is amorphous, so far as the molecular weight is sufficiently high. Oli-

Table 2. Drug weights and trapping in microspheres.

LA oligomer	Drug loaded ($\mu\text{g-ADR/mg-sphere}$)	% trapped
L-3,400	17.56	88.0
L-6,100	10.15	51.2
L-13,000	7.11	36.0
D, L-3,300	13.25	66.7
D, L-6,400	6.21	31.0
D, L-9,600	6.03	30.6

Preparation of Poly(lactic acid) Microspheres

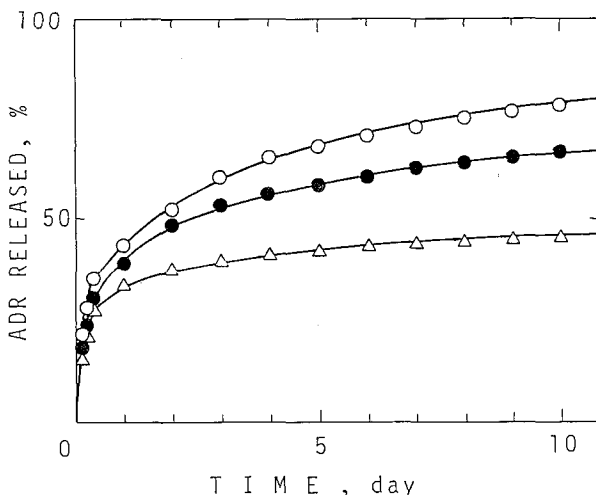


Figure 5. Release profiles of ADR from ADR/L-lactic acid oligomer microspheres. (○) $\bar{M}_w=3,400$ ($14.1 \mu\text{g-ADR/mg-sphere}$), (●) $\bar{M}_w=6,100$ ($14.4 \mu\text{g-ADR/mg-sphere}$), (△) $\bar{M}_w=13,000$ ($15.6 \mu\text{g-ADR/mg-sphere}$).

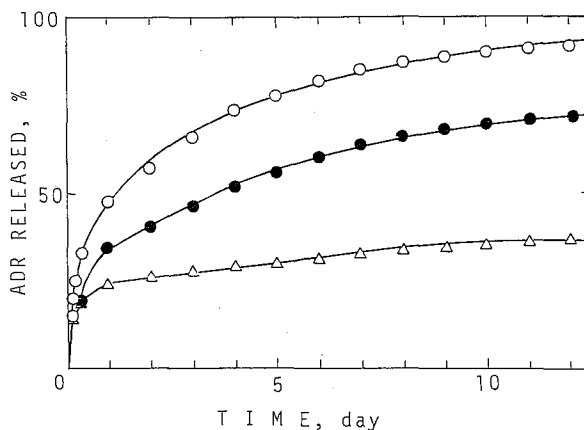


Figure 6. Release profiles of ADR from ADR/D, L-lactic acid oligomer microspheres. (○) $\bar{M}_w=3,300$ ($16.1 \mu\text{g-ADR/mg-sphere}$), (●) $\bar{M}_w=6,400$ ($17.2 \mu\text{g-ADR/mg-sphere}$), (△) $\bar{M}_w=9,600$ ($21.5 \mu\text{g-ADR/mg-sphere}$).

gomic poly(lactic acid)s used in this study may have low degrees of crystallinity and the difference of degradation rate between L- and D, L-polymer may be so small that the difference of the release rate between the two polymers must be quite small as shown in Figure 6.

We have also investigated the effect of monomer composition in poly(L-lactic acid-co-glycolic acid) on the release profiles of a fluorescent (rhodamine 6GX) from the copolymer microspheres.¹⁴⁾ The largest release rate was realized at the glycolic acid composition of 50 mole%, in accordance with the most rapid degradation of the copolymer. This result is in good agreement with that shown in Figure 5, again

suggesting that the degradation rate of the matrix polymer affects the release rate of drugs from the microsphere. However, in the case of ADR, the spacial distribution of the drug in the microsphere may not be sufficiently homogeneous. The small difference of the release profiles between poly(L-lactic acid) and poly(D, L-lactic acid) as described above may be also due to this distribution difference. In order to learn in more detail the effect of nature of the matrix polymer on the release profiles, a highly lipophilic drug such as aclacinomycin¹³⁾, which can be successfully entrapped in the poly(lactic acid) microspheres, may be more appropriate.

Morphological Change of Microspheres during Hydrolysis

Figure 7 shows the morphological change of the ADR microsphere accompanying the *in vitro* hydrolytic degradation. The microsphere was prepared from poly(L-lactic acid) with a molecular weight of 3,400. SEM observation reveals that the microsphere undergoes the hydrolytic degradation and produces small pores and cracks which probably are responsible for the release of the drug. After hydrolysis for 7 days, the microsphere did not have spherical shape any more, but still colored red due to the existence of ADR. This fact implies that the release of ADR after 7 to 10 days as shown in Figures 2, 6, and 7 are mostly caused by the degradation of the matrix polymer.

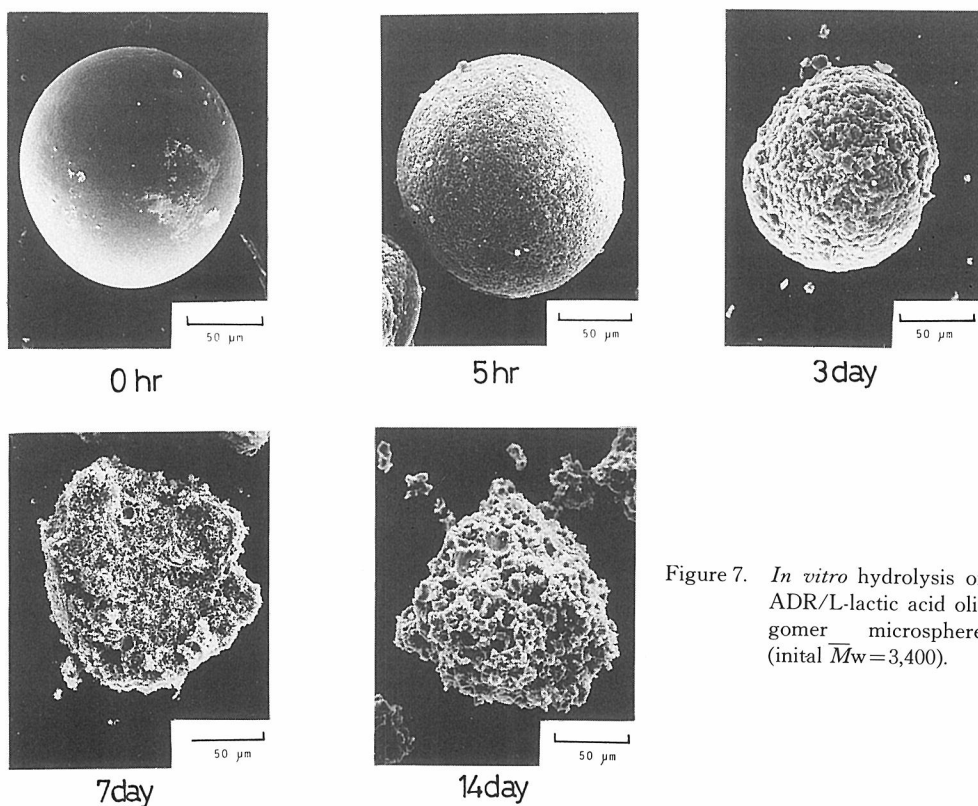


Figure 7. *In vitro* hydrolysis of ADR/L-lactic acid oligomer microsphere (initial $\overline{M}_w=3,400$).

Effect of Drug Distribution on the Release

As described above, large amounts of drugs loaded always leak out from the microspheres within first several hours. This phenomenon is called the "burst" release. In order to elucidate the cause of this phenomenon, we fabricated lactic acid oligomer beads containing ADR by the following method. One gram of lactic acid oligomer was melted on a steel plate heated to 60–70°C and then mixed mechanically with 10 mg of ADR. The apparently homogeneous mixture was shaped into spherical beads as large as 2 mm in diameter. The release profile from the ADR beads is shown in Figure 8. The initial burst is greatly reduced in comparison with that of microspheres. The relation between the release amount of ADR and the square root of the release period is almost linear as shown in Figure 9.¹⁵⁾ This may mean that the degree of dispersion of

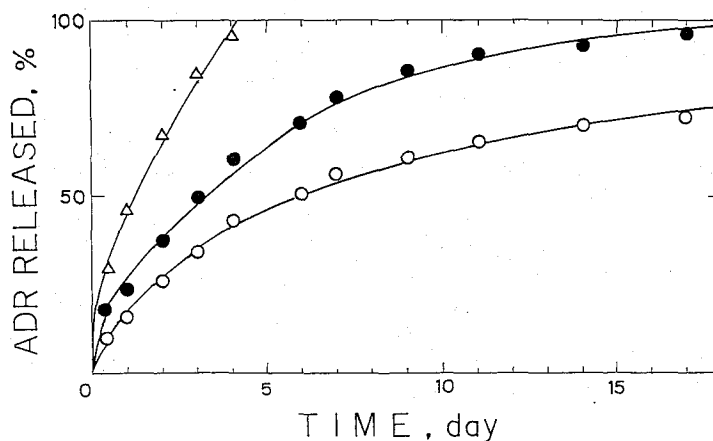


Figure 8. Release profiles of ADR from ADR/L-lactic acid oligomer beads ($\phi=2$ mm, ADR loading = 170 $\mu\text{g}/\text{bead}$). (Δ) $\bar{M}_w=3,400$, ($\bullet$) $\bar{M}_w=4,700$, ($\circ$) $\bar{M}_w=6,100$.

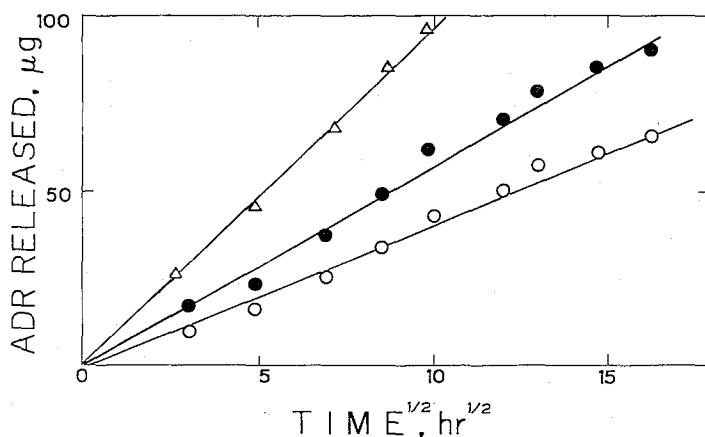


Figure 9. Higuchi plot of ADR release from ADR/L-lactic acid oligomer beads. (Δ) $\bar{M}_w=3,400$, ($\bullet$) $\bar{M}_w=4,700$, ($\circ$) $\bar{M}_w=6,100$.

the drug in the beads is more homogeneous than that of the ADR microspheres. In addition, it appears that the ADR microspheres have many pores and channels in the matrix. It follows that the most effective microsphere preparation from polylactides without the minimum burst release depends on how homogeneously the hydrophilic drugs are distributed in the microsphere.

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