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Degradation of DL-PLA-methadone microspheres during in vitro release

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Abstract

The in vitro degradation of polylactic acid of different weight average molecular weight (M_w) in microspheres containing variable amounts of methadone base (between 17 and 47%) was studied using the degradation index (DI), polidispersivity (pd) and molecular weights distribution. Both pd and DI are independent of initial methadone content and increase with M_w polymer, showing values of DI around 6.6 for the high M_w and 2.9 for the lower M_w. The distribution functions in the number of the molecular weights during the degradation assays indicate the presence of different break mechanisms for high and low molecular weight chains. A semiempiric model is proposed to explain the evolution of M_n during degradation in function of the number of bonds available for their breaking and hydrolysis rate constant. The first parameter ranges between 2.35 and 6.65 in function of M_w; the hydrolysis rate constant is independent of M_w and equal to 0.020 h^{-1} (SD = 0.011 h^{-1}).

Keywords: Microspheres; DL-PLA; Methadone; Mean degradation time; Mean release time; Degradation index; Polidispersivity

1. Introduction

Polylactic acid and its copolymers are among the most commonly biodegradable polymers used in the development and manufacture of biodegradable sustained release systems like implants and microspheres. These polymers undergo both acid and basic catalytic hydrolysis as well as enzymatic catalysis (Kulkarni et al., 1971; Pitt et al., 1981; Vert et al., 1981; Rosen et al., 1988; Le Ray et al., 1994), but the course of the polymer degradation depends largely on the heterogeneous nature of the degradation process. In general, it has been observed that the hydrolytic degradation of polyesters depends on properties related both to their molecular weight and chemical nature, as

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the presence of rubber, glass or crystalline areas, and the hydrophobicity (Makino et al., 1985; Park et al., 1995). Moreover, the increase of water accessibility (Hutchinson and Furr, 1989) and the decrease of glass transition temperature (T_g) (Omelzue and McGinity, 1992) as a consequence of the reduction of molecular weight, facilitates polymer degradation.

On the other hand, low molecular polymer fragments split from large chain barely diffuse out of the systems, more so when the polymerization index is higher than 4 (Himmelstein and George, 1993) or 15 (Park, 1994) according to different authors, remaining entrapped inside the microsphere or other devices, leading to higher catalytic polymer degradation rates in the center of the device (Joshi and Himmelstein, 1991; Park, 1994). Thereby, scission chain mechanisms will be influenced by these factors, the random scission being the most probable mechanism in heterogeneous systems but with different hydrolytic rates depending on the polymer region considered. However, Shih (1995) reported end group scission for poly(DL-lactic) acid (DL-PLA) and random scission for poly e-caprolactone in homogeneous systems.

Some basic drugs exhibit a specific basic catalytic effect on polylactic (PLA) and poly lactic-glycolic (PLGA) polymer degradation, both in heterogeneous (Maulding et al., 1986; Cha and Pitt, 1989) and in homogeneous systems (Lin et al., 1994). Cha and Pitt (1989) studied the factors responsible for the catalytic effect of meperidine, methadone, naltrexone and prometazine on L-PLA and LPLGA, finding that neither the drug pK_a, polymer/water partition coefficient nor the drug induced reduction in polymer T_g are correlated with the catalytic effect, but with the drug concentration in the polymer.

This paper reports the evolution of DL-PLA degradation during in vitro methadone release studies from microspheres reported previously (Delgado et al., in press), searching for the mechanisms involved in polymer degradation and the relationship between polymer degradation and drug release. For this purpose, we suggest the use of the true polymer molecular weight distributions instead of the raw chromatograms, and the degradation index (ID), linearly related to the extension of polymer chain scission, instead of number (M_n) or weight (M_w) average molecular weight evolution.

2. Materials and methods

2.1. Materials

The DL-PLAs were obtained by the ring-opening reaction as described by Kulkarni et al. (1966) and in agreement with previous data available (Munguía et al., 1992). Polystyrene standards were from Tokyo Soda Ltd. Methadone from Alcaliber S.A. Other chemicals and solvents were reagent grade (Merck).

2.2. Microspheres preparation

Microspheres were prepared by the solvent evaporation method which consists of a solution of DL-PLA and DL-methadone in methylene chloride and poured over 1 l of 0.1% polyvinyl alcohol aqueous solution and stirred at 8000 rpm for 5 min using a turbine homogenizer (IKA mod. Ultra-Turrax T-25, head type KR). Solvent evaporation was completed in the following 5 h at room temperature and atmospheric pressure using a paddle stirrer (Turú Grau, model D-6) at 250 rpm. The microspheres were separated by centrifugation and freeze-drying. The DL-methadone content was determined by spectrophotometric assay at $l = 290$ nm in methylene chloride.

Thirteen lots of microspheres were prepared in agreement with a second order composite rotable experimental design (Cochran and Cox, 1957), the two variables under study were polymer content (X_1) and polymer weight average molecular weight, M_w (X_2). The coded levels and the values of both variables have been published already (Delgado et al., in press, Table 1).

2.3. Particle size

Microspheres suspended in 0.1% Tween® 80 solution were measured by a Coulter Counter (Coulter Multisizer II) after bath sonication during 30 s to deagregate.

Table 1
Estimates of the parameters of the proposedsemiempiric model

Lot	M_n^0	n^0	k (h^{-1})
A	43 700	2.35	0.044
B	41 500	3.39	0.027
D	67 000	6.65	0.021
E	41 000	5.02	0.016
F	44 900	2.67	0.01
H	61 300	5.68	0.023
I1	48 700	3.46	0.017
I4	54 900	4.57	0.00

2.4. Degradation studies

Degradation studies were carried out simultaneously with the release studies (Delgado et al., in press). Microspheres (40 mg) were dispersed in 0.066 M phosphate buffer pH 7.4 (100 ml) containing 0.001% Tween® 80, ionic strength $\mu = 0.264$, and kept at 37°C in a water bath with magnetic stirring at 50 rpm. A container was used for each sample time and then withdrawn, the microspheres were filtered and dried for 24 h in dessecator and vacuo and measured in the gel permeation chromatography (GPC) system. The degradation assays lasted between 6 and 11 days, until at least 80% of methadone was released.

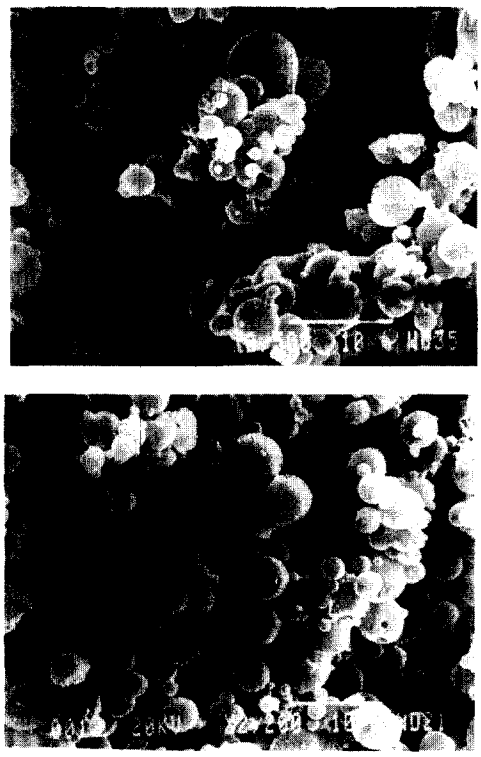

Fig. 1. Microphotograph of DL,PLA-methadone base microspheres, lot *A*, before (a) and after (b) methadone release.

2.5. Gel permeation chromatography (GPC) analysis

The different average molecular weight measurements were made using a Waters GPC system that consisted of a Model 510 pump, Rheodyne injector, four Ultrastyragel columns with exclusion limits of 10^5, 10^4, 10^3 and 500 Å, a differential refractometer (Model 410), and Maxima 820 Chromatography software, v. 3.30 for data acquisition. The operational conditions were as follows: solvent, tetrahydrofuran; column temperature, 31°C; injection volume, 20 ml; flow rate, 0.9 ml/min; and solute concentration 0.5% w/v. Polystyrene standards, with molecular weights 2800, 5500, 10 300, 43 900, 102 000, 190 000, 355 000 and 710 000, were used to calibrate the GPC system and all molecular weight values reported were relative to polystyrene. The polymer solutions were passed through 0.45 mm filters using a glass syringe.

In order to study the DL-PLA depolymerization process, we developed an equation to obtain the molecular weight distribution curves from the chromatograms. By definition (Pickett et al., 1966) the molecular weight distribution on log scale is

$$f = \frac{dW}{d \log M} \tag{1}$$

where W and M are the mass and molecular weight of a determined polymer fraction; V being the elute volume at time t, we can deduce that the above expression is equal to:

$$f = \frac{dW}{dV} \frac{1}{\frac{d \log M}{dV}} \tag{2}$$

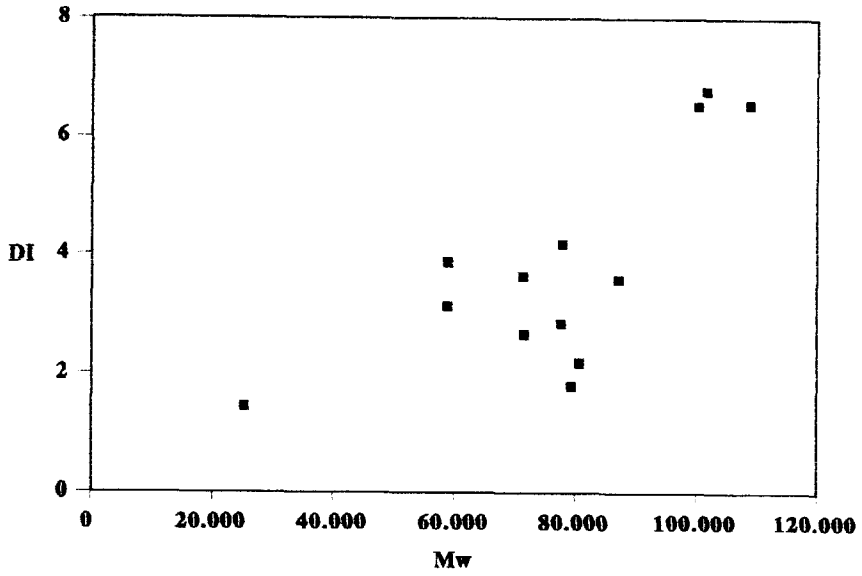

Fig. 2. Degradation index of different microsphere lots included in the experimental design at the end of methadone release.

M is related to the time t, in agreement with the calibration function,

$$M = 10^{b_0 + b_1 t + b_2 t^2} \tag{3}$$

On the other hand, the detector signal (h) is proportional to the concentration:

$$h = k \frac{dW}{dV} \tag{4}$$

If W is the weight fraction, we get:

$$1 = \frac{1}{k} \int_0^\infty h \, dV = \frac{Q}{K} \int_0^\infty h \, dt \tag{5}$$

where Q the flow. Let

$$H = \int_0^\infty h \, dt \tag{6}$$

finally we get,

$$f = \frac{h}{H} \frac{1}{b_1 + 2b_2 t} \tag{7}$$

To get the distribution function in number, each weight fraction is divided by the mean molecular weight of that class and is normalized in such a way that the area under the curve is equal to 1. Chromatograms were exported as DIF files and imported from Lotus 1-2-3 v. 3.1, where the last equation was implemented (3.5″ diskette with an example is available upon request).

3. Results and discussion

To quantify the polymer degradation the degradation index (DI) was used, which is defined as the proportion of broken bonds in relation to the initial number of polymers molecules (Glynn et al., 1976). The DI is calculated from the initial number average molecular weight ($M_n(0)$) and that presented by the polymer after a certain time ($M_n(t)$), through the expression:

$$\mathrm{ID} = \frac{M_n(0)}{M_n(t)} - 1 \tag{8}$$

All the microspheres lots elaborates appeared spherical (the number-volume average diameters range 9.0–18.8 mm) and the surface was smooth and apparently without pores as can be observed in one example in Fig. 1a. Despite the decrease seen in the DL-PLA weight average molecular weight (M_w) during the release assays (Delgado et al., in press, Table 2), in general the microspheres keep their structure as can be seen in Fig. 1b.

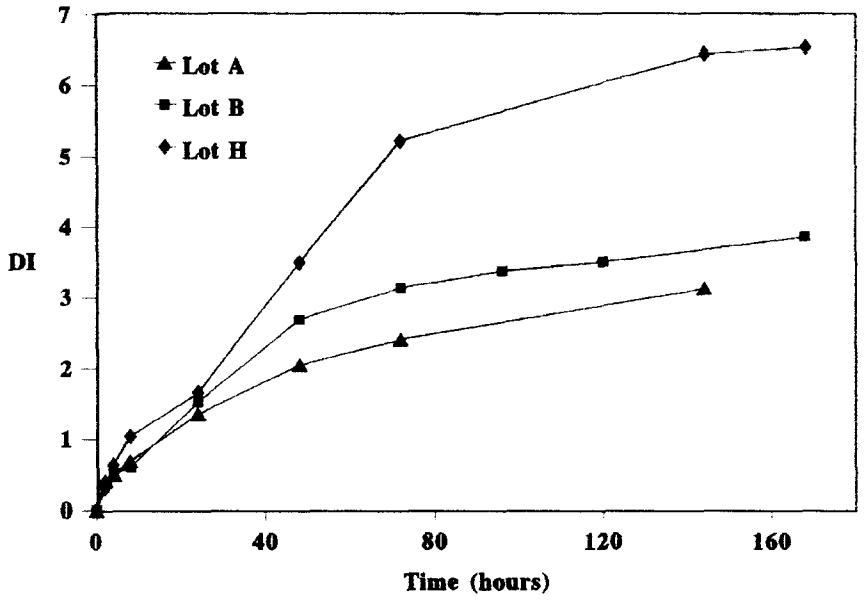

Fig. 3. DI during the degradation process for: lot *H* (M_r 100 300, 34.7% methadone); lot *A* (M_r 59 000, 38.3% methadone) and lot *B* (M_r 59 000, 17.2% methadone).

The polymer DI at the end of methadone release is higher in microspheres of larger M_w as is shown in Fig. 2. The thirteen lots which have been elaborated can be divided into two groups in function of the polymer M_w in the microspheres. One group would be made of microsphere lots whose polymers present, once the elaboration stage is finished, M_w between 100 000 and 110 000; they are the lots C (35.7% methadone), D (17.5% methadone) and H (34.7% methadone), showing a mean DI value of 6.6 (SD = $\pm$ 0.173). The other group is formed by the remainder lots with a M_w between 25 000 and 87 000; in this case the DI presents a mean value of 2.94 ($\pm$ 0.961), similar to the lots corresponding to the central point of the experimental design (mean = 2.790 $\pm$ 0.801); the within-lots variability being therefore almost the same as the between-lots. In both cases, although the polymer degradation is catalyzed by methadone, the degradation extent measured as DI is independant of the initial methadone content, as oppose to the results obtained by Cha and Pitt (1989) with the prometazine, where the degradation process was higher when the prometazine content was increased. This fact is illustrated in the Fig. 3, in which the evolution of DI during methadone release is presented for the following lots: lot H, with a polymer M_w of 100 300 and a percentage of methadone incorporated of 34.7%, and lots A and B, both prepared with the same

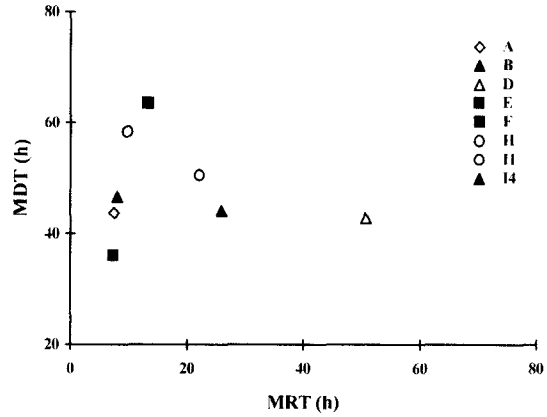

Fig. 4. Mean degradation time (MDT) of the polymer versus mean release time (MRT) of methadone from the microspheres for different lots of microspheres studies.

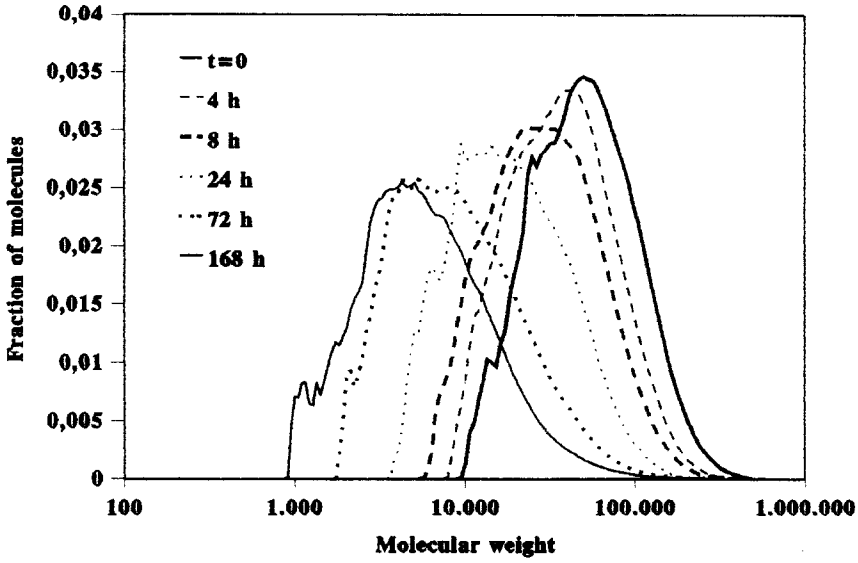

Fig. 5. Distribution function in number of molecular weights evolution during the degradation assay corresponding to lot *H* microspheres.

polymer which once elaborated present a M_w of approximately 59 000 and methadone charges of 38.3 and 17.2%, respectively . A first and faster degradation phase is appreciated, much more acute for DL-PLA of larger M_w and a second slower phase where the degradation rates tend to become the same. Formulations A and B independently of the initial methadone charge reach a value of DI of approximately 3.5 while formulation H doubles this DI value at the end of the assay. Furthermore, the polymer's degradation and the methadone release from the microspheres are independent processes because as can be seen in Fig. 4, there is no relationship between the mean degradation times (MDT) of polymers and the mean release times (MRT) of methadone from the different lots of microspheres studied, although as was mentioned before the methadone catalyze the degradation process.

The evolution of number distribution of molecular weights along the hydrolysis shows different breakage mechanisms for high and low molecular weight chains. Fig. 5 represents the evolution of molecular weights distribution in number for lot H. M_w was initially equal to 100 300, and after 4 h (DI = 0.63) only a shifting to the left was observed. This behavior can be explained through the selective scission of high molecular weight chains, particularly those with molecular weight above the intersection point of both distribution curves, i.e. above 45 000; probably, these scission must take place at non-random positions since the larger chains are under higher tension than shorter ones. After this point, the curves become flatter, forming a plateau and become wider and moving toward lower molecular weights which seems to suggest that the random scission begins to predominate due to the disappearance of molecules of high molecular weight. On the other hand, low M_w DL-PLA polymers did not show the first step as can be seen in Fig. 6, in which number distribution curves for lot E (initial M_w equal to 71 000) are depicted.

Finally, it must be taken into account that soluble fractions which could have been produced during the degradation process have not been evaluated, only on the bulk, with which we would get DI values slightly less than the real ones as long as microspheres morphology showed no apparent alteration and no small chains were de-

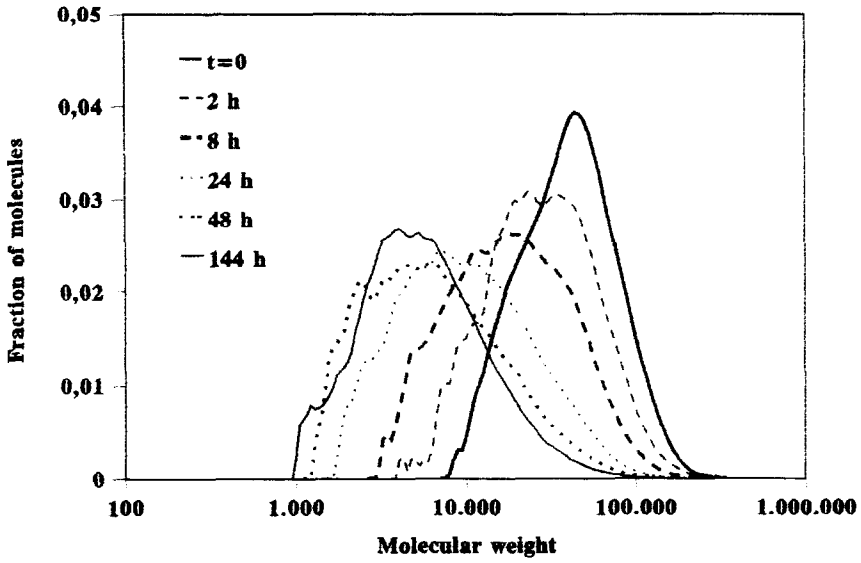

Fig. 6. Distribution function in number of molecular weights evolution during the degradation assay corresponding to lot *E* microspheres.

tected by GPC. On the other hand, we found that the polymer polidispersivity during degradation assays is also independent on initial methadone charge in microspheres, but pd values increase when the starting polymer M_w increases.

Because of the chemical structure of DL-PLA and the very large number of bonds available to the scission, a 'zero order' for DI would be expected. However, our experimental data show

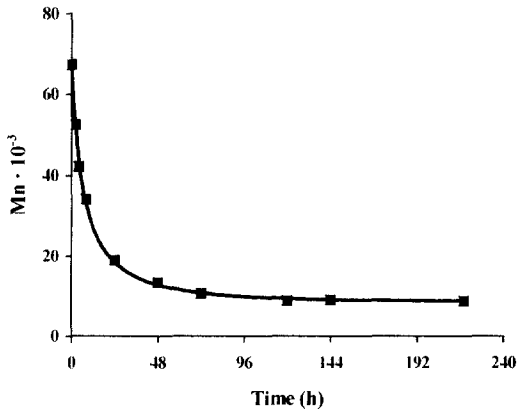

Fig. 7. Fitting the proposed semiempiric model to the degradation data obtained for lot *D*.

that during in vitro methadone release, only a limited number of bonds are available to scission and the M_n has a tendency towards a stable value because the methadone has been released and not to zero as would also be expected if a full depolymerization is on course, as was previously proposed by other authors (Pitt et al., 1992) for other biodegradable polymers. Keeping in mind these data and the fact that the degradation process is independent of the amount of methadone left in the microspheres, a semiempiric model to relate M_n with time was devised. Let n^0 and n the average number of bonds per molecule available to scission and the number of bonds broken at time t respectively; the scission rate is:

$$\frac{dn_i}{dt} = \int_{i=1}^{m} k_i(n_i^0 - n_i) \tag{9}$$

i is the index where the molecules are present at time zero (m) and k_i is the hydrolysis rate constant, and n_i^0 and n_i the number of bonds available to scission and the number of bonds broken respectively in the i-th molecule. Assuming k_i identical for all molecules,

$$\frac{dn}{dt} = mk(n^0 - n) \quad (10)$$

where n^0 and n are the initial and actual average number of bonds per molecule; integrating,

$$n = mn^0(1 - e^{-kt}) \quad (11)$$

By definition,

$$DI = \frac{n}{m} = n^0(1 - e^{-kt}) \quad (12)$$

and therefore,

$$M_n = \frac{M_n^0}{1 + n^0(1 - e^{-kt})} \quad (13)$$

Experimental data were fitted using the Mathemathica™ (Wolfram, 1988) function Nonlinear Fit. Fig. 7 shows the experimental data and fitted curve for lot D; in all cases, a good agreement between model and experimental data was observed; Table 1 summarizes the estimated parameters obtained for the lots studied. Except for the extreme values obtained for lots A and I4, k ranges between 0.012 and 0.027 h^{-1}, (average value = 0.020 h^{-1}), and no dependence on polymer M_w was observed; n^0 was proportional to initial M_w as would be expected from the fact that larger molecules contribute with more bonds to breakage.

4. Conclusions

As has been shown by the obtained results, the degradation of DL-PLA in microspheres containing variable amounts of methadone base, does not depend on the initial drug content in our experimental range, possibly due to the fact that the necessary amount to reveal its catalytic effect is below those used by us. The scission type along polymer degradation depends on the molecular weight of chain involved. Hydrolysis rate constant was independent of M_w, but not the number of bonds available to scission. Despite the extent of polymer degradation, resulting chains were too large to diffuse as was proved by microphotography, and therefore, drug release appears to be independent of polymer degradation.

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