Drug Development and Industrial Pharmacy, 22(7), 637-643 (1996)

An Investigation of the Synthesis of Poly(D,L-Lactic Acid) and Preparation of Microspheres Containing Indomethacin

M. C. Gohel,[1,*] M. M. Patel,[1] J. S. Kaul,[1] R. B. Patel,[2] S. R. Patel,[2] and T. R. Jani[2]

[1]*L. M. College of Pharmacy, Ahmedabad 380 009, India*
[2]*Cadila Healthcare Pvt. Ltd., Ahmedabad 380 050, India*

ABSTRACT

Seven batches of poly(D,L-lactic acid) (PLA) were prepared from D,L-lactic acid using tetraphenyltin or zinc acetate as a catalyst. The samples of PLA were characterized by terminal group analysis, gel permeation chromatography, infrared analysis, and differential scanning calorimetry. Polymerization conditions such as the time of reaction and the type of catalyst affected the molecular weight of PLA. Microspheres containing indomethacin were prepared by the oil/water (o/w) solvent evaporation technique using different formulations and process variables. The in vitro release of indomethacin in phosphate buffer was found to be dependent on the molecular weight of PLA and also on the type and amount of adjuvants used. The batch of microspheres containing 5% cholesterol released the drug at a slow controlled rate (29%, 65%, and 85% in 1, 7, and 24 hr, respectively). The results of F test for the microspheres revealed that there is no significant difference between the Higuchi model and the power law equation. The drug was released by a diffusion mechanism from the microspheres.

INTRODUCTION

Controlled-release devices composed of biodegradable and biocompatible polymers are gaining popularity in recent years. Poly(D,L-lactic acid) (PLA), a synthetic biodegradable polymer, has been used in sutures, fracture fixation, prosthetic devices, implants and microspheres (1-5). The degradation product of PLA is lactic acid, which is a natural metabolic product of all higher animals. Biodegradation of PLA can be tailored from few hours to few weeks by varying the molecular weight of PLA or by using copolymer such as

*To whom correspondence should be addressed.

poly(lactide-*co*-glycolide) (PLGA). Due to this flexibility, PLA has been in great demand in recent years. PLA is obtained from D,L-lactide via the ring-opening polymerization technique using a suitable catalyst (6,7).

The aim of this investigation was to prepare and characterize PLA using tetraphenyltin (TPT) or anhydrous zinc acetate (ZA) as catalyst. The reaction conditions were identified to obtain PLAs with a specific molecular weight range. The selected polymers were used for the preparation of microspheres of the model drug indomethacin. Indomethacin was selected as a model drug since it can produce gastric ulcers on oral therapy. Microspheres of indomethacin can be used to reduce side effects by tailoring the drug release.

EXPERIMENTAL

Materials

Indomethacin and Resomer® (R202, MW = 17,400) were received as gift samples from IDPL, India, and Boehringer Ingelheim, Germany, respectively. Tetraphenyltin (Aldrich Chem. Co.), zinc acetate (S.D. Fine Chem. Pvt. Ltd.), ZnO (Qualigens Fine Chem.), PVP (Kollidon), D,L-lactic acid (Merck), PVA (Polychem Pvt. Ltd.), and dialysis bag (Sigma Chem. Co.) were used as received. All other chemicals were of reagent grade.

Preparation of D,L-Lactide (8)

Finely powdered zinc oxide (5 g) was mixed well with lactic acid (250 ml) and the mixture was vacuum distilled at a temperature not exceeding 140°. During distillation, the pressure was stepwise decreased to 60–55 mm Hg over a period of 12 hr. About 70 ml of the distillate was collected. The creamish white residue was further distilled at 180° (4 mm Hg). The receiving flask was kept in an ice bath. About 90 g of the crude D,L-lactide was obtained. The D,L-lactide was washed with chilled ether and then recrystallized twice with ethyl acetate.

Polymerization of D,L-Lactide

A mixture of the recrystallized lactide (5 g) and either 0.02% w/w TPT or 1% w/w ZA (initiator) was heated in an evacuated sealed flask at 180° for time intervals ranging from 3 to 24 hr. The reaction conditions of the seven batches of PLA are shown in Table 1. ZA was added in powder form. TPT was dissolved in minimum amount of dry benzene and later benzene was distilled off under vacuum. Nitrogen gas was flushed before sealing the flask. The brownish polymer (PLA) was purified by dissolving it in dioxane, followed by slow precipitation in chilled water. The polymer was dried under vacuum at room temperature.

Table 1

Specifications for the Batches of PLA

Batch Code	Catalyst	Reaction Time (hr)	$\overline{M}_n$ ± 1000
PLA-1	ZA	3	11,000
PLA-2	ZA	6	13,000
PLA-3	TPT	3	12,000
PLA-4	TPT	6	17,000
PLA-5	TPT	9	26,000
PLA-6	TPT	12	31,000
PLA-7	TPT	24	38,000

Characterization of PLA

Terminal Group Analysis

The number-average molecular weight ($\overline{M}_n$) was measured by terminal group analysis (9). PLA (100 mg) was dissolved in 20 ml mixture of CH_2Cl_2 and CH_3OH (1:1 v/v). The solution was titrated with 0.1 N methanolic sodium methylate solution against bromocresol blue as indicator. The averages of three independent estimations are reported in Table 1.

Gel Permeation Chromatography (GPC)

Measurements were performed on a Waters Chromatograph GPC-II model equipped with 6μ Styragel® columns, with exclusion limits 10^6, 10^5, 10^4, 10^3, 500 Å × 2, and 100 Å × 2, respectively. Tetrahydrofuran (THF) was used as a solvent. The temperature of the column oven was maintained at 25° and the flow rate was kept as 2 ml/min. To calibrate the system, polystyrene monodisperse standards (Pressure Chem. Co.) were used. The reported value of the Mark–Houwink constant was used for computing the molecular weight of the samples (10). The average molecular weights of PLA-5 and -6 were found to be 12,000 and 15,000, respectively.

Melting Temperature Range

The melting temperature of each of the PLA samples was found to be in a close range. On an average, the

melting point was found in between 80° and 100°. There was, on an average, an increasing trend in the melting temperature range with increase in molecular weight of PLA.

IR Spectra

Infrared (IR) spectra of the polymers were recorded on a Perkin-Elmer 1600 series (FTIR). The major absorbance peaks were seen at 845 (C-H band), 1075-1160 (C-O stretch), 1345-1435 (C-H band), 1585 (asymmetric COO-stretch), 1740 (C=O ester), and 3650 (OH- stretch) cm^{-1} in the spectra of all the PLAs and standard PLA sample.

Differential Scanning Calorimetry (DSC)

A Perkin-Elmer DSC-7 was used. The heating rate was kept at 10°/min for determining the glass transition temperature of PLA-6 and the microspheres prepared from it.

Preparation of Microspheres

Microspheres containing indomethacin were prepared by the oil/water (o/w) solvent evaporation technique. PLA-2 or PLA-6 (300 mg) and indomethacin (75 mg) were dissolved in cold CH_2Cl_2 (3 ml). The organic phase was added at a slow controlled rate (0.3 ml/min) from a microburette under stirring to cold aqueous phase (300 ml, 15°, 500 rpm) containing 0.5% PVP. The resulting biphasic product was stirred for 20 min at 500 rpm, and then the solvent evaporation was continued by stirring the contents at 150 rpm for 3 hr under vacuum (150 mm Hg) at 37°. The hardened microspheres were filtered, washed with demineralized water (500 ml), and finally dried under reduced pressure. Few variations were tried in formulations to study the effect on microspheres formation. The compositions of different batches are shown in Table 2.

Indomethacin Content

The drug content in the microspheres were assayed by ultraviolet (UV) spectrometry (11). Duplicate samples of microspheres (5 mg) were accurately weighed and extracted with 1 ml of CH_2Cl_2. To 0.1 ml of the solution, 3.9 ml of C_2H_5OH:phosphate buffer (pH 7.2) mixture (1:1) was added. The solution was subjected to high-speed vortexing and then centrifuged. The absorbance of the aqueous phase was measured at 319 nm on a Hitachi double-beam UV/Visible spectrophotometer. The obtained absorbance values were converted into

Table 2

Formulation and Regression Parameters (Higuchi model) for Microspheres

	Batch No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Indomethacin (mg)	75	75	75	75	75	75	75	75	75	75	75	75
PLA (mg)	300	150	150 (R)	300 (X)	150	150	150	150	150	150	150	150
Additive (mg)	—	—	—	—	—	—	11.25 PEG	37.5 IPM	37.5 IBM	11.25 LEC	16.87 CHL	11.25 CHL
Solvent	DCM	DCM	DCM	DCM	DCM + CH	DCM + LA	DCM	DCM	DCM	DCM	DCM	DCM
Theoretical drug content (%)	20	33.3	33.3	33.3	33.3	33.3	31.7	28.5	28.5	31.7	31	31.7
Slope	0.469	0.875	0.738	2.075	1.155	1.884	1.313	4.605	3.689	2.592	4.323	2.034
Intercept	12.04	14.793	7.191	18.438	12.626	32.99	23.41	6.787	19.31	27.281	9.195	13.5
Corr. Coeff.	0.8122	0.909	0.947	0.897	0.911	0.811	0.782	0.9827	0.8916	0.8687	0.9705	0.951
t_{50} (hr)	108.8	26.9	56	3.85	17.4	1.35	6.83	1.46	1.15	1.26	1.48	5.35

Note. R = resomer, X = PLA-2. All other batches were prepared using PLA-6. PEG = polyethylene glycol, IPM = ispropyl myristate, IBM = isobutyl myristate, LEC = lecithin, CHL = cholesterol, DCM = dichloromethane, CH = cyclohexane, LA = lauryl alcohol.

concentration values using the equation generated by weighted least square regression analysis (absorbance = 0.017 × concentration -0.03).

In Vitro Drug Release

The dialysis tube diffusion technique was used. Phosphate buffer (900 ml, pH 7.2, 37°) was used as the dissolution medium in a USP XXI basket apparatus. Microspheres were suspended in 5 ml of the dissolution medium and then the dispersion was placed in a dialysis bag (12000 Mw C). The bag was placed in a basket rotated at 100 rpm. Samples of the dissolution medium were collected at an appropriate time interval and analyzed at 319 nm for determining the drug content, after filtering the sample through 0.45-μ membrane filter. The volume was adjusted to 900 ml every time after collecting the sample. The percentage of the drug released was calculated using the equation shown under indomethacin content. The values of t_{50} (time required for 50% drug release) were calculated from regression data of the Higuchi model (19).

RESULTS AND DISCUSSION

PLA was successfully prepared using TPT or ZA as catalyst. The data shown in Table 1 indicate that regardless of the catalyst used, the molecular weight of PLA increased with increase in time of polymerization. The value of $\overline{M}_n$ estimated by terminal group analysis would differ from the value of average molecular weight estimated by GPC analysis since in the present case the GPC data are analyzed using standard data of a quite different polymer, polystyrene. The $\overline{M}_n$ value is nearly double the value of average molecular weight estimated by GPC method of analysis. Hence, the difference in the molecular weights of the samples measured by terminal group analysis and GPC (based on standard molecular weight data of polystyrene samples) is not surprising. Among ZA and TPT, the latter seems to be a better catalyst for ring-opening polymerization of lactide.

A single peak was seen in the chromatograms of PLA-5 and PLA-6. Hence, it may be concluded that the preparative method is efficient enough to yield PLA of required purity.

The IR spectra of all the synthesized polymers have the major peaks corresponding to that of Resomer. The melting points of PLA-5, PLA-6, and Resomer were found to be around 105°. The glass transition temperatures (T_g) of PLA-6 and microspheres of batch 1 were found to be 56.3° and 54.5°, respectively. The small difference between T_g values suggests that the secondary attractive interactions between PLA and the drug are rather weak.

When an o/w system is used, the solvent evaporation process is most successful for drugs that are insoluble in an aqueous medium. Poor drug loading may be noticed if the drug is hydrophilic in nature (12). Therefore, indomethacin was chosen as the model drug in the present investigation.

PLA-2, PLA-6, and Resomer were used for the preparation of microspheres of indomethacin by the solvent evaporation method. Methylene chloride has higher water solubility and lower heat of evaporation, and is reported as the best solvent for the preparation of PLA microspheres by the solvent evaporation method (13). In the preliminary investigations, few batches of microspheres were prepared at different rates of stirring. Microspheres could not be isolated when the speed of agitation was kept below 500 rpm due to aggregation. Over the range of 500 to 700 rpm, nearly spherical microspheres with narrow size distribution were obtained. As expected, the size of the microspheres was found to be dependent on the rate of agitation. PVP (0.5%) or PVA (0.25%) was tried in aqueous phase. When PVP was used, morphologically superior microspheres were obtained. A twin-blade stirrer, with both blades at an angles of 45°, yielded superior microspheres as compared to those obtained with a propeller stirrer.

In the in vitro dissolution test, at 2 and 4 hr, 85% and 100% of indomethacin was released, respectively, from pure indomethacin powder. Batches 1 and 4 were prepared using 1:4 drug:polymer ratio using PLA-6 (higher MW) and PLA-2 (lower MW), respectively. The values of calculated t_{50}, shown in Table 2, indicate that the drug is released at a very slow rate from both the batches. From batch 4, only 75% of the drug was released in 16 hr. The remaining batches were prepared using a 1:2 drug:polymer ratio to hasten the dissolution of indomethacin.

To investigate the effect of MW of PLA on the drug release pattern, batches 2 and 3 were prepared using PLA-6 (GPC-$\overline{M}$ 15,000) and Resomer (GPC-$\overline{M}$ 17,000), respectively. The drug was released at a slower rate from the preparation containing Resomer (22% in 24 hr) as compared to that from the preparation containing PLA-6 (42% in 24 hr). This is expected as the drug release is dependent on the molecular weight of the polymer used.

Cyclohexane, a poor solvent for PLA, is less volatile in nature than dichloromethane. Batch 5 was prepared using organic phase containing 10% cyclohexane. The in vitro dissolution pattern was almost identical in nature to that of batch 2. When 10% lauryl alcohol was used (batch 6), a high burst effect was noticed; i.e., in the first hour, 61% of the drug was released. Even when PEG4000, a pore-forming agent, was used at a level of 5%, the drug was slowly released (38% in 1 hr, 57% in 24 hr). In batches 8 and 9, isopropyl myristate and isobutyl myristate were incorporated at a level of 25% of PLA, respectively, to modify the morphology of microspheres and also the dissolution rate. It was noticed that a higher burst effect was seen when the myristate with longer carbon chain length was incorporated in the microspheres. Similar results are reported by Juni et al. (14). Both the adjuvants enhanced the drug release (>80% in 7 hr). Microspheres with sticky characteristics were obtained when lecithin was tried in the formulation. The batch of microspheres containing 5% cholesterol released the drug at a controlled rate (29%, 65%, and 85% in 1, 7, and 24 hr, respectively). The drug was completely released in 8 hr when cholesterol was added at a level of 7.5%. Batch 13 was prepared by dissolving both indomethacin and PLA (1:3) in CH_2Cl_2 and the solvent was later evaporated at room temperature. The dried powder was then used in the in vitro dissolution study. The drug was also released at a controlled rate (23% in 1 hr, 67% in 6 hr, 91% in 24 hr).

The present study reveals that the dissolution pattern of indomethacin is affected by additives. The pores formed during the evaporation of the organic solvent might have been filled with the adjuvants.

In Vitro Release Kinetics

The possible mechanisms of release from PLA and PLGA microspheres are summarized by Jalil et al. (15). The zero-order model and square root of time model were fitted to the dissolution data of all the batches. The zero-order model was found to be inapplicable to the data since the drug release was nonlinear ($r < 0.888$) but the square root of time model was acceptably linear ($r < 0.982$).

Benita (16) and Parikh et al. (5) suggested that the drug release data should be examined for applicability of more than one kinetic model for judicious choice of mechanism of drug release. Hence, The goodness of fit test proposed by Bamba and coworkers (17) was used to determine the mechanism of drug release. The linear first-order rate (18), Higuchi (19), zero-order (time vs. % drug unreleased), Hixon–Crowell (20), and power law (21) equations were fitted to the data of batch 12 and 13. Regression analysis ruled out the Hixon–Crowell and zero-order models for both the batches; i.e., they showed poor correlation as compared to the remaining three models. From the results of F test (Table 3), it may be concluded that there is no significant difference between Higuchi model and power law

Table 3

Comparison of Fits of Data Using Least Square Equations (Batch 12)

Sr. No.	Time (min)	% Drug Released	First-Order CCPR	First-Order Residual Square	Higuchi Model CCPR	Higuchi Model Residual Square	Power Law CCPR	Power Law Residual Square
1	0	0.00	29.14	849.14	13.51	182.52	8.21	67.40
2	30	25.08	31.38	39.69	24.65	0.18	24.93	0.02
3	60	29.41	33.54	17.06	29.27	0.02	31.26	3.42
4	180	40.53	41.54	1.02	40.80	0.07	44.72	17.64
5	300	59.70	48.58	123.65	48.74	120.12	52.85	46.92
6	420	65.74	54.77	120.34	55.20	111.09	58.98	45.70
7	540	68.14	60.22	62.73	60.78	54.17	64.02	16.97
8	1320	76.69	82.72	36.36	87.42	115.13	85.70	81.18
9	1440	85.77	84.80	0.94	90.70	24.30	88.17	5.76
SSR/DF				178.70		86.80		40.72

Note. CCPR = calculated cumulative % drug released; F = *SSR/DF*; F table (*DF*: 7) = 3.79; F med./min. = 2.13 (insignificant); F high/min. = 4.38 (significant).

Table 4

Comparison of Fits of Data Using Least Squares Equations (Batch 13)

Sr. No.	Time (min)	% Drug Released	First-Order CCPR	First-Order Residual Square	Higuchi Model CCPR	Higuchi Model Residual Square	Power Law CCPR	Power Law Residual Square
1	0	0.00	31.09	966.59	9.24	85.38	3.37	11.36
2	30	15.82	34.65	354.57	24.39	73.44	18.36	6.45
3	60	23.25	38.04	218.74	30.67	55.06	25.94	7.24
4	120	36.17	44.29	65.93	39.54	11.36	36.65	0.23
5	180	54.43	49.91	20.43	46.35	65.29	44.85	91.78
6	240	56.14	54.96	1.39	52.09	16.40	51.77	19.10
7	300	63.27	59.50	14.21	57.15	37.45	57.87	29.20
8	360	67.67	63.59	16.65	61.72	35.40	63.37	18.49
9	420	72.11	67.26	23.52	65.93	38.19	68.43	13.54
10	480	77.90	70.56	53.88	69.84	64.96	73.14	22.66
11	540	80.77	73.53	52.42	73.52	52.56	77.56	10.30
12	600	82.35	76.20	37.82	77.00	28.62	81.74	0.37
13	1440	91.77	94.63	8.18	114.21	503.55	126.46	1203.40
SSR/DF				166.76		97.06		130.37

Note. CCPR = calculated cumulative % drug released; $F = SSR/DF$, F table (DF: 11) = 2.85; F med./min. = 1.34 (insignificant); F high/min. = 1.71 (insignificant).

expression. To strengthen the claim of drug release by a diffusion mechanism, a differential form of the Higuchi equation ($dQ/dt = K_2^2 \times S^2/2Q$) was fitted to the data of batch 12. A linear relationship ($r = 0.9583$) was observed between the rate of release (y axis) and inverse of % of drug release (x axis); hence, it may be concluded that the drug is released by a diffusion mechanism from the microspheres. The data of batch 13 (Table 4) best fitted into the Higuchi model.

The results presented in this investigation indicate that indomethacin-loaded PLA microspheres with controlled-release characteristics can be successfully prepared using the synthesized polymer. The formulation and also the process variables should be precisely controlled to obtain satisfactory microspheres.

REFERENCES

1. D. L. Wise, T. D. Fellman, J. E. Sanderson, and R. L. Wentworth, in *Drug Carriers in Biology and Medicine* (G. Gregoriadis, ed.), Academic Press, New York, 1979, p. 237.
2. R. R. M. Bos, F. R. Rozema, G. Boering, A. J. Nijenhuis, A. J. Pennings, A. B. Verwey, P. Nieuwenhuis, and H. W. B. Jansen, Biomaterials, 12, 32 (1991).
3. E. Albizzati, G. D. Cella, M. Maestri, M. Alessiani, and P. Dionigi, in *Proceedings of USA/Italy Joint Workshop on Polymers for Biomedical Applications*, Capri, Italy, 1989, p. 1.
4. R. C. Straw, S. J. Withrow, M. F. Cooper, B. E. Powers, P. D. Schwarz, M. G. O'Brien, V. J. Jameson, M. H. Lafferty, E. B. Douple, and J. H. Brekke, Front Osteosarcoma Res., 121 (1993); through Chem. Abstr., 121, 238234 (1994).
5. B. V. Parikh, S. M. Upadrashta, S. H. Neau, and N. O. Nuessle, J. Microencapsulation, 10(2), 141 (1993).
6. R. K. Kulkarni, E. G. Moore, A. F. Hegyeli, and F. Leonard, J. Biomed. Mater. Res., 5, 169 (1971).
7. N. Marcotte and M. F. A. Goosen, J. Controlled Release, 9, 75 (1989).
8. R. K. Kulkarni, K. Pani, C. Neuman, and F. Leonard, *Polylactic for Surgical Implants*, Tech. Rep. 6608, Walter Reed Army Medical Center, Washington, DC, 1966.
9. B. Wichert and P. Rohdewald, J. Controlled Release, 14, 269 (1990).
10. J. A. Van Dijk and J. A. M. Smith, J. Polym. Sci. Polym. Chem., 21, 197 (1983).
11. *The United States Pharmacopoeia,* 22nd ed., U.S. Pharmacopoeial Con., 1990, p. 1784.
12. G. Splenlehauer, M. Veillard, and J. P. Benoit, J. Pharm. Sci., 75(8), 750 (1986).
13. R. Bodmeier and J. W. McGinity, Int. J. Pharm., 43, 179 (1988).

14. K. Juni, J. Ogata, N. Matsui, and M. Kubota, Chem. Pharm. Bull., 33(4), 1609 (1985).
15. R. Jalil and J. R. Nixon, J. Microencapsulation, 7(3), 297 (1990).
16. S. Benita, Appl. Biochem. Biotechnol., 10, 255 (1984).
17. M. Bamba, F. Puisieux, J. P. Marty, and J. T. Carstensen, Int. J. Pharm., 2, 307 (1979).
18. J. G. Wagner, Drug Intell. Clin. Pharm., 4, 132 (1970).
19. T. Higuchi, J. Pharm. Sci., 52(12), 1145 (1963).
20. A. W. Hixon and J. H. Crowell, Int. Eng. Chem., 23, 923 (1931).
21. R. W. Korsemeyer, R. Gurny, and N. A. Peppas, Int. J. Pharm., 15, 25 (1983).