International Journal of Pharmaceutics, 86 (1992) 107–111

IJP 02794

Optimization of dl-PLA molecular weight via the response surface method

Obdulia Munguia, Araceli Delgado, José Fariña, Carmen Evora and Matías Llabres

Departamento de Ingenieria Quimica y Tecnologia Farmaceutica, Universidad de La Laguna, Facultad de Farmacia,
38200 La Laguna, Tenerife (Spain)

(Received 10 October 1991)
(Accepted 27 January 1992)

Key words: Poly(dl-lactic acid); Optimization; Response surface; GPC; Tetraphenyl tin; Molecular weight

Summary

The influence of time, temperature and catalyst concentration (tetraphenyl tin) on the molecular weight of poly(dl-lactic acid) (dl-PLA) was studied in terms of the surface response method. 15 combinations of the above three factors with six replications of the central point were selected in accordance with a central composite rotable experimental design. A gel permeation chromatography (GPC) molecular weight determination method was also validated. The molecular weight for the number distribution ranged from 7077 to 100 835; the residual variance coefficient for the quadratic model was 23% and the correlation coefficient 0.883. Surface response was maximal at 9 h, 161°C and 0.14% catalyst, while the predicted weight average molecular weight was 173 000. These findings were confirmed.

Introduction

Poly(dl-lactic acid) (dl-PLA) is often proposed as a biodegradable excipient for microsphere and implant preparations. It can be obtained from dl-lactide via the ring-opening reaction with an acid or organometallic compound as catalyst (Kulkarni et al., 1971; Marcotte and Goosen, 1989). Although the preparation of dl-PLA has been well documented, little information is available on the regulation of its molecular weight, as the variables involved – most importantly, type and concentration of catalyst, time, temperature and concentration of lactic acid – have not been examined in sufficient detail for their effects to be quantified (Suzuki and Price, 1985; Marcotte and Goosen, 1989).

Our aim was to evaluate the response surface for the molecular weights of dl-PLA in terms of catalyst (tetraphenyl tin) concentration, polymerization time and temperature and to identify the conditions under which the maximum molecular weight is attained, using the polymerization method proposed by Kulkarni et al. (1966).

Correspondence to: M. Llabres, Departamento de Ingenieria Quimica y Tecnologia Farmaceutica, Universidad de La Laguna, Facultad de Farmacia, 38200 La Laguna, Tenerife, Spain.

Materials and Methods

dl-PLA was obtained by the ring-opening reaction as described by Kulkarni et al. (1966). Tetraphenyl tin (Merck) was used as catalyst, the monomer being 3,6-dimethyl-1,4-dioxan-2,5-dione (dl-lactide, Aldrich) recrystallized until the melting point of 124–126°C for the racemic mixture was reached. All the dl-lactide used in this study came from a single-batch crystallization.

5-g lots were prepared in long-necked glass flasks and an oil bath was used to control the temperature. The polymer was obtained by extraction with dioxane solution (Merck) and precipitation with water. It was then fractionated, dissolved in dichloromethane (Merck) and precipitated with methanol (Merck).

Determination of the molecular weights

The molecular weights were determined by gel permeation chromatography (GPC) (a Waters chromatograph consisting of two Model 510 pumps, a Wisp 710B injector, a Model 410 differential refraction index detectors, oven for columns and Model 730 data module). Four columns of 10^2, 10^3, 10^4 and 10^5 Å pore size (ultrastyragel)® in series were used with tetrahydrofuran (Merck) as solvent; the flow rate was 0.9 ml/min and the column oven temperature was maintained at 31°C. To calibrate the system, polystyrene monodisperse standards of the following molecular weights were used: 2800, 10300, 102000, 190000, 355000 and 710000 (Tokyo Soda Ltd). The standard samples were made up at 0.04% (w/v) and those of the other samples at 0.5% with 60 μl of injected sample.

The molecular weight determination method was tested in two stages; first, the response linearity was established and then the method error was calculated. The linearity of the method was established by taking four readings of the retention time for each of the polystyrene standards used. The deviation of the linear model – retention time vs the logarithm of the molecular weight – was monitored by analysis of the variance (ANOVA) (Draper and Smith, 1966). To calculate the error of the molecular weight determination method, four different samples of dl-PLA were analysed five times; the coefficient of variation for the method was determined from the mean square of the ANOVA error term.

Experimental design

To obtain the response surfaces, the quadratic model

$$Y = b_0 + \sum_{i=1}^{3} b_i \cdot x_i + \sum_{i=1}^{3} b_{ii} \cdot x_i^2 + \sum_{\substack{i=1\\ j=1\\ i \neq j}}^{3} b_{ij} \cdot x_i \cdot x_j + e$$

was used, where i and j assume the values 1, 2 and 3 for the variables, time, temperature and catalyst concentration, respectively, Y denotes the response (molecular weight) and e is the error.

A central composite rotable design was used which contained 15 different combinations of catalyst, time and temperature. The combination of conditions corresponding to the central point of the design was replicated six times in order to confirm the validity of the model and reduce the estimate variance of the values predicted by the quadratic model (Cochran and Cox, 1980).

Table 3 lists the values selected for the three independent variables studied; these values were set out in the sequence −1.682, −1, 0, 1 and 1.682. For the temperature (X_2) a central value equal to 160°C was chosen, equivalent to zero-level, and an 12°C interval. The central value of the catalyst concentration was 0.1% with an interval of 0.05%. Time was spaced logarithmically in order to extend the time scale to 18 h and achieve an asymmetrical distribution for the polymerization times, as prior information indicated that the optimal polymerization time would be 3–4 h.

Results and Discussion

The findings from the polystyrene models confirm the linear relationship between the retention time (t_r) and the logarithm of the molecular weight (M_w); the equation of the regression straight line was;

$$t_r = 56.22 - 5.25(\log(M_w)) \qquad (1)$$

TABLE 1

Analysis of variance of linear regression of calibration line (see text for more details)

Source of variation	Degrees of freedom	Mean square	F
Total	23		
Regression	1	480.70	6162
Residual	22	0.078	
Lack of fit	6	0.086	1.15
Pure error	16	0.075	

and the correlation coefficient, $r = 0.998$. The hypothesis of lack of fit to the model, assayed via ANOVA regression (see Table 1), provided further confirmation of the validity of the model.

Table 2 summarizes the results of the method error determination in which mean values and typical deviations obtained for each model, the mean square of the error of the one-way variance analysis and the method variation coefficient are given. To avoid errors of calculation we used molecular weights divided by 1000 in this and the following ANOVA. As can be seen, the coefficients of variation fall between 3.4 and 5.0 which can be deemed satisfactory.

The results obtained in the polymerization of dl-lactide and the analysis of the response surfaces are given in Tables 3–5. Table 3 lists the values of the number average molecular weight

TABLE 2

Mean and standard deviation of the four samples used for validation purposes, mean square from one-way ANOVA and coefficient of variation or error expressed as a function of overall mean

Mean	Sample				s^2	CV
	A	B	C	D		
M_n	54.964 (3.82)	49.151 (2.45)	78.447 (4.47)	102.39 (3.12)	12.58	5.0
M_w	115.57 (5.27)	107.86 (2.17)	150.47 (6.29)	299.66 (4.62)	23.33	3.4
M_z	188.43 (7.70)	183.76 (4.66)	241.73 (8.59)	299.66 (9.11)	59.41	3.4

TABLE 3

Molecular weight obtained as a function of polymerization conditions

No.	Time (h,min)	Temperature (°C)	Concentration (%)	M_n	M_w	M_z
1	1,50	148	0.05	144	144	144
2	10	148	0.05	51539	93487	166650
3	1,50	172	0.05	53210	114097	187407
4	10	172	0.05	78118	157685	257804
5	1,50	148	0.15	7077	8702	9989
6	10	148	0.15	80636	152998	243825
7	1,50	172	0.15	98583	194635	320000
8	10	172	0.15	61933	126795	202752
9	1	160	0.10	20473	58167	146280
10	18	160	0.10	77758	164102	268922
11	4,15	140	0.10	22715	49045	87219
12	4,15	180	0.10	49767	109183	190256
13	4,15	160	0.02	34365	52013	76802
14	4,15	160	0.18	100835	182568	287547
15	4,15	160	0.10	76902 [a] (4081)	151178 [a] (26172)	225436 [a] (37434)

[a] Mean and S.D. of six samples.

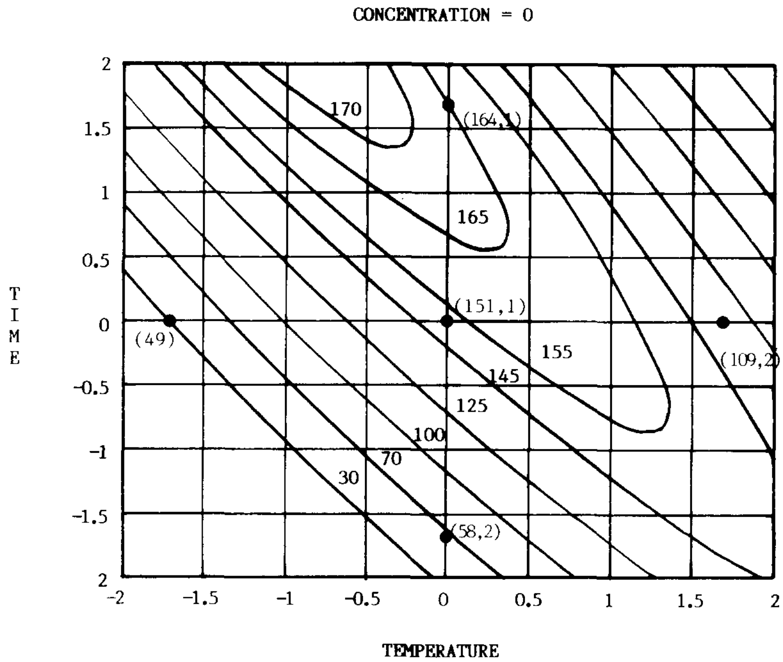

Fig. 1. Response surface of weight average molecular weight ($\times 10^{-3}$) as a function of coded variables time and temperature and zero level of catalyst concentration. Values in parentheses correspond to experimental points (●).

(M_n), weight average molecular weight (M_w) and Z averaging molecular weight (M_z). The combination of time, temperature and catalyst concentration no. 1 did not lead to the polymerization of dl-lactide as the only peak observed in the chromatogram was that corresponding to a molecular weight of about 144. The other combinations of the process variables afforded a broad range of molecular weights from 7077 to 100 835 for M_n, 8702 to 194 635 for M_w, and 9989 to 320 000 for M_z. The six replicates obtained for the central point of the experimental design ranged between 69 708 and 81 672 for M_n, 118 715 and 193 303 for M_w, and 170 727 and 272 455 for M_z.

The values for polydispersity equal to M_w/M_n varied from 1.51 to 2.84 and were not included in the statistical analysis, since the polymerization product had been fractionated.

Table 4 gives the results of the regression variance analysis for the three mean weights; as above, the molecular weights were divided by 1000 to reduce the risk of calculation error. The residual variances were similar for the three molecular weights when expressed coefficients of variation in relation to the mean of all the observations, and equal to 23, 27 and 27% for M_n, M_w and M_z, respectively. The calculated correlation coefficients, R^2, were 0.883, 0.836 and 0.826 for M_n, M_w and M_z, respectively. These data justify the use of the quadratic model for the analysis of the response surfaces, especially bearing in mind

TABLE 4

Analysis of variance of regression for linear quadratic model used for response surface analysis for each molecular mean

Source of variation	df	Mean squares		
		M_n	M_w	M_z
Regression				
Linear	3	2 792 [b]	11 045 [b]	26 897 [b]
Quadratic and cross terms	6	994.0 [a]	3 378 [a]	7 085
Residual	10	189.8	1 047	1 593
Lack of fit	5	363.0 [b]	1 408	3 785
Pure error	5	16.65	686.3	1 401

[a] Null hypothesis rejected at $\alpha = 0.05$ level.
[b] Null hypothesis rejected at $\alpha = 0.01$ level.

TABLE 5

Estimated coefficients of quadratic model used for response surface analysis

Coefficient	M_n	M_w	M_z
b_0	76.6	150.0	225.4
b_1	15.3 [b]	29.4 [b]	41.0 [a]
b_2	14.5 [b]	31.4 [b]	52.7 [b]
b_3	13.0 [b]	24.0 [a]	38.0 [a]
b_{11}	−8.88 [a]	−12.5	−6.22
b_{22}	−13.4 [b]	−23.8 [a]	−30.6 [a]
b_{33}	−2.34	−10.3	−15.2
b_{12}	−17.1 [b]	−34.0 [a]	−55.9 [a]
b_{13}	−4.92	−8.79	−15.0
b_{23}	−0.86	−1.07	−1.18

[a] Null hypothesis rejected at $\alpha = 0.05$ level.

[b] Null hypothesis rejected at $\alpha = 0.01$ level.

that the time-temperature profile inside the reaction flask could not be rigorously controlled in the polymerization process due to its heterogeneous nature.

Nonetheless, the lack of fit for M_n was significant; this is the result of the fact that the value obtained for the mean square of pure error was unexpectedly low, being the equivalent of a coefficient of variation of 5.3% with regard to the average of the six replicated samples, while that for the M_n determination method calculated in this study is equal to 5.0% (see Table 2).

For M_n and M_w, the null hypothesis was rejected for linear terms at a significance level of $\alpha \leqslant 0.01$, and $\alpha \leqslant 0.05$ for the quadratic coefficients and crossed products. For M_z the null hypothesis was rejected only for the linear terms due to the increase in the residual variance.

Table 5 sets out the coefficients of the quadratic model for each molecular weight together with the individual significance level. Fig. 1 depicts part of the response surfaces obtained.

The quadratic equation for M_n has a maximum for 9 h of polymerization at 161°C with 0.14% catalyst and the predicted molecular weight, 173300. To confirm this, three other samples of dl-PLA were prepared under identical experimental conditions; the molecular weights obtained were 185518, 204148 and 227527. The mean value, 205731 was 18.7% more than expected.

The findings of this study cannot be generalized given the heterogeneous nature of the polymerization reaction of dl-lactide and the variability in heat transfer from the oil bath to the product being polymerized. The presence of small amounts of lactic acid inhibits the process of polymerization (Hutchinson and Furr, 1985). Notwithstanding, from the data obtained, it may be concluded that the polymerization reaction can be adequately controlled by means of temperature, time and catalyst concentration, as can the ideal conditions for attaining the desired molecular weight for the polymer.

Acknowledgment

This research has been financed by the Comunidad Autónoma de Canarias (Spain) as part of Project no. 46/87.

References

Cochran, W.G. and Cos, G.M., *Diseños Experimentales,* Trillas, México, 1980, p. 372.

Draper, N.R. and Smith, H., *Applied Regression Analysis,* Wiley, New York, 1966, p. 1.

Hutchinson, F.G. and Furr, B.J.A., Biodegradable polymers for the sustained release of peptides. *Biochem. Soc. Trans.,* 13 (1985) 520–523.

Kulkarni, R.K., Moore, E.G., Hegyeli, A.F. and Leonard, F., Biodegradable poly(lactic acid) polymers. *J. Biomed. Mater. Res.,* 5 (1971) 169–181.

Kulkarni, R.K., Pani, K.C., Newman, C. and Leonard, F., Polylactic acid for surgical implants. *Arch. Surg.,* 93 (1966) 839–843.

Marcotte, N. and Goosen, M.F.A., Delayed release of water-soluble macromolecules from polylactide pellets. *J. Controlled Release,* 9 (1989) 75–85.

Suzuki, K. and Price, J.C., Microencapsulation and dissolution properties of a neuroleptic in a biodegradable polymer, poly(dl-lactide). *J. Pharm. Sci.,* 74 (1985) 21–24.