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Effects of polymerization variables on PLGA properties: molecular weight, composition and chain structure

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Summary

In order to study the influence of the variables which control the properties of polylactic-glycolic acid two experiments were conducted according to appropriate experimental designs. The responses studied were yield, weight average molecular weight (GPC), relative proportion of lactic and glycolic acids in the polymer (¹H-NMR) and the ratio of lactic-glycolic to glycolic-glycolic units (¹³C-NMR). The variables included in the first study were the relative proportion of lactide and glycolide in the mixture and the concentrations of lauryl alcohol and stannous octoate; the results showed a statistically significant influence of the relative proportions of lactide and glycolide in the mixture and the concentration of lauryl alcohol on the lactic/glycolic proportion in the polymer. The second study involved time, temperature and lauryl alcohol concentration as variables. The findings showed a statistically significant negative effect of temperature on weight average molecular weight and of temperature, time and time-temperature interaction on yield. Ratios between lactic-glycolic and glycolic-glycolic units ranged from 1:1 to 1:4. None of the variables studied had any effect on this characteristic.

Introduction

Lactic and glycolic acid copolymers (PLGA) have been much discussed because of their applications to the development of systems for the sustained release of drugs (Reed and Gilding, 1981; Kitchell and Wise, 1985; Ogawa et al., 1988a–c). Some of their advantages stem from the fact that they can affect the drug release rate

according to molecular weight, monomer proportions, crystallinity and the presence of polyglycol blocks (PGA).

This last feature has received particular attention due to the influence it has on the properties of the polymers affecting their solubility (Bendix, 1990) and degradative hydrolysis rate (Hutchinson, 1982) as the result of the crystallinity of the PGA blocks and the enhanced reactivity of the glycol-glycol bond. For this reason the study of the variables of the polymerization process which affect the formation of random copolymers, otherwise known as homogeneous copolymers, or block (heterogeneous) copolymers is of interest.

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Various authors have studied these variables (Bendix, 1990; Avgoustakis and Nixon, 1991) but they did not have an appropriate experimental design which would allow the different variables and their interactions to be studied in their entirety. This paper is an account of the results of two experiments to obtain PLGA by via a ring-opening reaction. The first used the relative ratio of monomers and the concentrations of catalyst and chain regulator as variables and the second, the temperature, time and concentration of chain regulator.

Materials and Methods

Synthesis

The PLGA copolymers were obtained by ring-opening polymerization of the appropriate cyclic dimers following the method described by Gilding and Reed (1979). dl-Lactide (Aldrich) was recrystallized in ethyl acetate at room temperature until the racemic mixture melting point was attained (124–126°C) and the glycolide (Boehringer Ingelheim) was used directly (m.p. 88–90°C). Stannous octoate and lauryl alcohol (both from Sigma) were used as catalyst and chain regulator, respectively.

Batches contained initial proportions (w/w) of 75:25 and 50:50 dl-lactide:glycolide in long-necked glass flasks and an oil bath was used to control temperature. The copolymer was extracted by dissolution in chloroform (Merck) followed by precipitation with methanol (Merck).

Characterization of the copolymers

The molecular weights were determined by gel permeation chromatography (GPC) (a Waters chromatograph consisting of two Model 510 pumps, a Wisp 710 B injector, a Model 410 differential refractive index detector, oven for columns and Model 730 data module). Four columns of 10^2 , 10^3 , 10^4 and 10^5 Å pore size (Ultrastrygel) 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 fol-

lowing molecular weights were used: 2800, 10 300, 102 000, 190 000, 355 000 and 710 000 (Tokyo Soda Ltd). The standard samples were made up at 0.04% (w/v) and those of the other samples at 0.5% w/v in filtered and degassed tetrahydrofuran, 60 µl of injected sample.

The copolymer composition was determined by $^1\text{H-NMR}$ (Gilding and Reed, 1979) using a Bruker AC spectrometer at 200 MHz. Samples were dissolved in CDCl_3 and the composition given, in moles of lactic-glycolic acid, was measured from the relationship of the areas of the signals obtained at δ 4.82 (methylene group of the glycol acid unit) and δ 5.21 (methine group of the lactic acid unit). The samples were prepared by dispersing 15 mg of polymer, in CDCl_3 and gently heating. Due to the low solubility of the polymer, the spectra had to be obtained by scan accumulation over different periods of time, of up to about 15 min.

The relative proportions of the lactic acid-glycolic acid units (LA-GA) and glycolic acid-glycolic acid (GA-GA) were assessed by $^{13}\text{C-NMR}$ (Bendix, 1990) on a Bruker AC at 50.32 MHz and Bruker AMX at 100.61 MHz using DMSO-d_6 solvent. Signals at 166.7 and 166.8 ppm were associated with the carbonyl groups of the LA-GA and GA-GA units, respectively; the relative proportions of the LA-GA and GA-GA units were determined from the relation of the relative intensities of these signals.

Experimental design

Two studies were carried out, one involving the concentrations of catalyst (C_{CAT}) and lauryl alcohol (C_{LA}) and the proportion of monomers in the mixture (LAC/GLI) as polymerization variables. A 2^2 factorial design was tried first, using the first and third variable, and then two further combinations were introduced increasing the concentration of lauryl alcohol in those mixtures with a high proportion of dl-lactide. Temperature (180°C) and polymerization time (2 h) remained constant. Although the introduction of two extra points into the original experimental design precludes statistical analysis by the conventional method (analysis of the variance, ANOVA), it does not prevent the linear model being evalu-

TABLE 1

Findings of the first study as a function of polymerization conditions (180°C and 2 h)

Lot	C_{CAT} (%)	C_{LA} (%)	LAC/GLI	1H -NMR	^{13}C -NMR	M_w	pd	% R
A-1	0.1	0.01	50:50	47:53	1:1.5	N.D.	N.D.	N.D.
A-2	0.1	0.01	75:25	72:28	1:2	12814	1.67	72
A-3	0.03	0.01	50:50	47:53	1:4	N.D.	N.D.	N.D.
A-4	0.03	0.01	75:25	70:30	1:2	11355	1.86	67
A-5	0.1	0.1	50:50	41:59	1:3	N.D.	N.D.	N.D.
A-6	0.03	0.1	50:50	42:58	1:2.5	N.D.	N.D.	N.D.

N.D., not determined; pd, polydispersity; % R, polymerization yield. See text for more details.

ated by multiple linear regression (Draper and Smith, 1966).

The second study was based on a factorial 2^3 design with temperature (160 and 180°C), polymerization time (2 and 4 h) and chain regulator concentration (0.01 and 0.05% of lauryl alcohol) as the variables studied. Two of the polymerization conditions were replicated to obtain an independent measurement of the variability of the process. The original proportion of monomers and the catalyst concentration, 0.03% stannous octoate, remained the same. Statistical analysis of the factorial design followed the standard pattern (Winer, 1971). In order to increase the degrees of freedom of the error term, the terms due to the interaction between the factors studied were omitted once it had been demonstrated that they were not statistically significant.

Results and Discussion

Tables 1 and 2 summarize the results of the first study and highlight the strong dependency of

the copolymer composition on the original mixture, quite independently of the greater reactivity of glycolide and the negative effect exercised by the lauryl alcohol on the lactic acid content (see Fig. 1) which indicates a preferred action on the reactivity of the lactide. The microstructure of the copolymer defined through the proportion of sequences LA-GA/GA-GA oscillated between 1:1.5 and 1:4.0, however, we were not able to find a statistically significant relationship between these alterations and polymerization conditions of the first study (variables, concentration of stannous octoate as catalyst, lauryl alcohol concentration and composition of the monomer mixture). Due to the low solubility of some of the lots in the solvent used for GPC only weight average

TABLE 2

Multiple linear regression for mol% LA

	Estimated coefficients	Standard error	Student's <i>t</i> -test	Probability
b_0	28	2.858	9.80	0.013
C_{CAT} (%)	0.333	0.882	0.38	0.742
C_{LA} (%)	-5.5	1.080	-5.09	0.037 ^a
mol% LAC	24	1.080	22.22	0.002 ^a

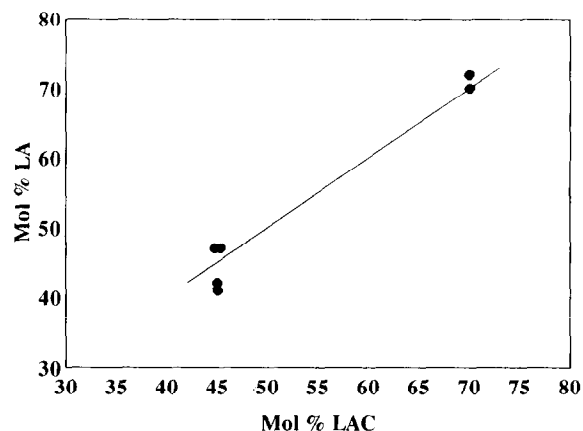
 b_0 , intercept.^a Null hypothesis rejected at α 0.05 level.

Fig. 1. Negative effect of lauryl alcohol on PLGA lactic acid content. Abscissa, composition of monomers mixture; ordinate, polymer composition. Points below line correspond to high lauryl alcohol level.

TABLE 3

Findings from factorial 2^3 design (LAC / GLI: 75 : 25 and C_{CAT} : 0.03%)

Lot	T (°C)	t (h)	C_{LA} (%)	M_w	pd	1H -NMR	^{13}C -NMR	% R
B-1	160	2	0.01	47 815	1.82	68 : 32	1 : 2	73
B-2	160	2	0.05	42 790	1.86	67 : 33	1 : 2	71
B-3	180	2	0.01	46 081	2.10	66 : 34	1 : 2	90
B-4	180	2	0.05	34 896	1.91	69 : 31	1 : 2	84
B-5	160	4	0.01	40 268	2.10	67 : 33	1 : 2.5	94
B-6	160	4	0.05	42 959	1.91	70 : 30	1 : 1.5	97
B-7	180	4	0.01	32 218	2.04	60 : 40	1 : 1.5	93
B-8	180	4	0.05	31 081	1.91	70 : 30	1 : 2	90
B-9	160	4	0.01	54 387	1.95	66 : 34	1 : 2	79
B-10	180	4	0.01	35 857	2.10	68 : 32	1 : 1	79

molecular weight (M_w) could be determined for lots A-2 and A-4.

Table 3 lists the findings from the second study. Lots B-9 and B-10 were obtained under identical conditions to lots B-5 and B-7, respectively, with the exception of the use of a different batch of dl-lactide.

The data obtained from lots B-1–B-8 were analysed by a factorial 2^3 ANOVA design as described above. The results are given in Table 4. Although M_w was seen to decline in relation to increases in temperature (mean values equal to 43 500 and 36 000 at 160 and 180°C, respectively), time (42 900 as opposed to 36 000 for 2 and 4 h, respectively) and lauryl alcohol concentration (41 600 instead of 37 900 for 0.01 and 0.05%, respectively), only the temperature was statistically significant. It is therefore to be expected that a greater temperature interval would produce a reduction in M_w , as has been commented elsewhere. For the response percent of moles of lactic acid (mol% LA), only with lauryl alcohol

concentration was a probability near to significance level attained (0.056 compared with $\alpha = 0.05$).

Polymerization yield, however, showed strong dependence on time ($p < 0.05$), temperature ($p < 0.05$) and interaction between two ($p < 0.05$) if replicated points are omitted, as can be seen in Fig. 2. Although the experimental design used was not suitable for process optimization, the data obtained indicate that a relatively low temperature (160°C) and relatively long time (4 h) are the best conditions for maximum yields. It may well be that they favour polymerization rather than depolymerization. No significant effect was recorded for lauryl alcohol.

The other variables, polydispersity, lactic/glycolic acid ratios in the chain or polymer microstructure measured by the quotient LA-GA/GA-GA were not affected by the variables under study.

The ^{13}C -NMR spectra are another matter. Hutchinson (1982) defined heterogeneous 'block'

TABLE 4

Analysis of variance of linear regression for factorial 2^3 design

Source of variation	Degrees of freedom	Mean square			
		M_w	pd ($\times 10^4$)	mol% LA	GA-GA
T (°C)	1	231.8 ^a	176.4	0.1	0.26
t (h)	1	44.7	86.0	5.8	0.15
C_{LA} (%)	1	72.8	286.0	45.8 ^b	0.0
Error	6	27.7	66.9	8.2	0.19

^a $p < 0.05$; ^b $p = 0.056$.

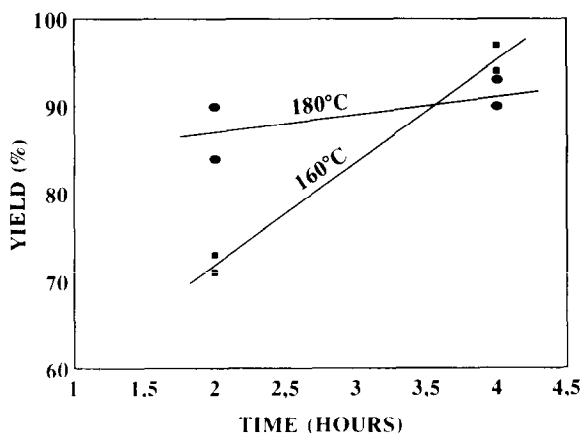


Fig. 2. Effect of time, temperature and time-temperature interaction on polymerization yield.

polymers as those which had a ^{13}C -NMR signal as a pair of doublets for the glycolide carbonyl group; the same criterion was also applied by Avgoustakis and Nixon (1991) but Bendix (1990), running a spectrometer at 75 MHz, did not observe any differences between the spectra of different PLGA even though the LA-GA relationship oscillated between 1:4.6 and 1:1, characteristic of heterogeneous and homogeneous polymers, respectively. Nor when the spectra were run at 50.32 and 100.61 MHz for values between 1:4.0 (lot A-3, see Fig. 3) and 1:1 (lot B-10, see Fig. 4) did we find the differences described by Hutchinson. In our view, Hutchinson's terminology is confusing as the doubling of the carbonyl

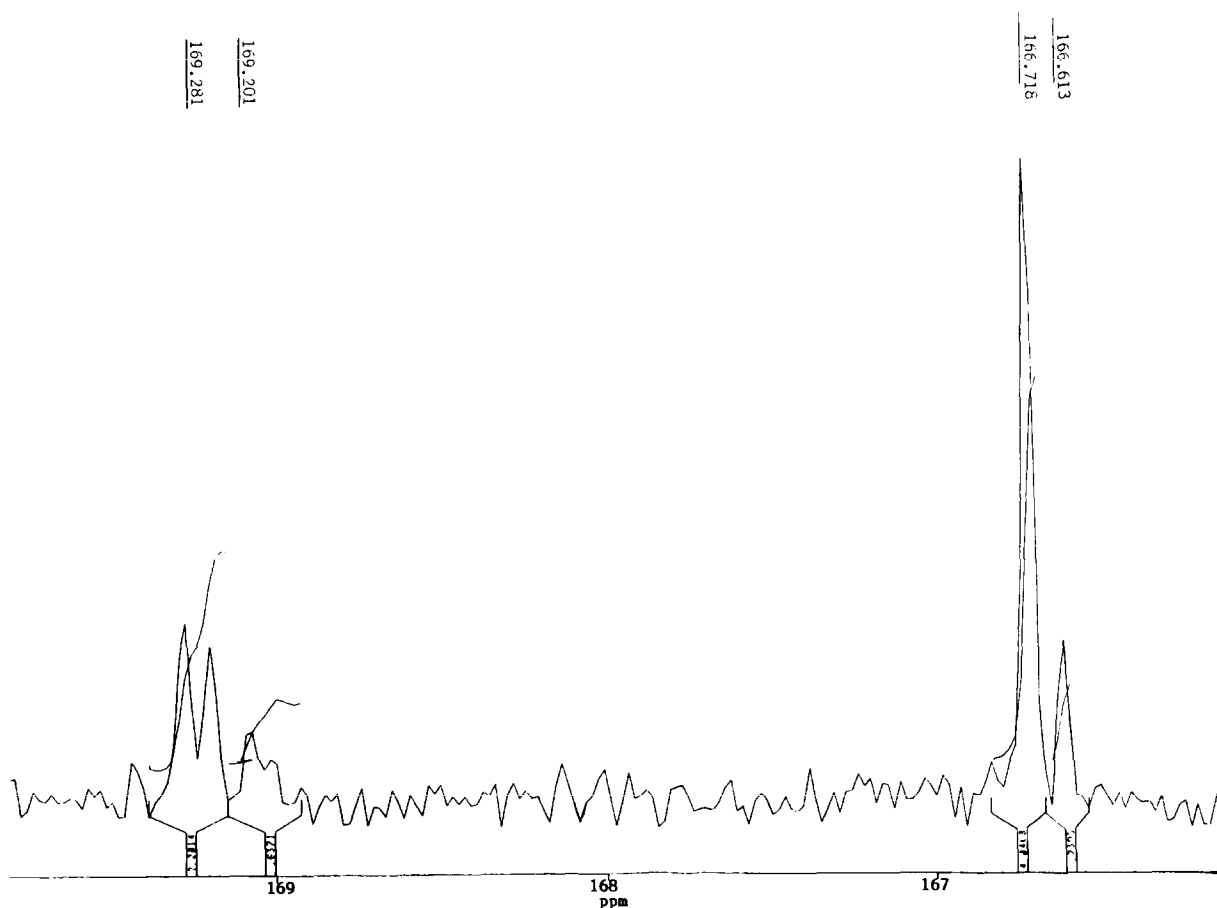


Fig. 3. ^{13}C -NMR spectrum of 50:50 LAC/GLI initial (w/w, lot A-3) copolymer in DMSO-d_6 .

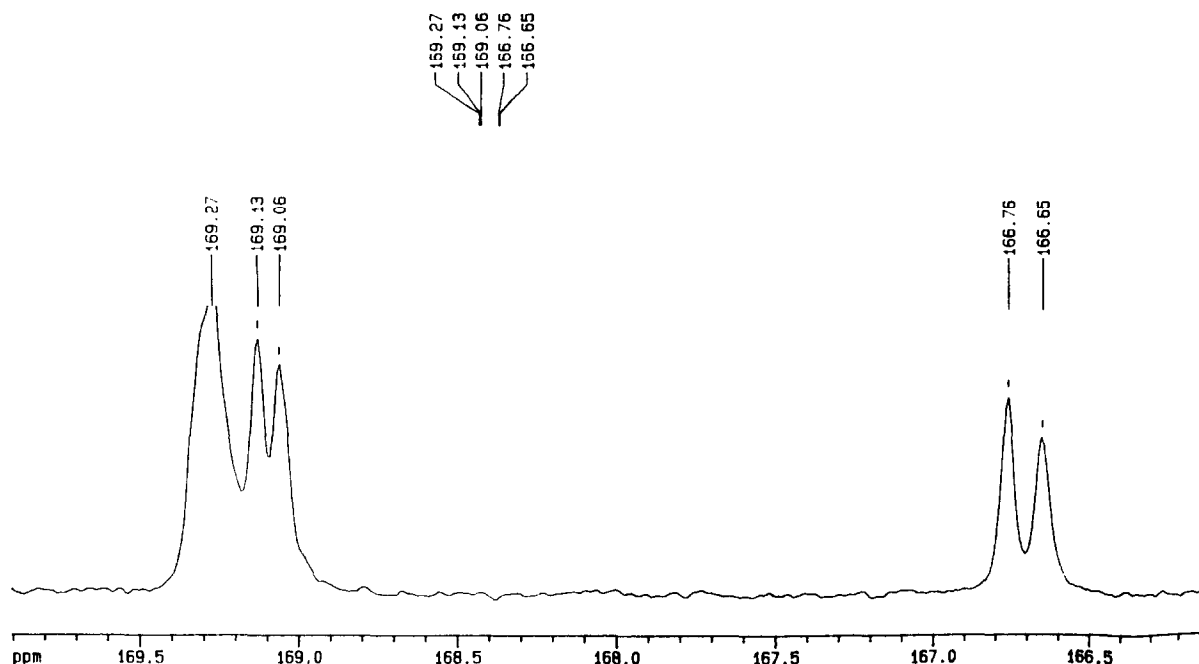


Fig. 4. ^{13}C -NMR spectrum of 75:25 LAC/GLI initial (w/w, lot B-10) copolymer in DMSO-d_6 .

group signal, as he reported it, is not likely and has not been observed under our experimental conditions. To summarize, the degree of polymer heterogeneity could only be determined from the signals corresponding to the LA-GA and GA-GA units.

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