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LACTOYL-LACTIC ACID DETERMINATION OF DL-LACTIDE AS STARTING MATERIAL IN THE SYNTHESIS OF POLYLACTIC ACID

A. Diaz, O. Munguía, J. Fariña and M. Llabrés

Departamento de Ingeniería Química y Tecnología Farmaceutica.

Facultad de Farmacia.

Universidad de La Laguna. 38200 La Laguna. Tenerife. Spain.

ABSTRACT

This paper reports the determination of lactoyl-lactic acid in dl-lactide using a kinetic interpretation of their peak heigths ratio obtained by HPLC. Lactoyl-lactic acid was the only acid compound identified by ^{1}H-MNR as impurity in dl-lactide stored in tigthy closed recipients as well as the only compound that appears during the hydrolysis of dl-lactide in mobile phase when process was studied along five hours. Well resolved and highly symetrical chromatograms showed that the hydrolisis do not take place inside the chromatography column and therefore a simple catenary model was used to relate the peak ratio to the mole fraction of lactoyl-lactic acid in the sample.

INTRODUCTION

The use of polylactic acid (PLA) in the manufacture of pharmaceutical controlled-release dosage forms, bone implant fabrication, and surgical suturing is justified by their biocompatibility, good handling and biodegradability (1-3), being its chemical(i.e. biodegradability) and mechanical properties highly related to PLA average molecular weights and molecular weights distribution. The ring opening polymerization reaction leads to the production of high molecular weight PLA (4), but as for other polymers, there is a dependence of polymer molecular weight distribution on the purity of the starting material (5-7).
Water and the hydrolisis products, lactoyl-lactic acid (LLA) and lactic acid, have been refered as inhibitors of the polymerization reaction. Avgoustakis et al. (8) related the specific surface of lactide crystals to the degree of alteration caused by humidity

lactide crystals to the degree of alteration caused by humidity traces. Zhang et al. (9) related the possible origin of the poor polymerization reproducibility with the presence of H_2O in the lactide or its hydrolysis products such as lactoyl-lactic acid or lactic acid. Hutchinson et al.(7) studied the inhibitory effect of lactic acid on the polimerization reaction.

In spite of the importance of these acid compounds on the polymerization yield and final molecular weight distribution of PLA, there is not any published method related to its quantification in the lactide. The main difficulties in developing an analytical method able to detect both lactoyl-lactic acid and lactic acid in the starting material is the rapid hydrolisis of lactide once it is dissolved. To overcome this problem we propose a method which account the fraction of lactide hydrolysed prior to the analysis.

EXPERIMENTAL

Both dl-lactide (LA) and lactoyl-lactic acid were determined using the Sawan & Barry s HPLC technique (11). A Water Millipore apparatus consisting of a pump, model 600E Multisolvent Delivery System, a UV-VIS detector, model 490E Programmable Multiwavelength Detector and data adquisition sofware, Maxima 820, was used. Mobile phase was acetonitrile (Merck)-water (20:80) with 0.1 % w/v of trifluoroacetic acid (Janssen) at 1 ml/min. A µ-Bondapack C-18 column (Waters Millipore) was used . The identity of eluted peaks was confirmed by ^1H NMR using a Bruker AMX spectrometer at 400 MHz with $CDCl_3$ as solvent.
Differents batches of dl-lactide (Aldrich) were recrytallized in ethyl acetate (Merck) until the racemic mixture reached its melting point (124-126°C). The lactides batches 1, 2, 3 and 4 were stored in tighted recipients, light protected at room temperature along 0, 75, 131 and 57 days respectively.

KINETIC MODEL AND STATISTICAL ANALYSIS

In aqueous solutions, lactoyl-lactic acid and lactic acid are produced from dl-lactide following a irreversible catenary model,

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

where A, B and C represent the dl-lactide, lactoyl-lactic acid and lactic acid moles respectively. Along the experiment conducted in this work, (five hours) no lactic acid was detected while lactoyl-lactic acid levels aproached a plateau; therefore , k_2 was set equal to zero. Let a and b the peak height measured by HPLC for dl-lactide and lactoyl-lactic acid respectively, γa and γb the ratio between peak height and molar concentration for each compound, and $\gamma = \gamma a/\gamma b$. Peak heights for dl-lactide and lactoyl-lactic acid are given by the equations

$$a = a_o \cdot e^{-k_1 \cdot t} \qquad (eq.1)$$

$$b = b_o + \frac{a_o}{\gamma} \cdot (1 - e^{-k_1 \cdot t}) \qquad (eq.2)$$

Kinetic parameters were estimated fitting simultaneously peak heights for dl-lactide and lactoyl-lactic acid by least squares non-linear regression method (12) using equations 1 and 2. Once proper estimates of k_1 and γ were available, the mole fraction of LLA present in the sample (i.e. $B_0/(A_0 + B_0)$) can be calculated using the equation,

$$f = 1 - \frac{e^{k_1 T}}{1 + \frac{r}{\gamma}} \qquad (eq.3)$$

where T is the elapsed time from lactide dissolution to its analysis and r is the peak heights ratio(LLA/LA).

The error involved in determination of f, is given by the equation,

$$var(f) = var(k_1)\left(\frac{\delta f}{\delta k_1}\right)^2 + var(\gamma)\left(\frac{\delta f}{\delta \gamma}\right)^2 + var(r)\left(\frac{\delta f}{\delta r}\right)^2 \qquad (eq.\ 4)$$

Variances for k_1 and γ were computed from four kinetic experiments carried out using dl-lactide batchs 1 and 2. Peak ratio variance at selected time 26.0 min, was estimated in a second experiment already designed to estimate both between-day and within-day components of variance(13). Six determination for each dl-lactide batches (3 and 4), were made in two different days.

RESULTS AND DISCUSSION

Figure 1 sets out the chromatograms for commercial dl-lactic acid and purified dl-lactide . Chromatogram A shows two peaks for dl-lactic acid (retention time, t_R, 3.8 min) and its impurity, eluted at 5.6 min, identified as lactoyl-lactic acid by ^1H NMR, ruling out the presence of another possible impurity. Chromatogram B also has two peaks lactoyl-lactic acid (t_R = 5.6 min) and dl-lactide (t_R = 9.5 min). Chromatogram C shows how the height of both peaks was affected by the time course, with the lactide peak diminishing and the lactoyl-lactic acid peak growing; after enough time (37 hours) a third peak elute at 3.9 min (lactic acid). Peaks for dl-lactide and LLA were well resolved and highly symetrical, excluding the possibility of hydrolysis along the chromatographic separation. We can conclude that the aforementioned kinetic model is adecuated to describe the hydrolisis of dl-lactide in dissolution.

Table 1 shows the estimated kinetic parameters(equations 1 and 2) for the different lactides batches, the reduced model (doing b_0 = 0) was tested and accepted (14).

Table 2 sets out the average and standard desviation obtained for each peak as well as their ratio, and table 3 shows the results

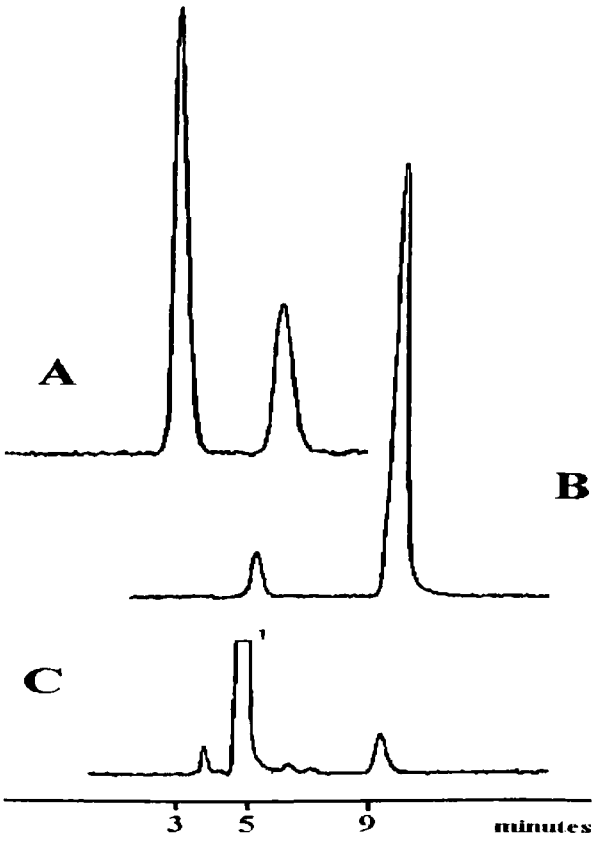

FIGURE 1. Chromatograms taken after the analysis of samples of commercial lactic acid (A) and purified dl-lactides before hydrolysis (B) and after (C) ([1] saturated peak).

TABLE 1. Estimated Kinetic Parameters for dl-Lactides Batches 1 and 2.

LACTIDE	DAY	$K * 10^3$ (min^{-1})	$A_0 * 10^{-3}$	γ
1	1	2.541	10.19	1.996
2	1	2.847	10.28	1.790
1	2	2.776	10.03	1.915
2	2	2.835	10.23	1.771
Mean Values±SD (n=4)		2.749±0.142	10.18±0.11	1.868±0.107

TABLE 2.- Response for dl-Lactides 3 and 4 (Given as Peak Height (μV) of Lactide and Lactoyl-Lactic Acid) after Analysis of 6 Replicates of each on Different Days.

Batch	Day	Peak Heights(μV)±SD		
		Lactide	LLA	r
3	1	9125±412	1063±71	0.1165±0.00385
3	2	9245±71	1056±71	0.1142±0.00713
4	1	9632±87	719±48	0.0747±0.00522
4	2	9641±115	715±35	0.0742±0.0032

TABLE 3.- ANOVA Results of the Comparison of dl-lactides batches 3 and 4.(p, α = 0.05).

SV	SS	DF	MS	F	p
DAY	$1.19.\ 10^{-5}$	1	$1.19.10^{-5}$	0.459	0.5059
LACTIDE	1.10^{-2}	1	1.10^{-2}	388	< 0.05
INTERACTION	$4.43.10^{-6}$	1	$4.43.10^{-6}$	0.171	0.6836
ERROR	$5.17.10^{-4}$	20	$2.59.10^{-5}$		
TOTAL	$1.06.10^{-2}$	23			

of the analysis of variance. As can be seen, from ANOVA findings null hypothesis about between-day variance has been accepted, concluding good stability of the HPLC method. However, the difference between both batches of dl-lactide was relevant and statistically significant, leading to the conclussion that this method could be used for lactoyl-lactic acid determination in lactide.

Figure 2 shows the predicted mole lactoyl-lactic acid fraction in sample (ordinate) in function of peak ratio (abcise) together with ± 2σ confidence curves computed from equation 3. A thereshold

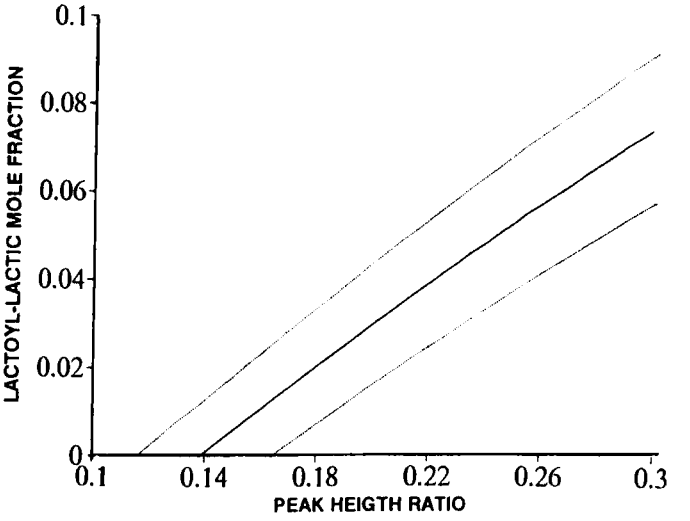

FIGURE 2. Predicted mole lactoyl-lactic acid fraction in sample (ordinate) in funtion of peak ratio (Abcise) together with ±2σ confidence curves computed from equation 3.

value for peak ratio equal to 0.132, is expected when no lactoyl-lactic acid is present in the sample and lactoyl-lactic acid is only produced by hydrolisis of dl-lactide in solution. However, detection limit is larger, because only when peak ratio is larger than 0.165 and the predicted mole fraction of lactoyl-lactic acid more than 0.01 aproximatelly, confidence limits exclude zero or negative values(see figure 2). All individual values for batch 3 and all except one for batch 4 fell below the detection limit, so the quantification of the lactoyl- lactic acid is not possible. However the proposed method lead us to differentiate two lactides with a diffference of 74 days in the storage time using the peak heigths ratio at 26 minutes.

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