

HYDROGEN ISOTOPE EXCHANGE AS A MEANS OF LABELING LACTIDES

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SUMMARY

Lactic acid-containing polymers obtained by the ring opening polymerization of lactides are now commercially available as bioresorbable polymers, or as devices that can be assimilated by the human body, or by living species present in the outdoor environment. Studying the mechanisms of lactide polymerization or polylactide degradation and monitoring the fate of the degradation compounds produced from the polymers when they degrade in complex media is thus very important and basically feasible via the labeling of polymer chains. Therefore, the possibility of exchanging some of the protons borne by lactide monomer molecules by deuterium or tritium was investigated. The substitution reactions involving deuterium were conducted and optimized using the High Temperature Solid State Catalytic Isotopic Exchange (HSCIE) method and applied to solid DL-lactide in the presence of various catalysts. The most active and stable catalyst was palladium on calcium carbonate. The analyses of the resulting deuterated compounds by ^1H NMR and mass spectrometry showed that substitution involved mainly the tertiary proton and that the reaction temperature and flask volume were critical factors. The experimental conditions that led to convenient hydrogen-deuterium exchange yields were then applied to hydrogen-tritium exchange. Highly radioactive DL-lactide having a specific activity of 150 GBq/mmol. (4.05 Ci/mmol.) was successfully synthesized.

Key Words : Isotope exchange, lactide, deuterium, tritium, labeling, PLA.

INTRODUCTION

Lactic acid-containing polymers derived from lactide ring opening polymerization are regarded as worthwhile sources of degradable organic materials that can be eliminated from a human body or assimilated by living species present in the environment (1-3). Under these conditions, monitoring the fate of the products that are to be resorbed or assimilated by living systems is very important, especially with regard to regulatory agencies throughout the world. On the other hand, the mechanism of lactide polymerization using stannous octoate or other initiating species are still questioned, especially regarding the presence of initiator residues, the effect of trans-esterification reaction on configurational chain structure and the configuration dependence of the mechanism of degradation (3). Polymer chain labeling is a method of choice to investigate these problems. However, labeling by fluorescent dyes or by γ -emitting heavy atoms like ^{125}I that are not normally present in lactic acid-containing aliphatic polyesters, can be the source of artifacts. Labeling polymer chains by replacing hydrogen or carbon atoms by heavier and/or radioactive isotopes is preferable. The use of radioactive ^{14}C atoms to label lactic acid-containing aliphatic polyesters has been reported many years ago (4, 5). However, the radioactivity was rather low and suitable for small animals only. Monitoring the fate of degradation products after dispersion in large complex media like the large animals used to model a human body or like the outdoor environment, requires much higher levels of radioactivity.

In this paper, we wish to report the results of a study aimed at finding a method to replace some of the protons present in DL- and L-lactide molecules by deuterium or tritium atoms. As the handling of high levels of radioactive tritium in a synthetic laboratory is not easy, the reaction was investigated primarily in the case of hydrogen-deuterium exchange. The reaction was optimized by varying the experimental conditions. The conditions leading to good exchange yields were applied so as to incorporate highly radioactive tritium atoms in DL-lactide molecules and thus open the route to highly radioactive lactic acid-containing polymers.

EXPERIMENTAL

DL and L-lactides were obtained from Purac (Gorinchem, Netherlands) and were subjected to sublimation at 57°C under vacuum (10^{-3} mBar) prior to use. 5% Pd/CaCO₃ was purchased from Acros Organics (Geel, Belgium), 10% Pd/Al₂O₃, 10%Pd/BaSO₄, 5% Pd/diatomaceous earth from Fluka (Buchs, Switzerland) and PtO₂ from Aldrich (USA). Commercial THF (Carlo Erba, Milan, Italy) was distilled in the presence of sodium metal prior to use. The deuterium gas was purchased from Euriso-Top (Gif-sur-Yvette, France). Pure tritium gas was produced by Commissariat à l'Energie Atomique (France) and distributed by Tritec (Switzerland).

Hydrogen-deuterium exchange reaction

Typically, 50 mg of DL- or L-lactide were dissolved in 1 cm³ of THF in a 16.5 cm³ (3.2 cm diameter) round bottom flask prior to addition of 250 mg of the 5% Pd/CaCO₃ catalyst. The mixture was allowed to stir until it became homogeneous. The solvent was evaporated to dryness in order to provide a thin layer of solid deposit on the whole of the flask wall. The round bottom flask was connected to a vacuum line. Prior to introduction of the gaseous hydrogen isotope, the mixture was either cooled to liquid nitrogen temperature for experiments conducted at 100 mBar pressure or kept at room temperature for the other pressures. The flask was sealed by a valve and immersed into a temperature-controlled silicone oil bath. At the selected reaction time, the flask was opened and 5 cm³ of THF added to the reaction mixture. The catalyst was removed by centrifugation and filtration using a 0.45 µm filter. The white powder that was obtained after solvent evaporation, was subjected to sublimation at 57°C under vacuum (10^{-3} mBar).

¹H NMR

¹H NMR spectra were recorded using a Bruker 250 MHz spectrometer. Samples were dissolved in DMSO-d₆ ($\approx 10\text{mg}/\text{cm}^3$).

The hydrogen-deuterium exchange yields were calculated from :

$$\text{Exchange yield} = \left(1 - \frac{YX_0}{XY_0} \right) \times 100 \quad (1)$$

Where X, X₀ and Y, Y₀ stand for the areas of the doublet reflecting CH₃-type protons and the quartet reflecting CH-type protons in the substituted and unsubstituted lactides respectively, assuming that only methine protons were substituted.

Mass spectrometry

Mass spectra were obtained using an HP 5989 spectrometer equipped with a Particle Beam interface which separates the eluted molecules from the solvent. The solvent vector was methanol. The ionization potential was 70eV and the source temperature was set at 220°C. Spectra were obtained by chemical ionization, the reaction gas was NH₃. The samples were dissolved in dichloromethane. The exchange yield was calculated using the following expression :

$$\text{Yield} = \frac{\left(\frac{\sum_{j=0}^n (W_{\%j} \times j)}{\sum_{j=0}^n W_{\%j}} \times 100 \right)}{2} \quad (2)$$

W_{%j} stands for the area of the j signal area as referred to the total of the j areas, j being the number of substituted hydrogens on the lactide molecules. Assuming again that substitution occurred at methine proton sites only, the maximum number of protons available for substitution was 2.

Circular dichroism (CD)

CD spectra were recorded on a CD Mark VI Jobin Yvon dichrograph. A known amount of L-lactide was dissolved in acetonitrile in order to produce a 1mg/cm³ concentration. The cell path length was 1 mm.

Radioactivity counting

Radioactivity was measured using a Packard Tri-Carb 1900 CA β-counter and Betamax™ (ICN) as liquid scintillation cocktail.

RESULTS AND DISCUSSION

Deuteration

The method of isotopic labeling has been extensively used in the field of biological molecules such as amino acids and peptides and contributed greatly to the identification of biochemical pathways (6, 7). Among the various methods which can allow one to introduce hydrogen isotopes into organic molecules, isotopic exchange in the solid state as proposed by Myasoedov et al. appeared very efficient in the case of amino acids and peptides (8-12). This method is based on the reaction of deuterium or tritium gas on a highly dispersed solid compound intimately mixed with metal catalyst such as rhodium, palladium, platinum on an inorganic carrier such as barium sulfate, potassium carbonate, aluminum oxide or coal.

The method was applied to DL-lactide. For the sake of experimental simplification, most of the experiments directed at obtaining high reaction yields and structural characteristics were performed with deuterium gas. In the first set of experiments, several palladium and platinum catalysts on different supports were used, namely 10% Pd/charcoal, 5% Pd/CaCO₃, 10% Pd/Al₂O₃, 10% Pd/BaSO₄, 5% Pd/diatomaceous earth, PtO₂, temperature and deuterium pressure being fixed at 90°C and 550 mBar, respectively (Table 1, run 1 to 6).

The extent of H→D exchange was determined using ¹H NMR spectroscopy. Spin decoupling allowed us to identify the protons affected by deuteration. Upon irradiation at the frequency of the methyl proton resonance, the quadruplet due to the coupled methyne proton was altered to a singlet thus suggesting that a single type of methyl proton that bore H protons only, remained after substitution. In contrast, upon irradiation at the methyne proton resonance frequency, the methyl proton resonance appeared as a doublet instead of a singlet, showing that nuclei other than hydrogen were present on the methyne carbon atoms of some of the lactides present in the recovered material (Fig. 1).

Table 1 : Effect of the principal parameters on the exchange reaction

Run	Catalyst	Catalyst/lactide ratio	Pressure mBar	Temperature °C	Time min	% exchange	Dimer ^{a)} presence	Chemical yield
1	10% Pd/Charcoal	5	550	90	60	4	1 ^{b)}	
2	5% Pd/CaCO ₃	"	"	"	"	5	0 ^{b)}	
3	10% Pd/Al ₂ O ₃	"	"	"	"	f ^{c)}	2 ^{b)}	
4	10% Pd/BaSO ₄	"	"	"	"	/	/	
5	5% Pd/diatom. earth	"	"	"	"	3	1	
6	PtO ₂	"	"	"	"	/	/	
7	5% Pd/CaCO ₃	0.5	"	"	180	3	0	
8	"	1	"	"	"	3	0	
9	"	2.5	"	"	"	2	0	
10	"	5	"	"	"	6	0	
11	"	7.5	"	"	"	5	1	
12	"	10	"	"	"	4	2	
13	"	5	250	"	"	4	0	
14	"	"	550	"	"	6	0	
15	"	"	750	"	"	7	1	
16	"	"	1000	"	"	9	1	
17	"	"	550	25	60	0	0	
18	"	"	"	90	"	5	0	
19	"	"	"	100	"	5	0	
20	"	"	"	110	"	8	1	
21	"	"	"	120	"	30	2	
22	"	"	"	90	"	5	0	
23	"	"	"	"	120	6	0	
24	"	"	"	"	180	6	0	
25	"	"	"	"	360	8	0	
26	"	"	"	"	480	16	1	
27	"	"	"	"	960	25	2	
28	"	"	100	120	45	4		62
29	"	"	"	"	60	19 ^{d)}		57
30	"	"	"	"	90	20 ^{d)}		52
31	"	"	"	"	60	25 ^{d)}		55
32	"	"	"	130	90	26 ^{d)}		48

a) Presence of lactoyllactate in the ¹H NMR spectra of the crude lactide after the exchange reaction.

b) 0 : no lactoyllactate detectable, 1 : lactoyllactate detectable, 2 : quantity more important.

c) / : total degradation of lactide.

d) cooled with liquid nitrogen before introduction of gas.

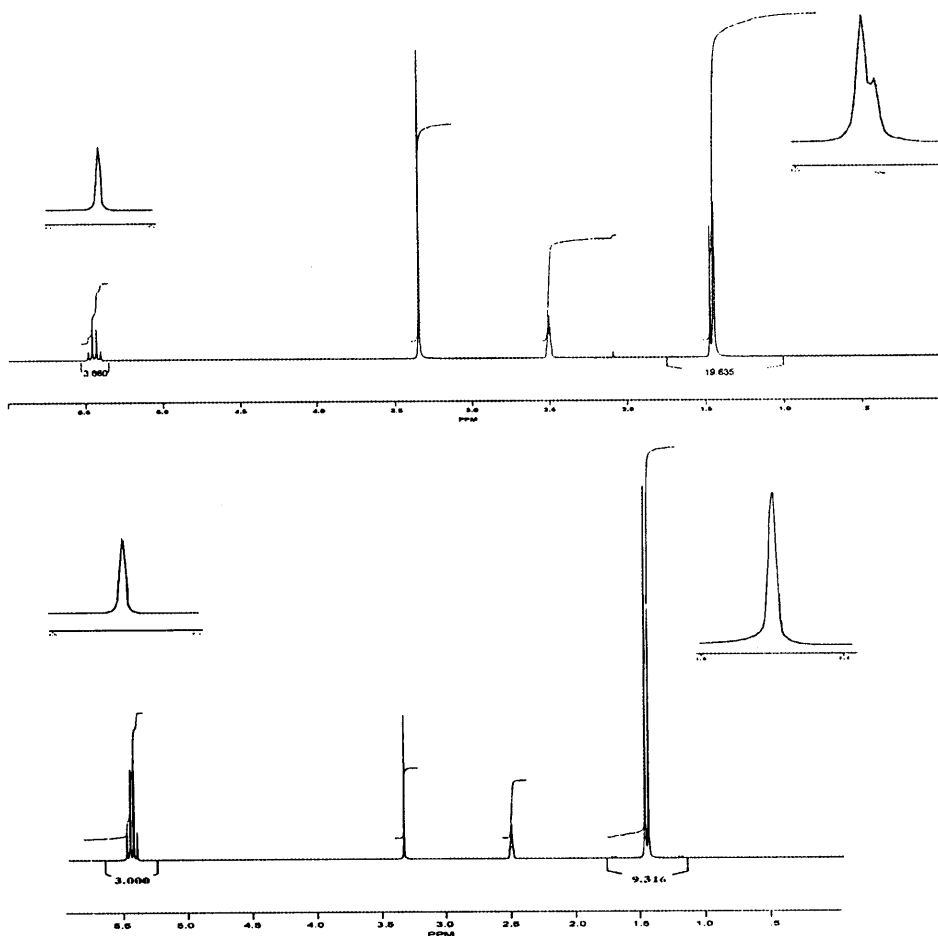


Figure 1 : Typical ¹H NMR spectrum of normal (bottom) and of partially deuterated (up) lactides. Inserts show the shapes of the resonances of spin-decoupled methyne (a) and methyl (b) protons.

These nuclei were likely to be deuterium. Therefore, the methyl proton resonance which was unchanged was taken as an internal reference to evaluate the substitution yield from the relative decrease of the methyne proton signal as referred to the methyl proton. Among all the tested catalysts, the 10% Pd/charcoal and the 5% Pd/CaCO₃ gave the best substitution yields. The 5% Pd/CaCO₃ catalyst was finally considered as the most convenient on the basis of a compromise between substitution yield and the yield of recovered labeled lactide. The effect of the catalyst/lactide ratio on the two yields was evaluated by considering the system where this ratio was 0.5, 1, 2.5, 5, 7.5, 10 in weight (Table 1, run 7 to 12). The substitution yield increased with the values of the ratio in the range 0.5-5. Beyond 5, the substitution yield leveled off whereas the yield

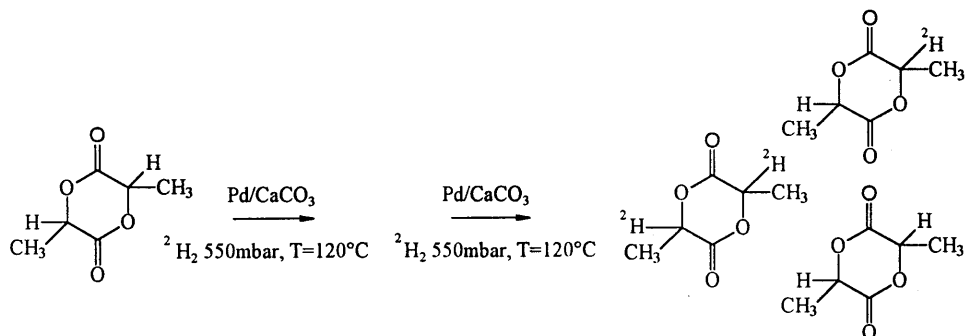
of labeled lactide decreased. When one increased the deuterium pressure from 250 to 1000 mBar keeping all the other parameters unchanged, the substitution yield increased slightly (Table 1, run 13 to 16). However, above 550 mBar, lactide degradation increased and lowered the corresponding yield. Last but not least, raising the temperature from 25 to 120°C at 550 mBar increased very much the reaction rate and led to comparable yields in 1 hour instead of 16 hours (Table 1, run 17 to 21). Therefore, temperature turned out to be the most critical parameter, as already observed by Myasoedov et al. for other compounds.

In a second series of reactions aimed at improving the substitution and the labeled lactide yields, the exchange reaction was conducted in flasks of various volumes, 1, 8.5, and 16.5 cm³ loaded with the same amount of solid lactide. The surface /amount of solid ratio appeared as a critical factor in so far as the substitution yield was concerned since the yields were 4.5, 19 and 26%, respectively. This finding was interpreted in terms of the larger exchange surface between the gas and the solid when the small amount of solid was distributed on the larger interior surface of the 16.5 cm³ flask.

In order to improve the substitution yield, two reactions both run at 120°C and 550 mBar over 45 minutes with an in-between purification stage were carried out. Intermediate purification appeared necessary otherwise the monomer was totally degraded in the second reaction.

Under these conditions, the extent of exchange was raised up to c.a. 45% but the chemical yield was lowered to 25%. Decreasing the deuterium pressure in the reaction flask also caused the substitution and degradation yields in the presence of 5% Pd/ CaCO₃ (Table 1, run 28 to 32) to change. At 100 mBar pressure, the exchange and the labeled lactide yields were respectively 25% and 55%, i.e. close to those observed for a 550 mBar pressure at 120°C over 45 min., provided that temperature and reaction time were raised to 130°C and 1 hour, respectively.

Mass spectrometric analysis was also used to quantify the deuteration yields. It showed that the molecular peak of normal lactide at 145 mass units was split in three peaks of different heights for the material collected after deuteration, as exemplified in scheme 1. The proportion of each of these lactides was 40%, 38% and 23% (Fig. 2). The substitution yield at an average of 42% estimated from equation (2) was in good agreement with the 45% obtained from ¹H NMR spectroscopy.



Scheme n°1 : Different substituted lactides identified in the reaction medium by mass spectrometry after purification.

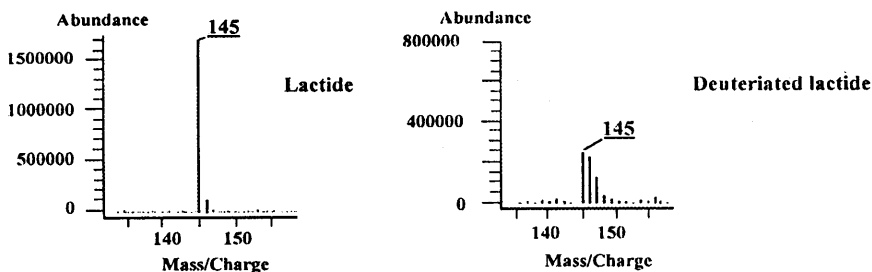


Figure n°2 : Typical mass spectra of lactide before and after partial hydrogen-deuterium exchange (molecular peak).

However, mass spectra did not provide information on whether methyl groups were really not substituted. Similar findings were obtained for the L-lactide diastereoisomer.

As the substitution affected the chiral carbon atoms present in the lactide molecules, racemization was possible in the case of the optically active L- or D-lactides. Therefore, the Circular Dichroism spectrum of native L-lactide was compared to that of the mixture of the 45% partially deuterated lactide. The two spectra being comparable, it was concluded that no racemization nor inversion of configuration occurred during the exchange and that the reaction did not proceed via an $\text{S}_{\text{N}}1$ mechanism.

Tritiation

In the case of tritium labeling, the use of 5% Pd/CaCO_3 led to rather high substitution yields although the experimental conditions were slightly different from those used for deuterium, the gas

pressure and the reaction time being reduced because of the radioactivity limits imposed by the national regulatory agency. For the same reason the reaction was carried out in one step only (Table 2). As in the case of deuterium gas, increasing the temperature and tritium pressure led to higher exchange yields as reflected by the specific activity of the resulting lactides. However, substitution yields were always much lower than those obtained with deuterium gas due to practical difficulties in handling radioactive tritium in a vented hood, a constraint which was not required in the case of deuterium. Despite this lower exchange yield, the recovered lactides were radioactive enough to permit blending with cold lactide to end up with a monomer of the desired high specific radioactivity which is necessary if one wants to monitor the fate of the derived polymer degradation by-products in complex living systems such as an animal body or an outdoor medium.

Table 2 : Results of lactide tritiation

	1 st Test	2 nd Test
Temperature	120°C	130°C
Time reaction	40	60
Specific activity GBq/mmol. (Ci/mmol.)	55.5 GBq/mmol. (1.5 Ci/mmol.)	150 GBq/mmol. (4.05 Ci/mmol.)
$^1\text{H} \rightarrow ^3\text{H}$ exchange ^{a)}	2.6%	7%

a) [tritium fixed to lactide/number of tertiary carbon atcm] × 100.

CONCLUSION

Hydrogen-deuterium exchange can be successfully performed on DL- and L-lactides by the Myasoedov method based on high temperature and solid state reaction. The exchange occurs without racemization. Conditions designed to obtain significant hydrogen-deuterium exchange and limited degradation were found by varying the catalyst and reaction temperature and pressure ; 5% Pd/CaCO₃ was selected as the most convenient catalyst. Although the reaction temperature and pressure influenced the exchange and degradation yields, it is the dispersion of the solid which

turned to be the most critical factor. This finding suggested the use of a rather large round bottom flask as reaction vessel in order to provide a sufficient volume to favor isotopic exchange. The method was also efficient in the case of hydrogen-tritium exchange but led to lower exchange yields because of experimental constraints imposed by the handling of highly radioactive tritium.

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References :

1. Lipinsky E. S., Sinclair R. G. - Chem. Eng. **82**: 26 (1986).
2. Conn R. E., Kolstad J. J., Borzelleca J. F., Dixler D. S., Filer L. J., La Du B. N., Pariza M. W. - Fd. Chem. Toxic. **33**: 273 (1995).
3. Vert M., Schwach G., Coudane J. - J. M. S. Pure Appl. Chem. **A32**: 787 (1995).
4. Kulkarni R.K., Pani K.C., Neuman C., Leonard F. - Arch. Surg. **93**: 839 (1996).
5. Gresser J. D., Wise D. L., Beck L. R., Howes J. F. - Contraception **17**: 253 (1978).
6. Fromageot P., Morgat J. L. - In Labelled compounds (Part A) (Buncel E., Jones J.R. eds), Elsevier, New York, (1987).
7. Willams P. G., Long M. A. - Chemistry in Australia 158 (1987).
8. Zolotarev Y. A., Petrenic B. V., Myasoedov N. F., Patent USSR 154 503, 21.07.83
9. Zolotarev Y. A., Kozik V. S., Zaitsev D. A., Dorokhova E. M., Myasoedov N. F. - Dokl. Akad. Nauk. SSSR. **308**: 1146 (1989) [Dock. Chem. 1989 308 (Engl. transl.)].
10. Zolotarev Y. A., Kozik V. S., Zaitsev D. A., Dorokhova E. M., Rosenberg S. G., Myasoedov N. F. - J. Label. Compds. **29**: 997 (1991).
11. Zolotarev Y. A., Kozik V. S., Zaitsev D. A., Dorokhova E. M., Myasoedov N. F. - J. Radioanal. Nucl. Chem. Art. **162**: 3 (1992).
12. Zolotarev Y. A., Laskatelev E. V., Kozik V. S., Dorokhova E. M., Rosenberg S. G., Borisov Y. A., Myasoedov N. F. - Russ. Chem. Bull. **46**: 4 (1997).