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NMR Analysis of Low Molecular Weight Poly(lactic acid)s

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ABSTRACT: ^{1}H and ^{13}C NMR analyses of L- and D,L-lactic acid oligomers were found considerably improved by recording spectra in DMSO-d_6 instead of $CDCl_3$. Systematic comparison of oligomer spectra led to the identification of a neighboring effect which was shown to be linearly additive. Assignment of all the ^{13}C NMR chemical shifts belonging to the different constitutive units was realized up to the octamer. Furthermore, the well-identified positions of the resonances of chain end units in the ^{1}H NMR spectra were used to determine the absolute average degree of polymerization of oligomers issued from polycondensation. Data were also used to study oligomers with covalently modified chain ends. Findings are of great interest for the study of the ultimate degradation stages of poly(lactic acid)s.

Introduction

Polymeric biomaterials are increasingly used in medicine for both permanent and temporary therapeutic applications. During the past two decades, much attention has been paid to degradable and biodegradable polymeric biomaterials. High molecular weight (HMW) bioresorbable polymers derived from L- and D,L-lactic acid (PLA) are now clinically used in surgery as sutures and devices for osteosynthesis and in pharmacology as drug delivery systems.[1,2] With these types of applications, it is critical that polymers be well purified and characterized. Structural characteristics are currently investigated by nuclear magnetic resonance (NMR) techniques, predominantely in $CDCl_3$ solutions. In this context, impressive work has been accomplished by different research groups.[3−7]

On the other hand, low molecular weight PLA are also of interest, and their uses in drug delivery systems have recently been proposed.[8,9] Low molecular weight PLA polymers are generally prepared by polycondensation of lactic acid.[8] Moreover, they can also be obtained as intermediate byproducts during the hydrolytic degradation of high molecular weight homo- and copolymers.[10] In the latter case, detection and characterization of lactic acid oligomers, especially *n*-mers with low *n*, can provide outstanding information on the rate and mechanism of degradation of these materials. Last but not least, oligomers may be used as macromonomers in polycondensation processes directly or after chemical modification.[11] Therefore, accurate characterization of oligomer structures, especially chain ends, is of great importance. NMR spectroscopy is one of the most convenient techniques to achieve this objective.

In this paper, we wish to report ^{1}H and ^{13}C NMR characteristics of PLA oligomers using DMSO-d_6. This solvent provided a very high resolution and allowed us to distinguish up to eight NMR contributions of repeating units differentiated by closest neighbor effects.

Experimental Section

Materials. Aqueous solutions (85−90%) of L-(+)-lactic acid from Janssen and D,L-lactic acid from Sigma were used. L-Lactide was purchased from Purac and purified by recrystallization from acetone. *N*-Methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald) from Aldrich was used as the precursor to diazomethane.

Methods. Low molecular weight poly(L-lactic acid), or PLA_{100}, and poly(D,L-lactic acid), or PLA_{50}, were prepared by step growth polycondensation of L- or D,L-lactic acid as described previously.[8] Esterification of PLA carboxyl end groups was achieved by using diazomethane according to a known method.[12] The methyl ester of L-lactyl-L-lactic acid was prepared by reaction of L-lactide with methanol in the presence of small amounts of *p*-toluenesulfonic acid as catalyst, according to Claborn.[13]

Measurements. NMR spectra were recorded on a Bruker AMX-360 spectrometer operating at 360 MHz (^{1}H) and 90.5 MHz (^{13}C). Samples were dissolved in DMSO-d_6 (∼100 mg/cm^3) and the spectra were recorded at 303 K. Chemical shifts (δ) were expressed in ppm with respect to the DMSO signals at 2.49 ppm (^{1}H) and 39.50 ppm (^{13}C). Samples were deuterated by addition of several drops of D_2O. The delay between scans was varied from 1 to 5 s in order to investigate its influence on the integral ratios.

Results and Discussion

Because of the high spectral resolution given by DMSO-d_6 as compared with $CDCl_3$, a systematic investigation of the ^{1}H and ^{13}C NMR spectra of the shortest PLA oligomers was carried out before examination of the case of mixtures of poly(lactic acid)s obtained by step growth polymerization.

I. ^{1}H NMR. (a) L-Lactic Acid Aqueous Solution. It is known that 90% lactic acid aqueous solutions contain a series of oligomers at equilibrium with lactic acid, the latter being the predominant species.[14] Such a solution was taken as a typical mixture of the smallest oligomers up to tetramer, and the corresponding ^{1}H NMR spectrum in DMSO-d_6 is shown in Figure 1a.

In contrast to the case of ^{1}H NMR spectra obtained in $CDCl_3$, nearly all the protons exhibited well-separated resonances which shifted upfield more or less depending on the location of repeating units with respect to chain ends. The most intense signals were assigned to C*H* (4.03 ppm) and C*H*$_3$ (1.23 ppm) from the monomer, L-lactic acid (unit a in Chart 1), while the signals with immediate lower intensity were attributed to the dimer, L-lactyl-L-lactic acid. This acid being constituted of two chemically different units, b and c, two sets of signals were expected for both C*H* and C*H*$_3$. Two C*H* quartets were actually detected at 4.92 and 4.19 ppm, along with two C*H*$_3$ doublets with comparable intensities at 1.38 and 1.27 ppm. Assignments of these signals were based on the difference between the two units within the

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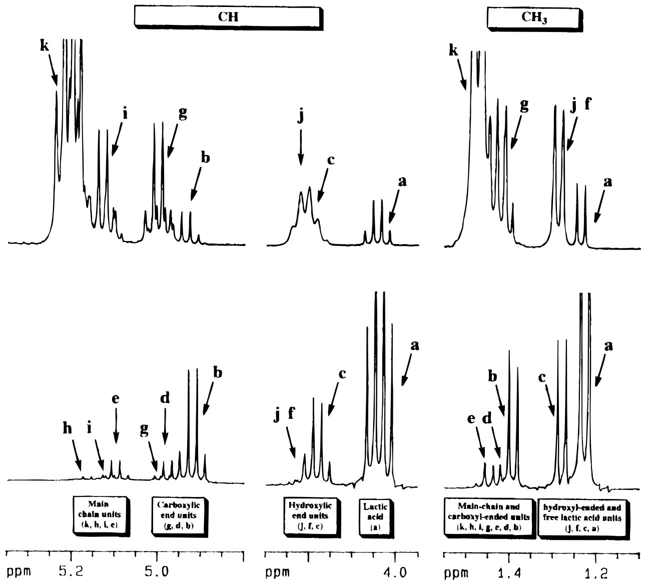
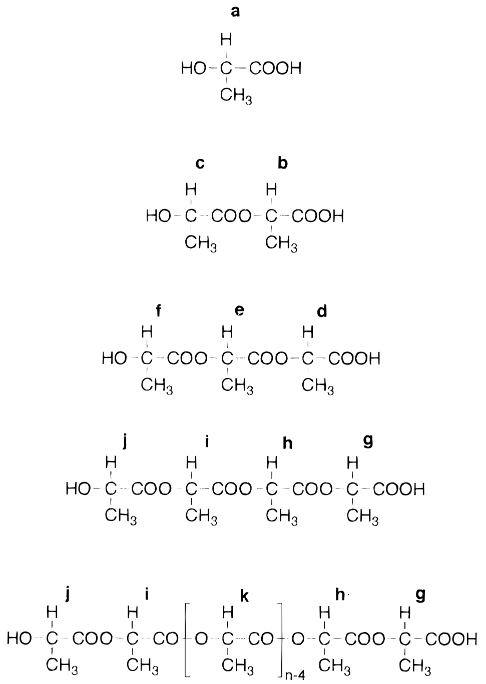


Figure 1. (a) ^{1}H NMR spectrum of 90% L-lactic acid aqueous solution and (b) ^{1}H NMR spectrum of low molecular weight PLA$_{100}$, in DMSO-d_6 (303 K, 360 MHz).

Chart 1. Labeling of the Different Constitutive Units of Lactic Acid Oligomers



dimer: one of them (b) being OH esterified and the other one (c) being OH free. It is well-known that esterification of an alcohol results in deshielding of the H$_\alpha$ resonance and, to a less extent, that of the H$_\beta$ resonance.[15] Therefore, downfield signals (4.92 and 1.38 ppm) were assigned to C*H* and C*H*$_3$ protons in unit b, and upfield resonances (4.19 and 1.27 ppm) to C*H* and C*H*$_3$ protons in unit c.

Besides the main resonances discussed above, several other signals of much lower intensities were detected which arose from d−f units in the trimer and from g−j type units in the tetramer. Taking into account relative intensities, these signals were correlated to the various types of units according to identification listed in Chart 1 (Table 1). However, at this stage, direct assignments to units h and i were not feasible. An indirect approach was selected which was based on the fact that recurrent differences were discovered between similarly located units within the various *n*-mers with respect to chain ends. The correlations were based on the known linear relationship between electronegativity of substituents and chemical shift.[15] It was assumed that inductive effects could be added by starting from the values of the assigned chemical shifts for C*H* in a−g and j type units.

Interpolated chemical shift values for CH_h and CH_i (5.16 and 5.11 ppm, respectively) appeared very close to experimental data (5.17 and 5.11 ppm), thus showing the validity of Chart 2 for the assignment of resonances in lactic acid oligomers. A similar analysis was made for C*H*$_3$ resonances, which also led to good fits.

(b) PLA$_{100}$ Oligomers. The ^{1}H NMR spectrum of a low molecular weight PLA$_{100}$ obtained by polycondensation of L-lactic acid is shown in Figure 1b. The most intense signals were those located at 5.19 and 1.46 ppm which corresponded respectively to C*H* and C*H*$_3$ in the repetitive central k type units, in good agreement with calculated data in Chart 2. The spectrum in Figure 1b showed resonances similar to those of the mixture of smaller oligomers (Figure 1a). However, intensities of the various resonance signals were relatively different, in agreement with the greater sizes of oligomers present in low molecular weight PLA$_{100}$.

The well-resolved contributions of both the hydroxyl and carboxyl end units were of great interest for estimating absolute $\overline{\mathrm{DP}}_n$ values. They were also of interest for evaluating the content in lactic acid present as a residue in the oligomer mixtures. Moreover, a relationship was found between the type of unit and the coupling constant (^3J). As shown in Table 1, the values of ^3J depended on the position of the units: lower values corresponding to units closer to the hydroxyl chain end.

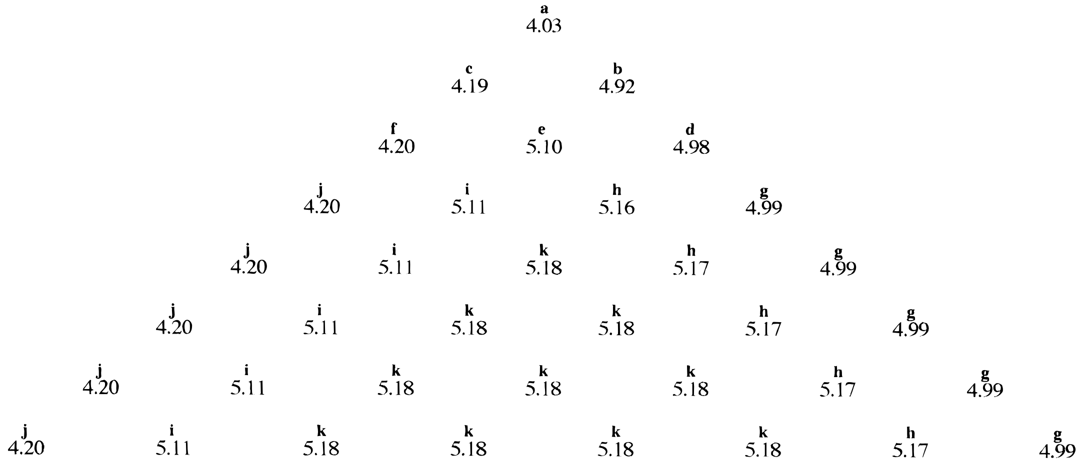
(c) D,L-Lactic Acid Series. It is well-known that NMR spectra of polymers are sensitive to neighbor effects and to configurational structures as well. Changing from the L to the D,L series led to complicated features. The ^{1}H NMR spectrum of a commercial D,L-lactic acid solution is presented in Figure 2a. Comparison between the spectrum of L-lactic acid (Figure 1a) and D,L-lactic acid (Figure 2b) showed the emergence of double sets of signals corresponding to C*H* in unit b and C*H* and C*H*$_3$ in unit c. The splitting of these signals arose from the stereosensitivity due to the presence of two pairs of lactyllactic acid enantiomers which are diastereomerically different, respectively, the LL/DD pair and the LD/DL pair. In contrast, there was no splitting in the case of the CH$_3$ resonance of unit b, even at 600 MHz. The reason for this absence of splitting is still

Table 1. ^{1}H NMR Chemical Shifts (δ, in ppm) and Coupling Constants (^3J, in Hz) of C*H* and C*H*$_3$ Protons in a−k units[a] of L-Lactic Acid Oligomers (DMSO-d_6, 303 K, 360 MHz)

	monomer	dimer		trimer			tetramer				polymer
	a	b	c	d	e	f	g	h	i	j	k
δ(C*H*)	4.03	4.92	4.19	4.98	5.10	4.20	4.99	5.17	5.11	4.20	5.19
δ(C*H*$_3$)	1.23	1.38	1.27	1.40	1.44	1.27	1.40	1.45	1.44	1.27	1.46
^3J	6.9	7.1	6.9	7.1	7.0	6.9	7.1	7.0	7.0	6.9	7.0

[a] For **a**−**k** identification, see Chart 1.

Chart 2. Interpolation Analysis of Methine Protons in a−g and j units



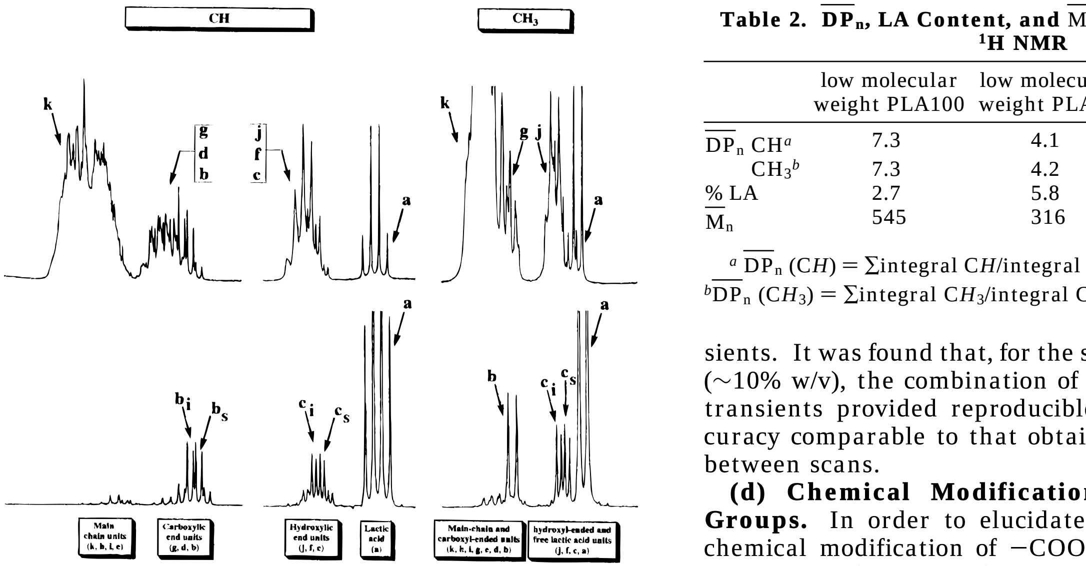


Figure 2. (a) ^{1}H NMR spectrum of 90% D,L-lactic acid aqueous solution and (b) ^{1}H NMR spectrum of low molecular weight PLA$_{50}$, in DMSO-d_6 (i = isotactic, LL/DD; s = syndiotactic, LD/DL) (303 K, 360 MHz).

Table 2. $\overline{DP}_n$, LA Content, and $\overline{M}_n$ Data Obtained from ^{1}H NMR

	low molecular weight PLA100	low molecular weight PLA50	low molecular weight methylated PLA50
$\overline{DP}_n$ CH[a]	7.3	4.1	7.9
CH$_3$[b]	7.3	4.2	8.0
% LA	2.7	5.8	
$\overline{M}_n$	545	316	605

[a] $\overline{DP}_n$ (CH) = Σintegral CH/integral CH(OH end + lactic acid). [b] $\overline{DP}_n$ (CH_3) = Σintegral CH_3/integral CH_3(OH end + lactic acid).

unexplained. In order to assign each split signal, complementary spectra were recorded from mixtures of commercial L- and D,L-lactic acid in different proportions. Variation of relative intensities allowed us to attribute conclusively all the upfield resonances to heterodimers composed of L,D- and D,L-lactyllactic acid isomers, and the downfield ones to L,L and D,D homodimers.

The ^{1}H NMR spectrum of low molecular weight PLA$_{50}$ (Figure 2b) was complicated because of the overlapping of resonances belonging to different stereoisomers of each n-mer. Therefore, identification of all these signals was impossible, except for CH_a (4.03 ppm) and CH_{3a} (1.23 ppm) of lactic acid. Nevertheless, all other signals appeared in differentiated groups, similarly to those shown in Figure 1b for PLA$_{100}$ polycondensates.

Integration of the different groups of signals was used to calculate the absolute $\overline{DP}_n$ and $\overline{M}_n$ and the lactic acid content in low molecular weight PLA samples. The results are presented in Table 2.

The influence of the delay between scans on the integral values was examined by recording spectra at different combinations of it and the number of transients. It was found that, for the selected concentrations (~10% w/v), the combination of a delay of 1 s and 64 transients provided reproducible results with an accuracy comparable to that obtained for higher delays between scans.

(d) Chemical Modification of −COOH End Groups. In order to elucidate the influence of the chemical modification of −COOH end groups on the chemical shift values of the corresponding α-methine protons in a, b, d, and g type units, a model compound, the methyl ester of L-lactyl-L-lactic acid, was synthesized by acid catalyzed methanolysis of L-lactide.[13]

A shift of 0.11 ppm downfield was observed in the ^{1}H NMR spectrum of this compound for CH_b, whereas no shift of the CH_c resonance was detected. It is interesting to note that in this spectrum, a residual quartet remained at 5.43 ppm (^3J = 6.7 Hz) which was easily assigned to unreacted L-lactide. On the basis of these findings and recurrent values from one oligomer to the other, a similar 0.11 ppm downfield shift for CH resonances of a, b, d, and g type units in methylated higher oligomers was predicted. The ^{1}H NMR spectrum of a low molecular weight methylated PLA$_{50}$ is shown in Figure 3. The comparison with Figure 2b shows that resonances of the CH protons in b, d, and g type units did shift downfield to overlap with those due to main chain units (e, h, i, and k). Moreover, the CH_a resonance also shifted downfield to 4.14 ppm to overlap with the CH signals of hydroxyl end groups (c, f, and j type units). As a consequence, the accuracy of the $\overline{DP}_n$ estimation, which was based on the relative integrals ratio of these groups of signals, decreased (Table 2). However, the cleared zones between 4.9 and 5.0 ppm (for units b, d, and g with free COOH) and around 4.03 ppm (for lactic acid, unit a) should be usable to study partially degraded PLA polymers.

II. ^{13}C NMR. ^{13}C NMR spectra of a 90% L-lactic acid aqueous solution and of a low molecular weight PLA$_{100}$

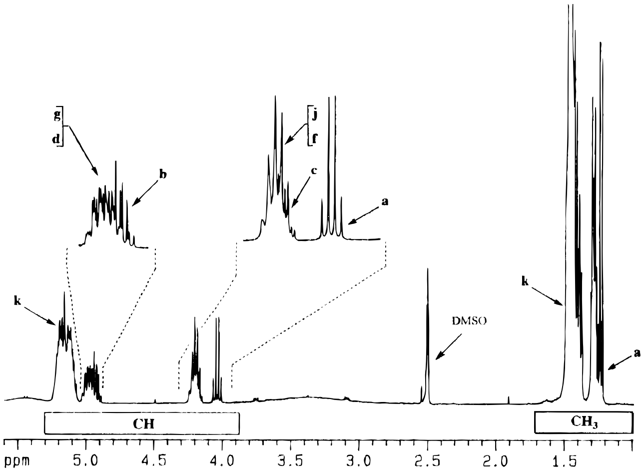


Figure 3. ^{1}H NMR spectrum of methyl esters of low molecular weight PLA$_{50}$ in DMSO-d_6 (303 K, 360 MHz).

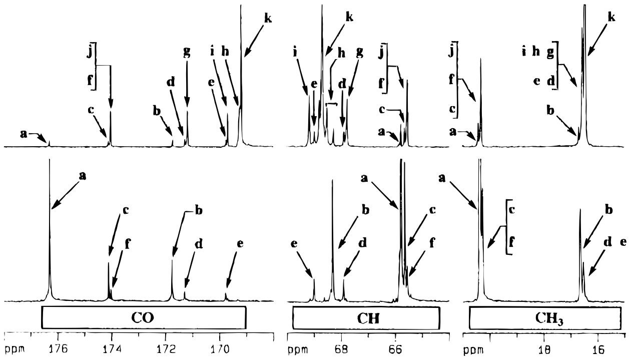


Figure 4. Selected regions of the ^{13}C NMR spectra in DMSO-d_6 of 90% L-lactic acid aqueous solution (a) and of low molecular weight PLA$_{100}$ (b) (303 K, 90 MHz).

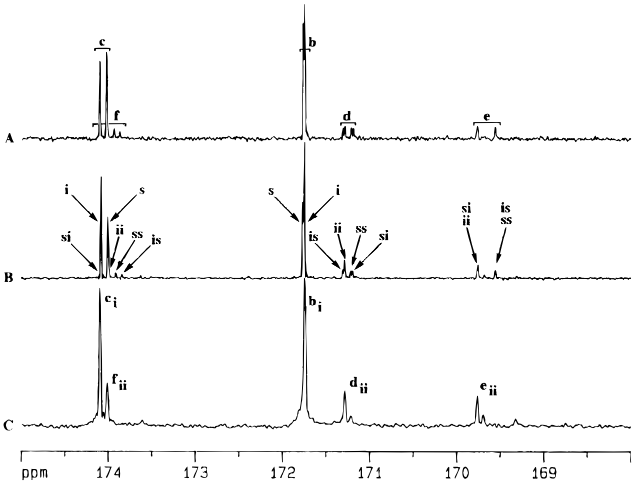
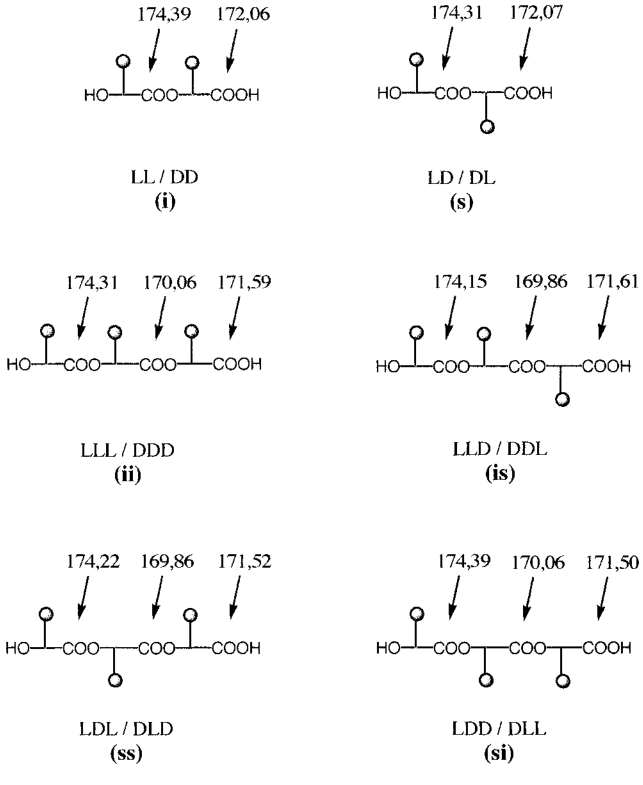


Figure 5. Comparison of the *C*O region of the ^{13}C NMR spectra in DMSO-d_6 of 90% D,L-lactic acid aqueous solution (a), a 4:1 mixture of 90% D,L- and L-lactic acid aqueous solutions (b), and 90% L-lactic acid aqueous solution (c) (i = isotactic; s = syndiotactic) (303 K, 90 MHz).

Chart 3. Assignments of the *C*O Resonances in Dimer and Trimer Stereoisomers



are shown in Figure 4. For both, *C*H resonances were found to be the most sensitive to location within polymer chains. The comparison of the two spectra and the use of a Chart 2 type analysis allowed the correlation of all the *C*H resonances to the different units present in the oligomers, up to the octamer. Assignments were also performed for *C*O and *C*H$_3$ resonances (Table 3).

As in the case of ^{1}H NMR, changing from the L to the D,L series led to the splitting of each signal due to stereosensitivity, as shown in Figure 5a for a 90% D,L-lactic acid aqueous solution. Complete assignment of the *C*O resonances in all dimer and trimer stereoisomers was realized (Chart 3) by comparing the spectra of commercial L- and D,L-lactic acids with that of a 4:1 mixture of the two solutions (Figure 5). For this, the differences between the δ values of homo- and heterodimers and the number of signals due to the *C*O atom of the e type units of the various trimer stereoisomers were considered. Analysis of the δ values in the case of the trimer allowed us to conclude that, for this particular oligomer, the greatest influence on the *C*O chemical shift of the two end units was due not to the neighboring unit located in the center but to the other end unit. Therefore, the relative 1,3 long distance interaction appeared more important than the 1,2 or 2,3 ones, suggesting a particular conformation which might be investigated by molecular modeling.

Table 3. ^{13}C NMR Chemical Shifts (δ, in ppm) of *C*O, *C*H, and *C*H$_3$ in a−k Units^a of L-Lactic Acid Oligomers (DMSO-d_6, 303 K, 90 MHz)

	monomer	dimer		trimer			tetramer				polymer
	a	b	c	d	e	f	g	h	i	j	k
δ(*C*O)	176.31	171.97	174.36	171.52	170.00	174.28	171.43	169.52	169.94	174.28	169.44
δ(*C*H)	66.04	68.53	65.89	68.15	69.24	65.83	68.07	68.88	69.40	65.80	68.95
δ(*C*H$_3$)	20.67	16.94	20.57	16.82	16.70	20.57	16.82	16.70	16.70	20.57	16.70

[a] For **a**−**k** identification, see Chart 1.

Conclusion

In this paper, we reported ^{1}H and ^{13}C NMR spectra of various lactic acid oligomers obtained from DMSO-d_6 solutions. All assignments were made on the basis of systemic identification and comparison of the resonance signals. These assignments were possible because of the high spectral resolution found in DMSO-d_6 as compared to the usual NMR spectra obtained in chlorinated solvents. The well-resolved contributions of chain end groups in the ^{1}H NMR spectra can now be used to determine the absolute $\overline{\mathrm{DP}}_\mathrm{n}$ values of low molecular weight PLA. Furthermore, DMSO-d_6 and ^{1}H NMR will be of great importance to investigate the ultimate stages of the degradation of PLA polymers through monitoring of the chain end units.

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