

Compositional Analysis of Copoly (DL-Lactic/Glycolic Acid) (PLGA) by Pyrolysis-Gas Chromatography/Mass Spectrometry Combined with One-step Thermally Assisted Hydrolysis and Methylation in the Presence of Tetramethylammonium Hydroxide

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Rapid and precise compositional analysis of copoly (DL-lactic/glycolic acid) (PLGA) was performed by pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) combined with one-step hydrolysis and methylation in the presence of tetramethylammonium hydroxide (TMAH). Pyrolysis of PLGA with TMAH gave two characteristic products, derivatives of DL-lactic acid and glycolic acid, which directly reflect the average molar composition of PLGA. The analytical results for PLGA samples with various compositional ratios were in good agreement with those obtained by ¹H-NMR spectrometry, and the precision was satisfactory.

Key words compositional analysis; copoly (DL-lactic/glycolic acid); pyrolysis; gas chromatography/mass spectrometry

Homopolymers and copolymers based on DL-lactic acid and glycolic acid are of interest in the medical and pharmaceutical fields and been applied to devices for wound closure, orthopedics and controlled drug release because of their degradability and excellent toxicological profiles.^{1–5} The *in vitro* degradation of these polymers in aqueous media proceeds *via* random hydrolysis of the ester bonds in the polymer chain. The degradation rate depends mainly on the molecular weight and compositional ratio of DL-lactic acid to glycolic acid in the case of copoly(DL-lactic/glycolic acid) (PLGA).^{4,5} For determination of the compositional ratio, ¹H-NMR has generally been used. In the ¹H-NMR spectra of PLGA, the lactide and glycolide parts are clearly separated, and the ratio of the corresponding integrals directly gives the average molar composition. However, this method requires a special instrument and is far from convenient.

Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) has been widely used to study the chemical structures and the thermal degradation mechanisms of polymers.^{6–8} The molecular mass of some polymers was reduced and the volatility of the products was increased by degradation. In most cases, the obtained pyrogram is complicated, and interpretation of the data is quite difficult; however, a newly developed Py-GC/MS technique involving one-step thermally assisted hydrolysis and alkylation greatly simplifies the resulting pyrogram.^{8–11} Using this technique, compositional analysis of polymeric materials used in engineering has been reported.¹² This is based on the peak intensity ratio of the pyrolytic products, which reflects the average molar composition of the starting polymer. The advantages of this method are that only a small amount of sample (from several micrograms to several tens of micrograms) is required, and that sample pretreatment such as dissolution in a solvent is not required.

In this study, Py-GC/MS combined with one-step thermally assisted hydrolysis and methylation was applied to the compositional analysis of PLGA used in medical and pharmaceutical applications as an alternative to the ¹H-NMR

method. The utility of the methylating reagent and pyrolysis conditions were examined to obtain characteristic products that directly reflect the average molar composition of the starting PLGA. To assess this method, the precision was examined by replicate analysis of PLGA samples with various compositional ratios.

Experimental

Materials and Reagents The poly DL-lactic acid (PLA) and PLGA with various compositions used in this study are listed in Table 1. They were all purchased from Sigma Chemical Co. (St. Louis, Mo, U.S.A.). Tetramethylammonium hydroxide (TMAH, 10% in methanol) used as the methylating reagent, DL-lactide and L-lactide were purchased from Tokyo Kasei Kogyo, Co., Ltd. (Tokyo, Japan). DL-Lactic acid (90.5%) and glycolic acid (99.8%) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Conditions for Py-GC/MS A vertical furnace-type double shot pyrolyzer (Frontier Lab Co., Ltd., Koriyama, Japan) mounted on top of a Shimadzu model QP5050A GC/MS with an electron impact ion source was used. About 5 μg of a sample was placed inside the sample cup (5.0 mm height×3.8 mm i.d.) with 1 μl of the TMAH solution and pyrolyzed at a fixed temperature between 300 °C and 700 °C under a flow of carrier gas. The column used was a 30 m×0.25 mm i.d. fused silica column coated with a 0.25-μm film of polydimethylsiloxane (DB-1, J & W Scientific Co., Ltd., Folsom, CA, U.S.A.). The split injection mode was used with an approximate splitting ratio of 1 : 50. The carrier gas was helium; the average linear velocity in the column was about 35 cm/s; and the injector temperature was 270 °C. The column temperature was programmed to hold at 35 °C for 5 min, then increase to 175 °C at a rate of 8 °C/min, then to 260 °C at a rate of 35 °C/min and finally to hold at 260 °C for at least 16 min. Mass chro-

Table 1. PLA and PLGA Samples with Various Compositions Used in This Study

Sample	Composition (mol %) ^{a)}	
	DL-Lactic acid	Glycolic acid
A	100	0
B	85	15
C	75	25
D	65	35
E	52	48

a) ¹H-NMR results, certified by the vendor.

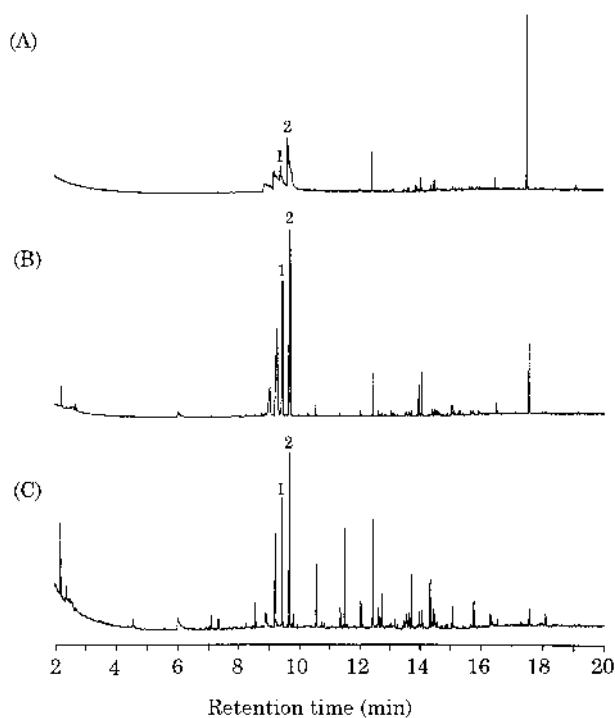


Fig. 1. Pyrograms of Sample E Pyrolyzed without Addition of TMAH (A) 300 °C, (B) 500 °C, (C) 700 °C. Peaks: 1=L-lactide, 2=DL-lactide.

matograms were recorded in the range of m/z 45 to 300 in electron ionization (EI) and chemical ionization (CI) mode at 70 eV, and the ion source was maintained at 280 °C. Isobutane was used as the reagent gas for the CI mode.

Results and Discussion

Py-GC/MS in the Absence of TMAH Figure 1 shows the pyrograms obtained from sample E (DL-lactic acid: glycolic acid=52:48) at 300, 500 and 700 °C without addition of TMAH. The pyrogram at each temperature showed many pyrolytic product peaks including some oligomers whose intensity increased at higher pyrolytic temperatures; however, their reproducibility was poor. It is known that the major components formed upon pyrolysis of PLA are *meso*-lactide and DL-lactide, cyclic dimers consisting of D-lactic acid and/or L-lactic acid.¹³ The two main products (retention times of 9.4 and 9.7 min) were identified as *meso*-lactide (D-lactide or L-lactide) and DL-lactide by mass spectrometry in the CI and EI mode. Further, the mass spectra and retention times of these products were in good agreement with those of L-lactide (9.4 min) and DL-lactide (9.7 min) standards. Pyrolysis without addition of TMAH did not give characteristic products reflecting the average molar composition of the original PLGA sample.

Py-GC/MS in the Presence of TMAH Figure 2 shows the pyrograms obtained from sample E at 300 and 500 °C in the presence of TMAH. The pyrolysis with addition of the methylating reagent generated only two pyrolytic products within 5 min, and no other peaks were recognized after that. The peaks became broader at 300 °C due to slower thermal reaction; however, they became sharper at 500 °C. From the CI and EI mass spectra shown in Fig. 3, these pyrolytic products were identified as methyl methoxyacetate (MMA, 3.3 min) and methyl 2-methoxypropanoate (MMP, 4.2 min),

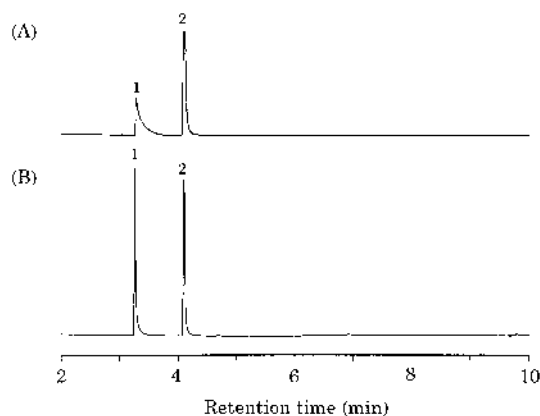


Fig. 2. Pyrograms of Sample E Pyrolyzed in the Presence of TMAH (A) 300 °C, (B) 500 °C. Peaks: 1=MMA, 2=MMP.

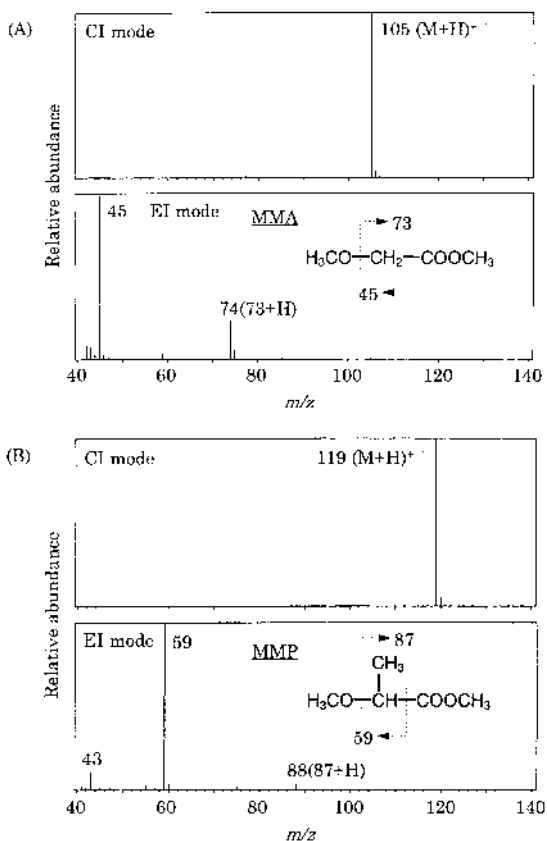


Fig. 3. Mass Spectra of Pyrolytic Compounds Generated from PLGA in the Presence of TMAH

Retention time of compounds: (A) 3.3 min, (B) 4.2 min.

respectively. On the basis of these results, a pyrolysis temperature of 500 °C was chosen. The suitable amount of TMAH solution was found to be 1 μ l because a peak for methoxyacetate formed upon incomplete methylation was observed at 2.9 min with less than 0.5 μ l. Figure 4 shows the pyrograms obtained from samples A—E pyrolyzed at 500 °C in the presence of TMAH.

Determination of the Sensitivity Ratio of MMA to MMP In order to determine the sensitivity ratio of MMA to MMP on GC/MS, the peak intensities of these compounds derived from DL-lactic/glycolic acid mixtures with known ratios were compared. Pyrolysis of DL-lactic/glycolic acid mix-

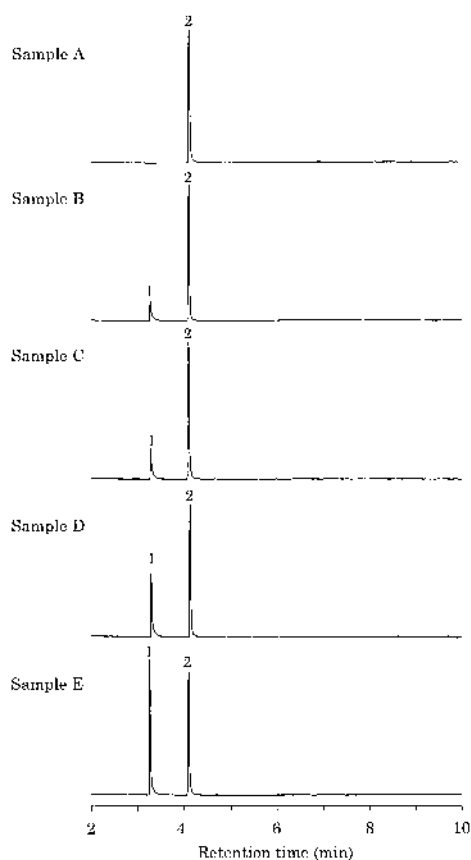


Fig. 4. Pyrograms of PLA and PLGA Samples with Different Compositions Pyrolyzed at 500 °C in the Presence of TMAH

Peaks: 1=MMS, 2=MMP.

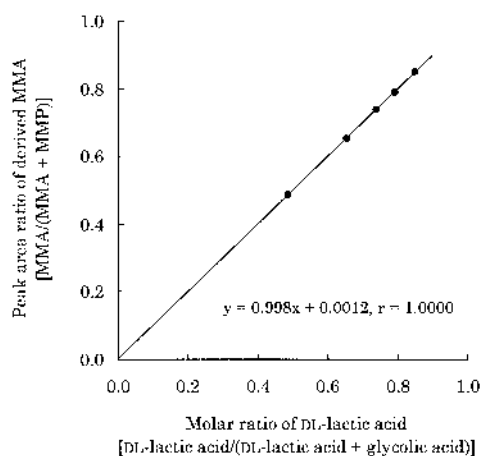


Fig. 5. Relationship between Molar Ratios of DL-Lactic Acid and Peak Area Ratios of Derived MMA

tures whose molar ratios are 85 : 15—50 : 50, at 500 °C in the presence of TMAH yielded the peaks of MMA and MMP whose intensity ratios almost corresponded to those observed for PLGA samples. Figure 5 shows the relationship between the molar ratios of DL-lactic acid and the peak area ratios of derived MMA. In the entire range of the molar ratios of DL-lactic acid, the plot was linear and passed through the origin. The sensitivity ratio of MMA to MMP obtained from the slope of the obtained straight line was 0.998, indicating that

Table 2. Compositional Analysis of PLGA Samples by Py-GC/MS in the Presence of TMAH

Sample	Composition (mol %)			
	Py-GC/MS ^{a)}		¹ H-NMR ^{b)}	
	DL-Lactic acid	Glycolic acid	DL-Lactic acid	Glycolic acid
B	85.1 (0.6)	14.9	85	15
C	74.9 (0.8)	25.1	75	25
D	65.2 (1.0)	34.8	65	35
E	51.1 (1.4)	48.9	52	48

a) Mean value of 6 analyses. b) Certified by the vendor. Values in parentheses are RSDs (%).

the method has the same molar sensitivity for the two compounds.

Compositional Analysis of PLGA Samples The molar ratios of DL-lactic acid to glycolic acid in samples B—E determined by this method are listed in Table 2 together with reference values obtained by ¹H-NMR. The total analytical period could be shortened to 20 min when the analytical column flash started 5 min after starting analysis. Sensitivity corrections were not made because the sensitivity ratio of MMA to MMP was almost 1 (0.998, Fig. 5). The determined molar ratios of each sample were in good agreement with those certified by ¹H-NMR by the vendor. The precision of this method was satisfactory; the relative standard deviations (RSDs) of the DL-lactic acid composition were 0.6—1.4% for 6 repeated runs.

Conclusions

The Py-GC/MS technique combined with one-step thermally assisted hydrolysis and methylation in the presence of TMAH was found to be applicable to the compositional analysis of PLGA samples. The pyrolytic products, MMA and MMP, which directly reflect the average molar composition of PLGA were formed in the presence of TMAH. The compositional molar ratios of DL-lactic acid to glycolic acid in the several PLGA samples examined were in good agreement with those obtained by the ¹H-NMR method. This new method was proved to be both convenient and precise.

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