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Characterization of Commercial PLGAs by NMR Spectroscopy

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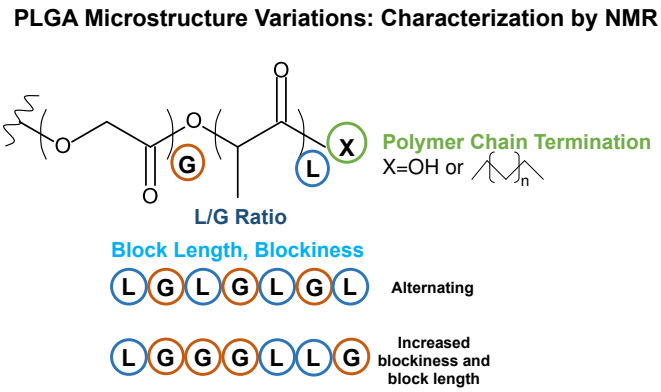
Abstract

Poly(lactic-*co*-glycolic acid) (PLGA) is among the most common of biodegradable polymers studied in various biomedical applications such as drug delivery and tissue engineering. To facilitate the understanding of the often overlooked PLGA microstructure on important factors affecting PLGA performance, we measured four key parameters of 17 commonly used commercial PLGA polymers (Resomer®, Expansorb®, Purasorb®, Lactel®, and Wako®) by NMR spectroscopy. ^{1}HNMR and ^{13}CNMR spectra were used to determine lactic to glycolic ratio (L/G ratio), polymer end-capping, glycolic blockiness (**Rc**), and average glycolic and lactic block lengths ($\mathbf{L_G}$ and $\mathbf{L_L}$). In PLGAs with a labeled L/G ratio of 50/50 and acid end capping, the actual lactic content slightly decreased as molecular weight increased in both Resomer® and Expansorb®. Whether or not acid- or ester-, termination of these PLGAs was confirmed to be consistent with their brand labels. Moreover, in the ester end-capped 75/25 L/G ratio group, the blockiness value (**Rc**) of Resomer® RG 756S (**Rc**: 1.7) was highest in its group; whereas for the 50/50 acid end-capped group, Expansorb® DLG 50-2A (**Rc**: 1.9) displayed notably higher values than their counterparts. Resomer® RG 502 ($\mathbf{L_L}$: 2.6, $\mathbf{L_G}$: 2.5) and Expansorb® DLG 50-2E ($\mathbf{L_L}$: 2.5, $\mathbf{L_G}$: 2.6) showed the lowest block lengths, suggesting they may undergo a steadier hydrolytic process compared to random, heterogeneously distributed PLGA.

Key words: PLGA, blockiness, block length, degradation, NMR spectroscopy.

Graphical Abstract



1. Introduction

Polymeric biomaterials are widely used in commercial biomedical products in the past several decades.[1, 2] Poly(lactic-*co*-glycolic acid) or poly(lactide-*co*-glycolide) (PLGA) is a thermoplastic co-polyester comprised of various ratios of its monomers and is hydrolyzed *in vivo* into non-toxic lactic and glycolic acid, that are metabolized in the tricarboxylic acid cycle and eliminated via carbon dioxide and water.[3-5] Due to its biocompatibility, biodegradability, and ease of processing, PLGA has been further developed in areas such as tissue engineering (e.g., bone regeneration,[6-9] wound dressing,[10-12] vascular grafting[13, 14]), medical imaging,[15, 16] and drug delivery,[17-19] especially for long-acting release formulations.[20-22] Several widely used PLGA-based commercial medical devices [23] and drug products have also been developed, such as Supralimus®,[24] a coronary sirolimus-eluting stent coated with a blend of PLGA/PLA/PVP, and the Lupron Depot® microspheres and Zoladex® implant,[25, 26] both of which are luteinizing hormone-releasing hormone analog, continuous long-acting release formulations.

Nuclear magnetic resonance (NMR) spectroscopy, the physical phenomenon of nuclei in a magnetic field first observed by F. Bloch and E.M. Purcell who received the Nobel Prize in physics in 1952,[27, 28] has been explored in various areas such as pharmaceutical analysis and clinical diagnostics.[29] Similar to other forms of spectroscopy, NMR spectra results from the transitions between different energy states of the dipole-carrying atomic nuclei isotopes (^{1}H, ^{13}C, ^{15}N and ^{31}P).[30, 31] NMR technology is highly specific, precise, and non-invasive, and thus, is well-established for structural elucidation and confirmation of small to large molecules.[32-34] Combined with quantitative analysis, NMR methods have been further used in pharmaceutics and pharmacokinetics to determine composition, impurities, and metabolites.[35, 36] Another sub-field in which NMR is heavily applied is polymer science. Typically, copolymers are comprised of different repeating monomers which possess a wide range of mechanical and biological properties.[37, 38] As an example, NMR can be used to determine the polymer molecular weight, component ratios, monomer sequences, and microstructure,[39] all of which affect both *in vitro* and *in vivo* behavior of polymers. Furthermore, several studies have used NMR spectroscopy to characterize copolymers such as PLGA to determine their solubility, amphiphilicity and hydrolysis mechanism/rate.[40-42] As an example, Garner *et al*.[43] have reported an analytical protocol to confirm the key properties of PLGAs used in commercial drug products, namely the ratio of lactic and glycolic acid (L/G ratio) and nature of polymer end-capping.

Microscopic PLGA properties such as L/G ratio, molecular weight and end-capping, are primary polymer factors impacting the subsequent behaviors of PLGA formulations such as their degradation and drug release behavior.[44] Beyond these polymer properties, there are manufacturing and formulation variables such as formulation processes, size/geometry, presence of excipients, and the drug itself that all serve a considerable role in product performance, but the scope of this research is to investigate fundamental polymer properties.[45, 46] The PLGA microstructures such as monomer sequence distribution along the polymer chain, described by the block length and blockiness, is often overlooked and rarely reported, though it is expected to have an effect on the hydrolytic degradation profile. The blockiness of PLGA measures the relative occurrence of glycolyl-glycolyl compared to glycolyl-lactyl linkages while the block length measures the average length of the glycolic and lactic linkages in one segment.[47] The increased reactivity of glycolic monomers has been previously studied and Vey *et al*. determined that the glycolic unit (G-G bond) consistently hydrolyzes 1.3 times faster than the lactic unit across different L/G ratio polymer films submerged in phosphate buffer.[48-50] Due to the increased reactivity of the glycolic-glycolic linkage, the increase in blockiness or block length will lead to an accelerated hydrolysis of PLGA. It was recently reported that sequenced PLGAs with a "true" alternating microstructure,[51] exhibited a slower swelling, more gradual loss of molecular weight and a longer preservation of morphology compared to the more 'blocky' counterparts synthesized by ring-opening polymerization (ROP).

As a commonly used non-toxic biomaterial in many FDA-approved products, various commercial PLGA polymers across different brands and with different features (MW, L/G ratio, and end-capping) have been widely investigated. However, for many of these commercial PLGA polymers, properties such as block length and blockiness remain unclear and have yet to be systematically analyzed. Hence, in this work, using NMR spectroscopy as an accurate method, we investigated 17 commonly-used PLGAs to compare separated into 4 different groups of similar composition, and obtained four critical chemical properties, namely L/G ratio, end-capping, blockiness and block length. We compared the different polymer portfolios of 5 manufacturers, Expansorb®, Resomer®, Purasorb®, Lactel®, and Wako®. Not all manufacturers had the same breadth of polymers available, e.g., there are not two polymers from Expansorb® to compare with Lactel®, thus not all brands were compared in each group. The potential impact of these data on physicochemical properties such as degradation is discussed and will be investigated directly in future publications and depth analysis of erosion behavior and drug release properties of these polymers. Hence, these results

could help bridge the gap between the parameters obtained from PLGA microstructures and subsequent performance, providing guidance for further biomedical and drug delivery applications.

2. Materials and methods

2.1. Materials

All Expansorb® polymers were provided by Merck KGaA (Germany). Resomer® and Purasorb® polymers, Wako® 7515 and Lactel® DL-PLG B6007-2 were purchased from Sigma Aldrich (US), Corbion (The Netherlands), Wako Pure Chemical Industries, Ltd (Japan) and Lactel (US), respectively. All polymers used in this investigation were the racemic (D,L) form of lactide or lactic acid. **Table 1** summarizes the polymers utilized/analyzed in this study. Deuterated chloroform, deuterated benzene, and ColorSpec® NMR tubes (5 mm × 7 in, parameter 800 MHz frequency) were acquired from Sigma Aldrich (US). Hexafluoro-isopropanol was from Supelco Ltd (US).

2.2. Nuclear magnetic resonance (NMR) spectroscopy

NMR scanning was performed using a Varian vnmr-500 MHz (11.7 Tesla) Premium Shielded NMR spectrometer (US) running Vnmrj software (the NMR Facility of the University of Michigan Chemistry Department, US) for ^{1}HNMR and ^{13}CNMR. NMR spectra are available in the Supporting Information.

2.2.1. Lactic/glycolic ratio (L/G ratio) calculation

Each PLGA sample was weighed out (~5 mg) and dissolved in deuterated chloroform ($CDCl_3$) (0.5 mL), then pipetted into an NMR tube. The spectrum of every sample was collected by ^{1}HNMR spectroscopy (16 scans, 0.5 s relaxation delay, and 45-degree pulse angle). L/G ratios were determined by comparing proton intensities at chemical shifts 5.2 ppm and 4.8 ppm.[43] The peak at 5.2 ppm represents a single proton of the lactic unit while the peak of 4.8 ppm represents two protons of the glycolic unit. Hence the mole fractions of lactic ($\mathbf{m_L}$) and glycolic ($\mathbf{m_G}$) units were calculated from peak integrations (**p**) of each component, as shown below:

$$\mathbf{m_L} = \frac{p_L}{p_L + (p_G/2)} \qquad \mathbf{m_G} = \frac{p_G/2}{p_L + (p_G/2)}$$

2.2.2. End-capping analysis

Each PLGA sample was weighed (~15 mg), dissolved in deuterated chloroform ($CDCl_3$) (0.75 mL), and then the resulting polymer solutions pipetted into an NMR tube. The end-capping of PLGA polymers were confirmed by ^{13}CNMR. The relative molecular weight of the ester end-cap is smaller than the PLGA polymer, thus, the signal collection should be performed with the maximized signal-to-noise ratio. A Z-restored spin-echo pulse sequence was used with a 30-degree observation pulse, a 3-s inter pulse delay, and a 0.55-s data acquisition time and a total of 12,000 scans were acquired over 12.5 h.[52] Generally, PLGA polymers have two types of end caps: ester and acid end caps. In the ^{13}C NMR spectrum, methyl units at the end of the alkyl chain appear very far upfield at a chemical shift of 14 ppm, which is used to confirm an ester end-capping of PLGA polymer. Acid end-capping was confirmed by the absence of this ester-specific peak at 14 ppm in their NMR spectrum.[43]

2.2.3. Blockiness analysis

The blockiness was determined by high-resolution ^{13}CNMR.[43] These spectra were collected from 50 mg/mL polymer solutions (25 mg) in $CDCl_3$ (0.5 mL). These spectra were analyzed for the different monomer sequence distributions. This was done by using a pulse sequence without NOE enhancement, and employing a 30-degree ^{13}C observe pulse, a 2.0 s inter-pulse delay, a 4.6 s relaxation delay, and a 0.4 s acquisition time. Total acquisition time was approximately 4 h and 50 mins to give an accurate signal-to-noise ratio.

Blockiness represents the heterogeneity of PLGA and is determined by the glycolic unit sequence distribution according to the glycolic carbonyl group located in the chemical shift 166-167 ppm in high-resolution ^{13}CNMR spectrum. The upfield G-G peak ($I_{G\text{-}G}$) represents the glycolyl-glycolyl carbonyl; while the downfield G-L peak ($I_{G\text{-}L}$) represents a glycolyl-lactyl carbonyl. Blockiness values can be calculated according to two methods. One is calculated by the definition of Skidmore *et al.*,[53] in which **Rc** value is obtained by the ratio of $I_{G\text{-}G}$ to $I_{G\text{-}L}$; while the other is reverse **Rcms** (the ratio of $I_{G\text{-}L}$ to $I_{G\text{-}G}$) by the definition of Hausberger and DeLuca[44] representing the ratio of the two carbonyl peaks. Here, we use the **Rc** value to describe blockiness.

$$\mathbf{Rc} = \frac{I_{G\text{-}G}}{I_{G\text{-}L}} \qquad \mathbf{Rcms} = \frac{I_{G\text{-}L}}{I_{G\text{-}G}}$$

2.2.4. Block length analysis

The block lengths were determined by high-resolution ^{13}CNMR.[54] Each PLGA sample was weighed (20 mg) and dissolved in a mixture of hexafluoro-2-propanol (10 mg/mL) for enhanced dissolving capacity and deuterated benzene for the lock signal (V/V=5/1), and then solutions were pipetted into a NMR tube. The number of transients was 5000, with a relaxation delay of 10 s. The average sequence lengths in monomer units **L** and **G**, which could be calculated from the relative dyad splitting intensities of the carbonyl carbon of the lactyl-lactyl, lactyl-glycolyl, glycolyl-glycolyl and glycolyl-lactyl signals ($I_{G\text{-}G}$, $I_{G\text{-}L}$: signal intensities of glycolyl-glycolyl and glycolyl-lactyl bonds; $I_{L\text{-}L}$, $I_{L\text{-}G}$: signal intensities of lactyl-lactyl and lactyl-glycolyl bonds at chemical shift ~172 ppm and ~169 ppm):

$$\mathbf{L_L} = \frac{I_{LL}}{I_{LG}} + 1 \qquad \mathbf{L_G} = \frac{I_{GG}}{I_{GL}} + 1$$

3. Results and discussion

3.1. Lactic/glycolic unit ratio (L/G ratio) calculation

In the molecular properties of PLGA polymers, the L/G ratio is an important factor determining its macroscopic properties. Lactic acid units are more hydrophobic than glycolic units, so the hydrophobicity of PLGA polymer increases with an increasing L/G ratio in the non-crystalline groups of racemic PLGA 50/50 to 100/00, leading to different properties and behaviors.[43] For instance, in T.G. Park's study of PLGA degradation,[55] PLGA 50/50 microspheres (polymer manufacturer: Medisorb) exhibited very sharp T_g peaks around 120-130^{o}C at day 14 and broad T_g peaks at day 33; whereas PLGA 70/30 microspheres (polymer manufacturer: Polysciences) showed multiple small T_g peaks at day 14. These results imply that crystallization was generated due to different degradation processes associated with an increasing lactic unit content. The lactic-lactic bond is also more stable than the glycolic-glycolic bond as further supported by the relative stability of the lactyl-lactic acid dimer in water relative the linear dimer of

glycolic acid, the latter of which is not detectable in released PLGA degradation products.[56, 57] The L/G ratio could be readily and accurately calculated based on the proportion of specific peak intensities at chemical shifts 4.5-5.5 ppm as described in section 2.3. Briefly, three representative ^{1}HNMR spectra of PLGA polymers with different L/G ratios are shown in **Figure 1,** where **A, B** and **C** represent the spectrum of Expansorb® DLG 50-5A, Expansorb® DLG 75-2A and Expansorb® DL 100-2A, respectively. Except for the cluster of proton peaks at chemical shift 1.6 ppm (iii), Expansorb® DLG 50-5A(**A**) displayed two main groups of peaks at 5.2 ppm (i) and 4.8 ppm (ii), indicating the respective protons of methine of lactic units and methylene of glycolic units. Compared to spectrum **A**, the intensities of peaks at chemical shift 4.8 ppm (ii) proportionally decreased in **B** and then disappeared in **C**, implying the decrease in glycolic monomer used during synthesis from **A** to **C**. These results matched the labelled L/G ratios of these PLGA polymers, Expansorb® DLG 50-5A (50/50), Expansorb® DLG 75-2A (75/25), and Expansorb® DL 100-2A (100/0). We analyzed three groups of L/G ratios: 50/50, 75/25, and 100/0. The lactic content in the commercial PLGA polymers was calculated by ^{1}HNMR spectroscopy and is displayed in **Figure 2** and **Table 1**.

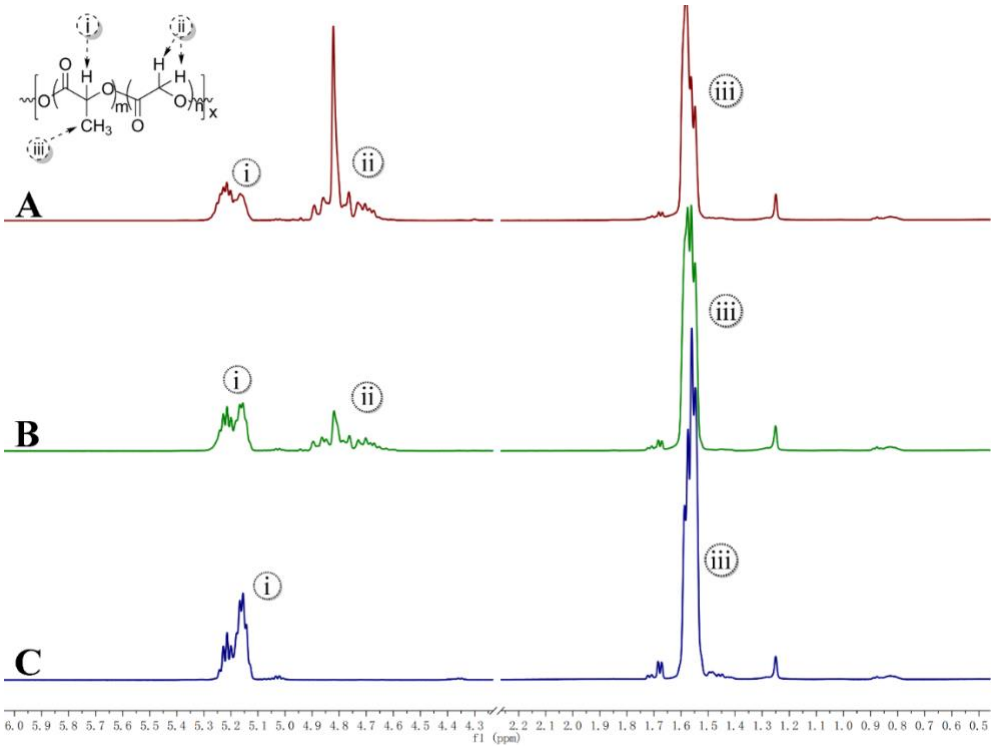


Figure 1. Characteristic proton peaks of PLGA polymers in ^{1}HNMR spectra ($CDCl_3$, 500 MHz). A, B and C represent the spectra of three PLGAs with L/G ratio of 50/50, 75/25 and 100/0, respectively (Expansorb® DLG 50-5A, Expansorb® DLG 75-2A and Expansorb® DLG 100-2A). Labels corresponding to the peaks of (i) and (iii) are the protons of methine and side methyl groups in lactic mer units, respectively; (ii) protons of methylene in glycolic mers.

Table 1. Macromolecular properties of commercial PLGA polymers.

Brand & product	Labelled lactic acid %	Molecular weight[a] kDa	Inherent viscosity[b] dL/g	Lot No.	End-cap	Average block length	
						L_L	L_G
Expansorb® DLG 50-2E	50	17	0.21	C100011425	Ester	2.5	2.6
Expansorb® DLG 50-2A	50	13	0.19	PP10056489	Acid	4.4	4.2
Expansorb® DLG 50-5A	50	48	0.47	PP10059967	Acid	4.0	4.0
Expansorb® DLG 75-9E	75	136	0.95	C100011427	Ester	6.7	2.4
Expansorb® DLG 75-2A	75	8	0.12	PP10056560	Acid	6.9	2.5
Expansorb® DL 100-2A	100	14	0.18	PP10059963	Acid	NA	NA
Resomer® RG 502	50	14.7	0.20	BCCB0256	Ester	2.6	2.5
Resomer® RG 502H	50	14	0.22	BCBZ7916	Acid	3.0	3.0
Resomer® RG 504H	50	52.9	0.57	BCBX4108	Acid	2.9	2.9
Resomer® RG 756S	75	NR	0.90	BCBZ4420	Ester	5.8	1.7
Resomer® RG 752H	75	13.4	0.22	BCBw4713	Acid	8.5	2.6
Resomer® R 202H	100	16.9	0.24	BCBV6665	Acid	NA	NA
Purasorb® PDLG 5004A	50	NR	0.37	1110001124	Acid	3.4	3.2

Purasorb® PDLG 7502A	75	NR	0.19	1802003617	Acid	7.8	2.7
Purasorb® PDL 02A	100	NR	0.22	1703003820	Acid	NA	NA
Wako® 7515	75	NR	0.18	TWO.1257	Acid	10.6	1.4
Lactel® DL-PLG B6007-2P	75	NR	0.81	A17-068	Ester	5.5	2.1

[a]MW reported from manufacturer certificate of analyses, determined by GPC. [b]Expansorb® i.v.: 0.5% in chloroform, 25°C. Resomer® inherent viscosity (i.v.): 0.1% in chloroform at 25°C. Purasorb® PDLG 5004A i.v.: 0.5 g/dL in chloroform at 25°C. Purasorb® PDLG 7502A, and Purasorb® PDL 02A i.v.: 1 g/dL in chloroform at 25°C. Lactel® i.v.: 0.5 g/dL in chloroform at 30°C. Wako i.v. method not reported. NR=not reported.

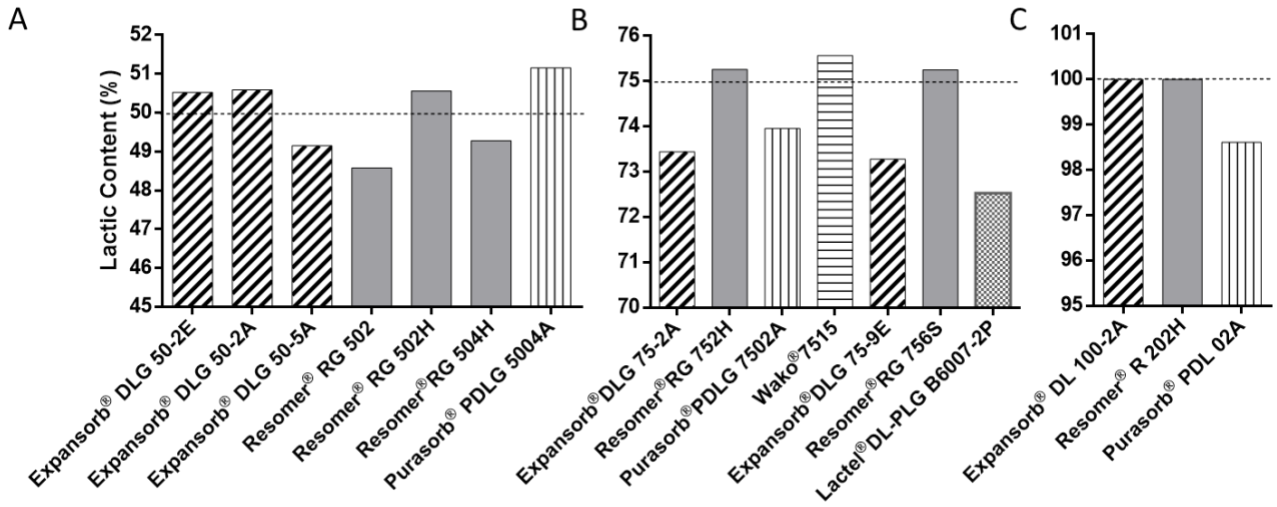


Figure 2. Calculated molar lactic acid content (%) of different branded PLGAs as assessed by ^{1}HNMR spectroscopy ($CDCl_3$, 500 MHz). A, B and C represent these groups of PLGAs with L/G ratio of 50/50, 75/25 and 100/0, respectively.

From the data set described, some notable trends were observed, although clearly a higher quantity of lots and/or polymer molecular weights would be needed for more definitive conclusions. The first trend observed was that for L/G ratio of 50/50 (**Figure 2A**), the lactic content decreased as the molecular weight (as reported in the product

certificate of analysis) increased when compared within the same manufacturer. For example, the L/G ratio of Resomer® RG 502H (14 kDa) > Resomer® RG 504H (52.9 kDa) and Expansorb® DLG 50-2A (13 kDa) > Expansorb® DLG 50-5A (48 kDa). A second trend was observed where Resomer® polymers with ester end-capping displayed a higher lactic content than their comparable polymers with acid end-capping, e.g., L/G of Resomer® RG 502 > Resomer® RG 502H. For L/G ratios of 75/25 (**Figure 2B**), Resomer® polymers were found to most closely match the listed L/G ratio, e.g., Resomer® RG 756S (75.25/24.75) and Resomer® 752H (75.26/24.74). In the L/G ratio 75/25 ester end-capped group, although the lactic content did not follow a trend with its MW or inherent viscosity (i.v.), the lactic contents were lowest in these high molecular weight polymers, e.g., Resomer® RG 756S (i.v.=0.90 dL/g, 75.25/24.75) > Expansorb® DLG 75-9E (0.95 dL/g, 73.28/26.72) > Lactel® DL-PLG B6007-2P (0.81 dL/g, 72.53/27.47). For polymers with an L/G ratio of 100/0 (**Figure 2C**), Resomer® R 202H and Expansorb® DL 100-2A displayed exactly 100% lactic content, although surprisingly, Purasorb® PDL 02A revealed a low level of glycolic impurities (98.61/1.39). We also observed that the lactic content slightly decreased as the molecular weight of PLGA increased, which could be attributed to the synthesis of the PLGA polymer. In either the ring-opening or direct polycondensation, the PLGA product mainly undergoes three steps: chain initiation, chain growth and termination. During polymerization, glycolic monomers are more reactive and add to the growing polymer chain easier than lactic monomers.[58] As the chain-length grows the molecular weight of the intermediate increases, leading to an increase in steric hinderance at the hydroxyl reaction site.[59] Thus, the higher the molecular weight and the longer the reaction proceeds, there may be an increase in smaller chains forming where the more reactive glycolic monomers are preferentially added compared to lactic monomers.[60]

When other factors such as molecular weight and concentration are similar among polymers, the L/G ratio could affect the solubility of low molecular weight PLGA monomers, as the affinity with water increases as the content of more hydrophilic glycolic monomers increases (L/G ratio decreased).[43] Moreover, L/G ratio is well known to influence the degradation rate of PLGA *in vitro* and *in vivo*.[55, 61] As lactic content increases, this creates a more hydrophobic and sterically hindered environment, thus hydrolysis of the PLGA is delayed.[62, 63]As reported,[55] PLGA polymers with different L/G ratios could degrade with very complicated morphological changes, such as decreases in T_g, appearance of double glass transitions and evolution of crystalline melting peaks. Firstly, the ester bonds of the glycolic unit (glycolic-glycolic acid) are preferentially cleaved compared to those of the lactic unit (lactic-lactic acid) due to its

inherent higher reactivity with water and/or its greater hydrophilicity; secondly, compared to the more hydrophobic PLA (PLGA 100/0), the amorphous PLGA is considered to possess a greater extent of water hydration. For these reasons, it could be concluded that PLGA with low L/G ratio would undergo a faster degradation than those of high L/G ratio, containing PLA. However, PGA (PLGA 0/100) does not display a more rapid degradation *in vitro* or *in vivo*. The typical rank of degradation is PLGA 50/50 > PGA (PLGA 0/100) > PLA (PLGA 100/0).[64] PGA with all glycolic units displays higher crystallinity (melting point is ~220 °C) and chain packing (density is ~1.58 g/cm^3) compared with both PLGA and PLA polymers, leading to better mechanical strength and slower degradation.[65, 66]

3.2. End-group analysis

There are two common types of end groups of commercial linear PLGA, terminating in carboxylic acid or aliphatic esters, with the other end of the polymer chain terminated by a single hydroxyl group. In synthesis of PLGA polymer, hydroxyl containing compounds such as water, lactic acid, or an alcohol, are used for initiators in acid end-capped polymer, while an aliphatic alcohol, such as 1-dodecanol is used as the initiator for obtaining an ester end-capped polymer.[43, 67] As mentioned in section 2.4, in ^{13}CNMR spectrum, the distinct difference between acid- and ester-modified end caps of PLGA is the carbon peak of the methyl group of the aliphatic chain in the ester end-cap at a chemical shift of 14 ppm. The side methyl unit of lactic monomers should appear at a chemical shift of 16 ppm, so there is a clear split between the different methyl groups.[52] As shown in **Figure 3**, in the ^{13}CNMR spectrum of Expansorb® DLG 50-2E (**B**), the first methyl peak appears very high upfield (red arrow, chemical shift of 14 ppm), suggesting an ester-modified end cap in its structure; while no signal was observed at the same point in the ^{13}CNMR spectrum of Expansorb® DLG 50-2A (**A**), suggesting an acid end group. All the 17 commercial PLGA polymers were analyzed by ^{13}CNMR spectroscopy and the data is presented in **Table 1**. Five polymers, including Lactel® DL-PLG B6007-2P, Expansorb® DLG 75-9E, Resomer® RG 756S, Resomer® RG 502 and Expansorb® DLG 50-2E, were verified that they possessed ester-modified end caps; while the remaining PLGA polymers had the acid end group. These results all coincided with their brand labels.

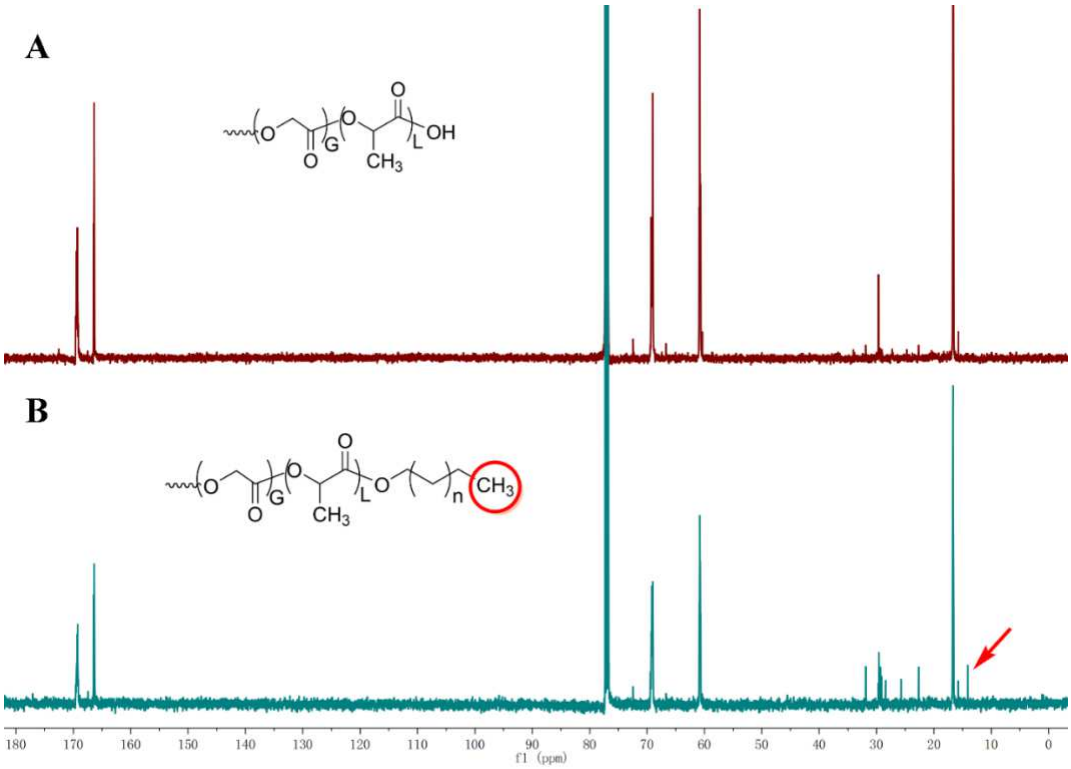


Figure 3. ^{13}CNMR ($CDCl_3$, 500 MHz) spectra of two representative PLGAs with acid-/ester-modified end-cap. A is Expansorb® DLG 50-2A and B is Expansorb® DLG 50-2E. The red arrow directs carbon signal at upfield chemical shift (14 ppm) of the methyl group at the terminal of aliphatic ester bond.

End-capping is considered an important index influencing the behavior of PLGA hydrolysis. Compared to the PLGA polymers with an acid end group, ester end-capping decreases the rate of hydrolysis. This can be explained by two aspects:[68] first, ester-modification masks the carboxyl group at the chain terminus, enhancing the hydrophobicity and decreasing water uptake of PLGA polymers; second, the ester end-cap impedes the acid-mediated autocatalysis of the acid end-capped PLGA polymers. For example, Tracy *et al.*[69] described that the ester-capped PLGA polymer degraded 2-3 times slower *in vitro* and 3-4 times slower *in vivo* than the uncapped (acid-capped) PLGA polymer with similar molecular weights. Most research[70, 71] shows that ester end-capped PLGA-based formulations generally display a substantially slower hydrolysis rate and slower drug release compared to PLGA with acid end capping, but this process is obviously affected by multiple parameters.

3.3. Monomer sequence analysis

Numerous reports have discussed the influence of PLGA composition (L/G ratio and end-capping) and molecular weight on its behavior, however, little is known about the effects of the intrachain sequence. Most PLGA polymers are obtained by ring-opening polymerization. However, in this process, the monomers of lactide and glycolide usually do not undergo a uniform distribution (random or alternating sequenced) with each other, and actually generate multiple short randomly distributed blocks of lactyl-lactyl (L-L) and glycolyl-glycolyl (G-G) linkages along the polymer chain.[73] With all other factors equal, the sequence distribution, defined as the term "blockiness", may result in differences leading to variations in PLGA solubility and degradation behavior. The "blockiness" can be calculated according to two descriptions, one by the definition of Skidmore *et al*.,[53] in which **Rc** value is obtained by the ratio of $I_{G\text{-}G}$ to $I_{G\text{-}L}$; while the other is the **Rcms** value (the ratio of $I_{G\text{-}L}$ to $I_{G\text{-}G}$) by the definition of Hausberger and DeLuca.[44] In this paper, we chose the former as the term investigated below since the former result could more straightly present impact of G-G units on PLGA polymer hydrolysis and degradation. As mentioned in section 2.5, based on the different chemical molecular environments of the carbonyl carbons in G-G (blue) and glycolyl-lactyl (G-L, red) bonds (shown in **Figure 4**), the blockiness value (**Rc**) can be calculated by dividing the peak intensities located at chemical shift 166-167 ppm.[43] In this cluster of two peaks, the upfield peak represents the carbonyl of a glycolic unit connecting to another glycolic unit ($I_{G\text{-}G}$) while the downfield peak ($I_{G\text{-}L}$) represents the carbonyl of a glycolic connected with a lactic unit.

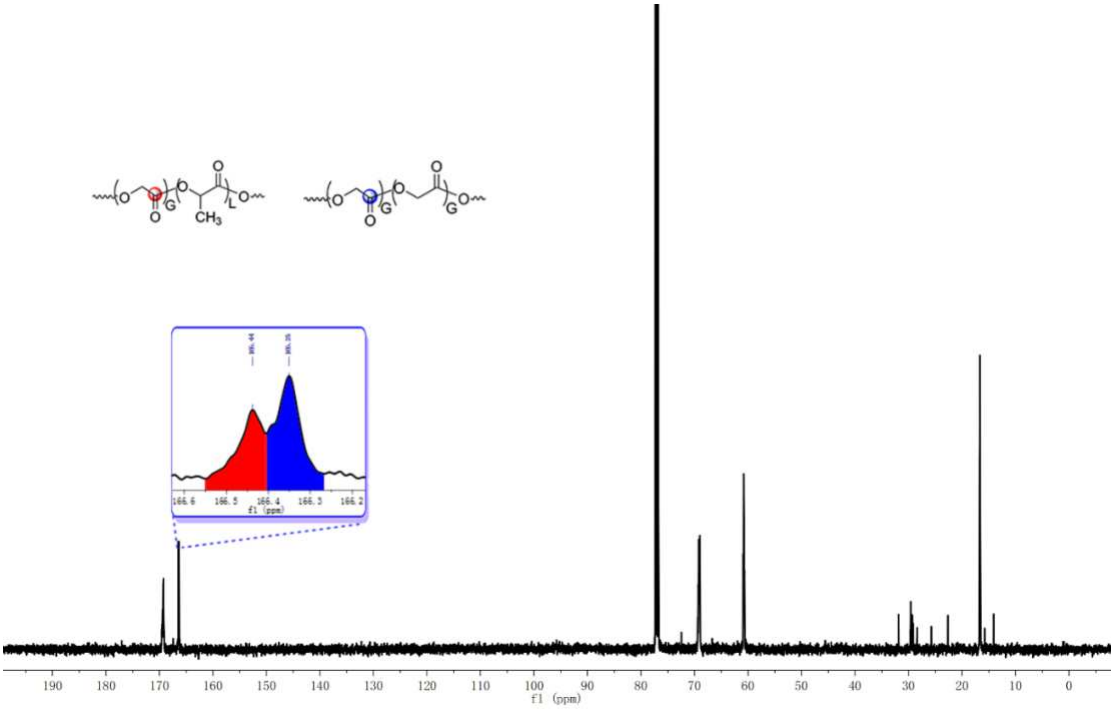


Figure 4. ^{13}CNMR spectra ($CDCl_3$, 500 MHz) of carbonyl regions of glycolic mer units of the representative Expansorb® DLG 50-2E. The red area is intensity of the carbonyl peak of glycolic mers adjacent to lactic mers, while the blue zone represents the intensity of the carbonyl peak of a glycolic mers adjacent to another glycolic mers.

Blockiness describes the monomer sequence distribution and is affected by multiple factors, especially L/G ratio. Therefore, it is important to consider blockiness value within groups of PLGAs of the same class of MW, racemic lactic acid/lactide, end-capping, L/G ratio, etc. Blockiness values (**Rc**) were calculated by ^{13}CNMR spectroscopy and are presented in **Figure 5**. In the group of high molecular weight, ester-capped PLGA polymers with L/G ratio of 75/25, the blockiness value of Resomer® RG 756S (**Rc**=1.7) was notably higher than that of Expansorb® DLG 75-9E (**Rc**=0.5) and Lactel® DL-PLG B6007-2P (**Rc**=0.6). In the lower molecular weight range (9-15 kDa) acid-capped PLGA 75/25, Expansorb® DLG 75-2A (**Rc**=1.3) had the highest blockiness compared to others, and Resomer® RG 752H (**Rc**=0.7) and Wako® 7515 (**Rc**=0.6) displayed a slightly higher blockiness than Purasorb® PDLG 7502A (**Rc**=0.5). Moreover, in the L/G ratio of 50/50 acid-capped group, the order of blockiness in same weight range (44

kDa-58 kDa) was Purasorb® PDLG 5004A (**Rc**=1.6) > Resomer® RG 504H (**Rc**=1.3) > Expansorb® DLG 50-5A (**Rc**=0.7). Moreover, there was a slight increase of blockiness between Expansorb® DLG 50-2E (**Rc**=1.4) and Resomer® RG 502 (**Rc**=1.5). Notably, the blockiness of Expansorb® DLG 50-2A (**Rc**: 1.9) was significantly higher than that of Resomer® RG 502H (**Rc**=1.4). Additionally, the three PLA polymers, Purasorb® PDL 02A, Resomer® R 202H and Expansorb® DL 100-2A, naturally had no blockiness due to the absence of glycolic units in their structure. These results verified the increase of blockiness occurs as L/G ratio decreases, as mentioned above. Except for Resomer® RG 756S (**Rc**=1.7) and Expansorb® DLG 75-2A (**Rc**=1.3), 50/50 PLGA polymers, such as Expansorb® DLG 50-2A (**Rc**=1.9) and Expansorb® DLG 50-5A (**Rc**=0.7), displayed a higher blockiness than those with L/G ratio of 75/25, such as Resomer® RG 752H (**Rc**=0.7) and Lactel® DL-PLG B6007-2P (**Rc**=0.6). The probability of increased G-G blocks decreases as the lactic content increases from 50/50 to 75/25 of L/G ratio.[54] Moreover, in the L/G ratio of 75/25 group, Resomer® PLGA and Expansorb® polymers showed an inverse relationship of blockiness values in their low and high molecular weight ranges, e.g., Resomer® RG 756S (**Rc**=1.7) is higher than Expansorb® DLG 75-9E (**Rc**=0.5), while Expansorb® DLG 75-2A (**Rc**=1.3) is higher than Resomer® RG 752H (**Rc**=0.7). From this data, we can conclude that blockiness values of the PLGA polymers differed, although these products were labelled with the same L/G ratio or molecular weight even within the same brand, increasing the complexity of sourcing PLGA for biomedical products.

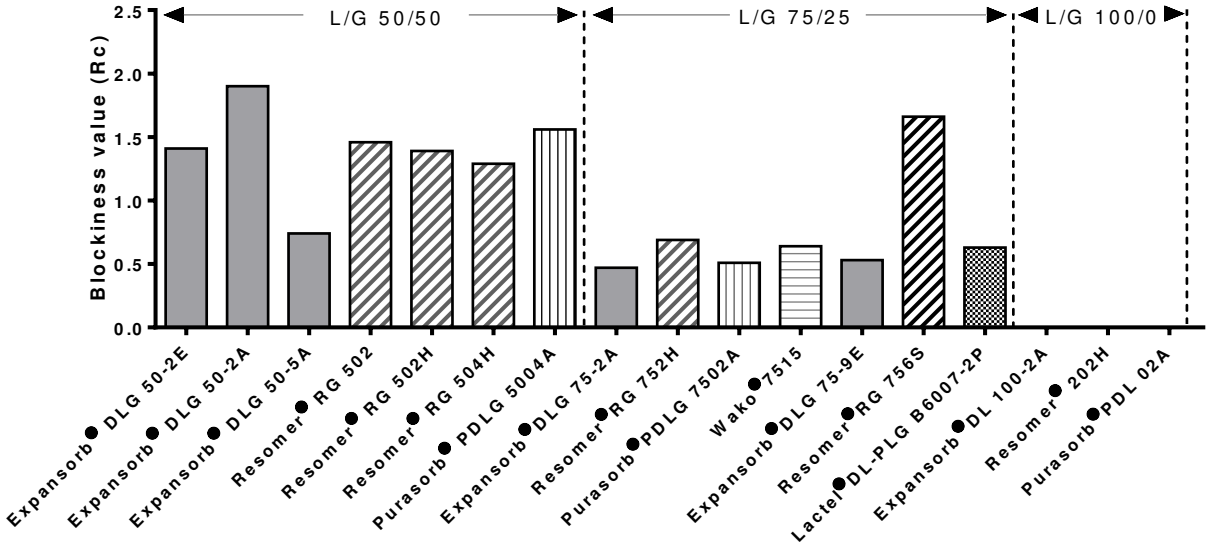


Figure 5. Blockiness values of PLGA polymers by ^{13}CNMR spectroscopy ($CDCl_3$, 500 MHz).

As the blockiness value increases, higher numbers of glycolic monomer units are grouped together and are less connected with lactic monomers, resulting in more intrachain heterogeneity of PLGA polymers.[44] A larger number of G-G blocks would be expected to increase water uptake of PLGA (presuming the absence of crystallite formation), and in turn, increase ester-bond cleavage. Thus, the blockiness could affect PLGA physicochemical properties such as degradation.[55, 62] Meyer [47, 51] *et al*. revealed that there was a difference in the hydrolysis process between random PLGA with higher heterogeneity and higher block lengths relative to more homogenous PLGA polymers with a 50/50 L/G ratio. The hydrolysis of the random and more 'blocky' PLGA polymer underwent a very fast initial step due to the rapid degradation rate of the more sterically accessible G-G bonds compared to lactic-rich blocks, and then the degradation speed gradually slowed as lactic-rich blocks remained. The hydrolysis of the sequenced PLGA with low or no blockiness was steady during the whole period. These results implied that PLGA polymers with high blockiness could lead to a fast loss of molecular weight or uneven erosion in the process of degradation, which might affect the long-acting zero-order release behavior of encapsulated drugs. Therefore, as seen in **Figure 5**, Resomer® RG 756S (**Rc**=1.7) displayed higher blockiness than Expansorb® DLG 75-9E (**Rc**=0.5) and Lactel® DL-PLG B6007-2P (**Rc**=0.6), implying that the former may possess a relatively more rapid hydrolysis rate than the other two polymers.

3.4. Block length analysis

Almost all commercial PLGA polymers have a non-statistical distribution of the co-monomers, which implies that there are different block lengths of glycolic or lactic monomers. Technically, block lengths are influenced by the synthetic parameters (such as reaction type and time) which stems from the increased reactivity of glycolide compared to that of lactide.[73] The average block length of either lactic units or glycolic units, associated closely with the blockiness, could affect the physicochemical properties of PLGA polymers, such as solubility and crystallinity, leading to different degradation and hydrolysis behaviors.[74] As the glycolic sequence length increases, the solubility of PLGA polymers has been reported to decrease in organic solvent,[59] e.g., the amorphous PLLGA (46.3 (L-lactide)/53.7) with an $\mathbf{L_G}$ value of 4.3 was reported insoluble in chloroform at 25°C.[54] As described in section 2.6,[54,

[75] the block lengths of both lactic and glycolic unit sequences can be accurately calculated from the peak intensities of the dyad splitting of carbonyl carbons in the ^{13}CNMR spectroscopy (seen in **Figure 6**). If there was a truly random distribution of lactic and glycolic units in PLGA 50/50, the theoretical values of block lengths (both $\mathbf{L_L}$ and $\mathbf{L_G}$) should be near or equal to 2.[76] Lower values are also theoretically gotten when a "transesterification" process occurs during the period of reaction.

As shown in **Table 1**, both $\mathbf{L_L}$ and $\mathbf{L_G}$ of these commercial PLGA polymers were typically >2, showing the non-ideal random distributions of the lactic and glycolic units. In the group with an L/G ratio 50/50, low molecular weight PLGA polymers (~12 kDa) including both Resomer® RG 502 ($\mathbf{L_L}$=2.6, $\mathbf{L_G}$=2.5) and Expansorb® DLG 50-2E ($\mathbf{L_L}$=2.5, $\mathbf{L_G}$=2.6) displayed lower block lengths than the other polymers evaluated. For a moderate molecular-weight range (32 kDa-58 kDa), Resomer® RG 504H displayed the lowest block lengths ($\mathbf{L_L}$=2.9 and $\mathbf{L_G}$=2.9) compared to Expansorb® DLG 50-5A ($\mathbf{L_L}$=4.0, $\mathbf{L_G}$=4.0) and Purasorb® PDLG 5004A ($\mathbf{L_L}$= 3.4, $\mathbf{L_G}$=3.2). These PLGA polymers with shorter block lengths of both lactic and glycolic units would be expected to undergo a steadier rate of hydrolysis compared to a PLGA with random heterogeneity. Moreover, in the group of low molecular weight PLGA polymers with L/G ratio of 75/25, there were significantly larger $\mathbf{L_L}$ values than $\mathbf{L_G}$ values, especially for Wako® 7515 ($\mathbf{L_L}$=10.6, $\mathbf{L_G}$=1.4), Resomer® RG 752H ($\mathbf{L_L}$=8.5, $\mathbf{L_G}$=2.6) and Purasorb® PDLG 7502A ($\mathbf{L_L}$=7.8, $\mathbf{L_G}$=2.7). An increase in lactic block length ($\mathbf{L_L}$) due to the increased proportion of lactic acid in the L/G ratio is expected, whereas the glycolic block lengths ($\mathbf{L_G}$) are more informative of the PLGA sequence distribution. Wako® 7515 PLGA is produced by polycondensation (PC) in contrast with the most common ring opening polymerization (ROP) method. Here, it is interesting to point out that the Wako polymer had the lowest glycolic block length within its class. Both ROP and PC can result in long block lengths due to the increased reactivity of glycolide or glycolic acid,[59, 67] yet it has been shown that very low block lengths can be achieved in PC by increasing the reaction time and although lower block lengths can also similarly be achieved in ROP, even a dramatically long reaction time does not necessarily result in a completely homogeneously sequenced structure.[77] Furthermore, combined with high blockiness, the long block length of glycolic units ($\mathbf{L_G}$) meaning more reactive G-G bonds would be expected to contribute to the increased hydrolytic rate in the initial step of PLGA degradation. For instance, when all other variables are considered equal or similar, which is usually difficult to achieve, Expansorb® DLG 50-2A (**Rc**: 1.9, $\mathbf{L_G}$: 4.2) would be expected to have increased

initial degradation rate compared to Expansorb® DLG 50-5A (**Rc**: 0.7, $\mathbf{L_G}$: 4.0) and Resomer® RG 502H (**Rc**: 1.4, $\mathbf{L_G}$: 3.0), respectively.

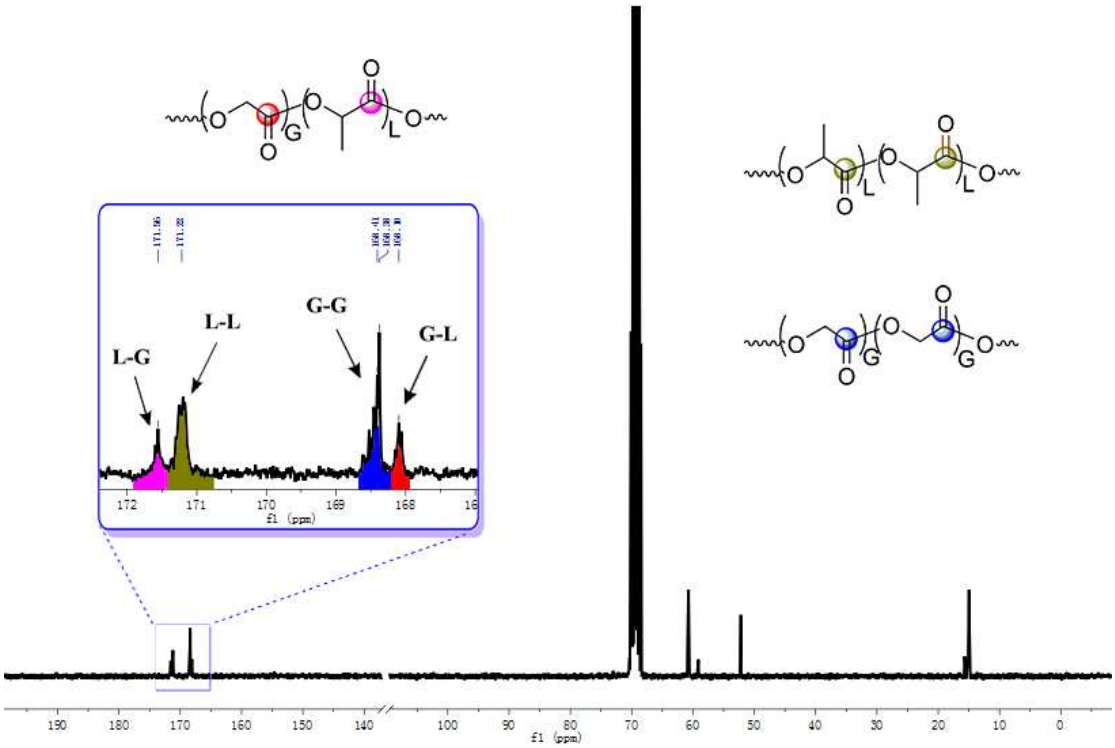


Figure 6. ^{13}CNMR spectra (hexafluoro-2-propanol/benzenze-d_6, 500 MHz) of the carbonyl regions of PLGA polymer (representative is Expansorb® DLG 50-5A). Shown as the arrows, the areas of pink (**L-G**), brown (**L-L**), blue (**G-G**) and red (**G-L**) represent the intensities of the carbonyl peak of lactic mer units adjacent to glycolic mers units, lactic adjacent to lactic, glycolic adjacent to glycolic, and glycolic adjacent to lactic, respectively.

4. Conclusion

Four important properties describing the chemical microstructures of 17 commercial PLGA polymers across 5 different brands and varying L/G ratio, end-capping, and molecular weights have been investigated by ^{1}H and ^{13}CNMR spectroscopy. Overall, polymers with similarly labeled properties from different manufacturers are quite comparable.

It was observed that within groups of similar L/G ratio and molecular weights, different brands of PLGA polymers possessed distinct blockiness and block lengths, which could lead to diverse behaviors. Based on these measured values, it is anticipated that when other parameters are in the same range, the PLGA polymers with low blockiness and shorter block lengths of both glycolic and lactic monomers may show more uniform erosion and steady degradation rates. In theory, these methods could be used as a guide for polymer selection across multiple fields, especially long-acting release of drugs. Further research on the correlation between these properties and PLGA degradation process and PLGA drug release *in vitro* and *in vivo* will be reported by our lab soon.

Supporting Information

^{1}HNMR Spectra of Commercial PLGA Polymers

Declarations

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Conflicts of interest

The authors declare no competing financial interest.

Availability of data and material

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Code availability

Not applicable

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

All authors whose names appear on the submission approved the version to be published.

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Figures

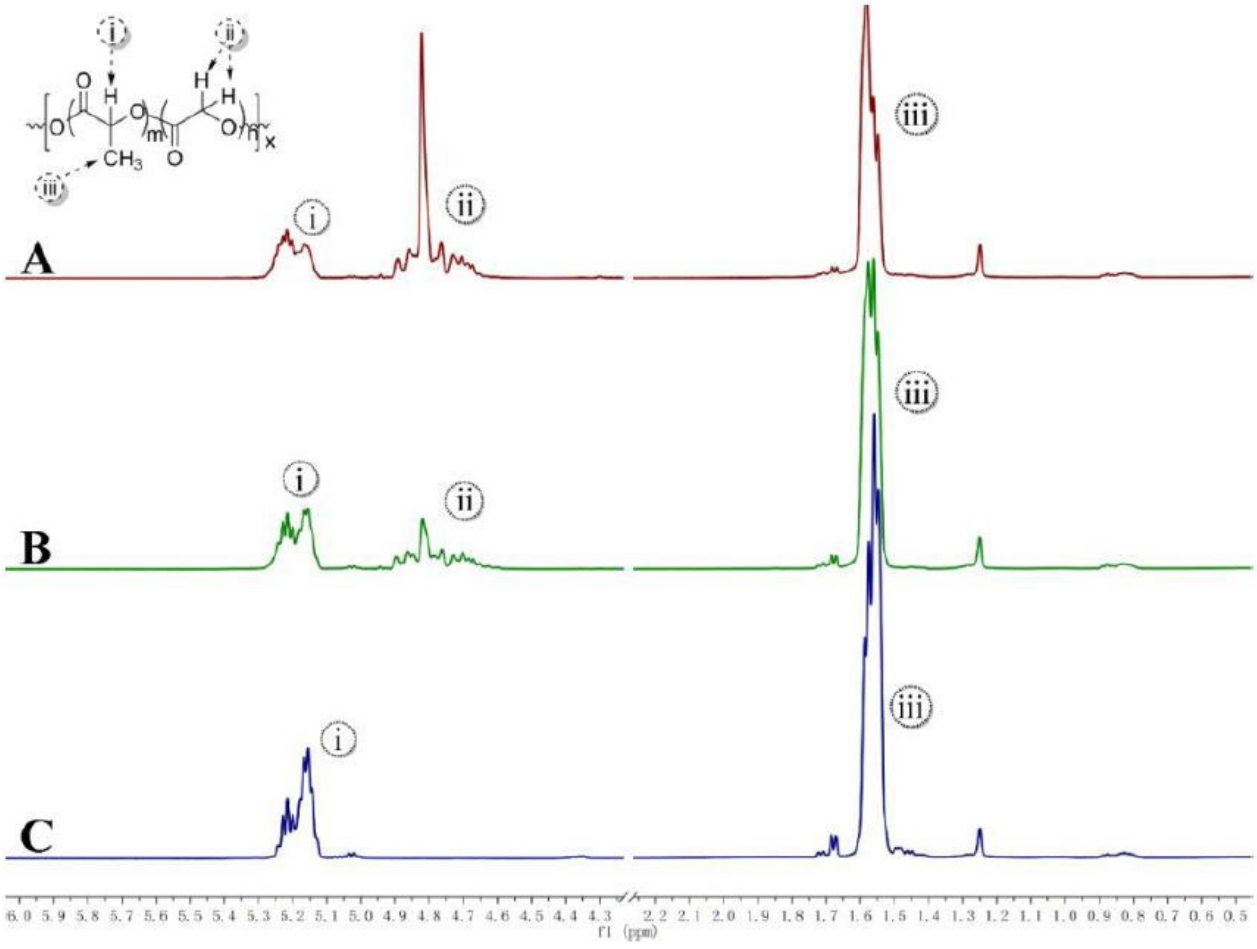

Figure 1

Characteristic proton peaks of PLGA polymers in 1HNMR spectra (CDCl3, 500 MHz). A, B and C represent the spectra of three PLGAs with L/G ratio of 50/50, 75/25 and 100/0, respectively (Expansorb® DLG 50-5A, Expansorb® DLG 75-2A and Expansorb® DLG 100-2A). Labels corresponding to the peaks of (i) and (iii) are the protons of methine and side methyl groups in lactic mer units, respectively; (ii) protons of methylene in glycolic mers.

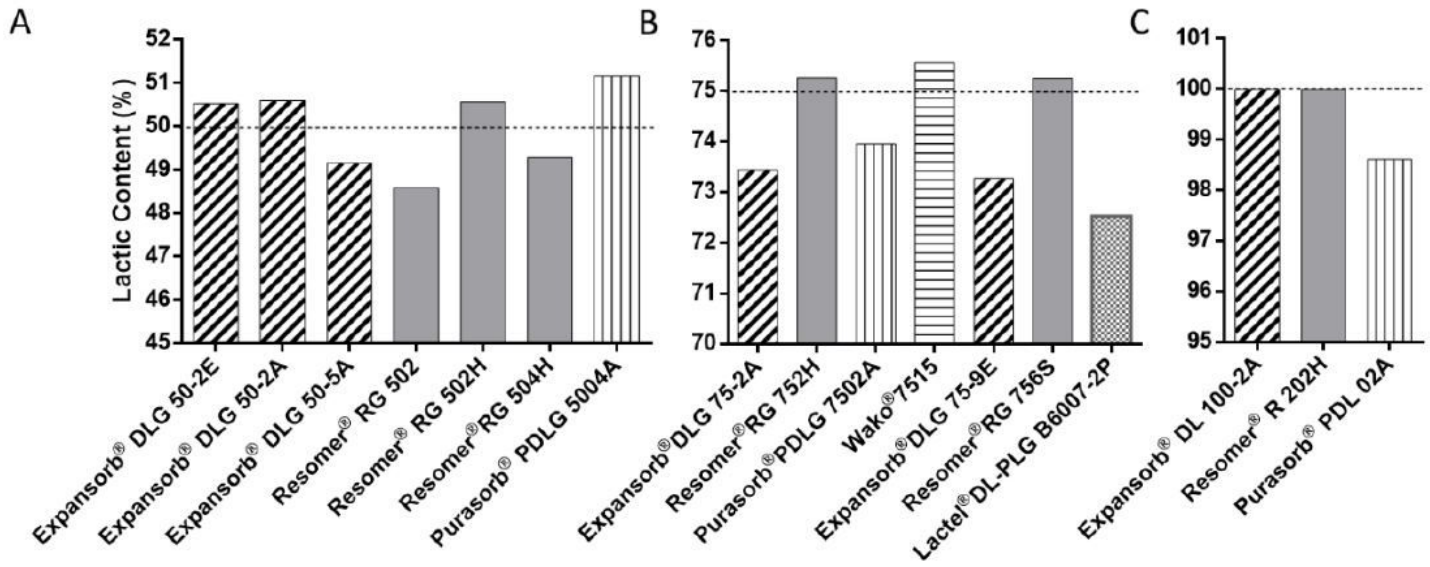

Figure 2

Calculated molar lactic acid content (%) of different branded PLGAs as assessed by 1HNMR spectroscopy (CDCl3, 500 MHz). A, B and C represent these groups of PLGAs with L/G ratio of 50/50, 75/25 and 100/0, respectively.

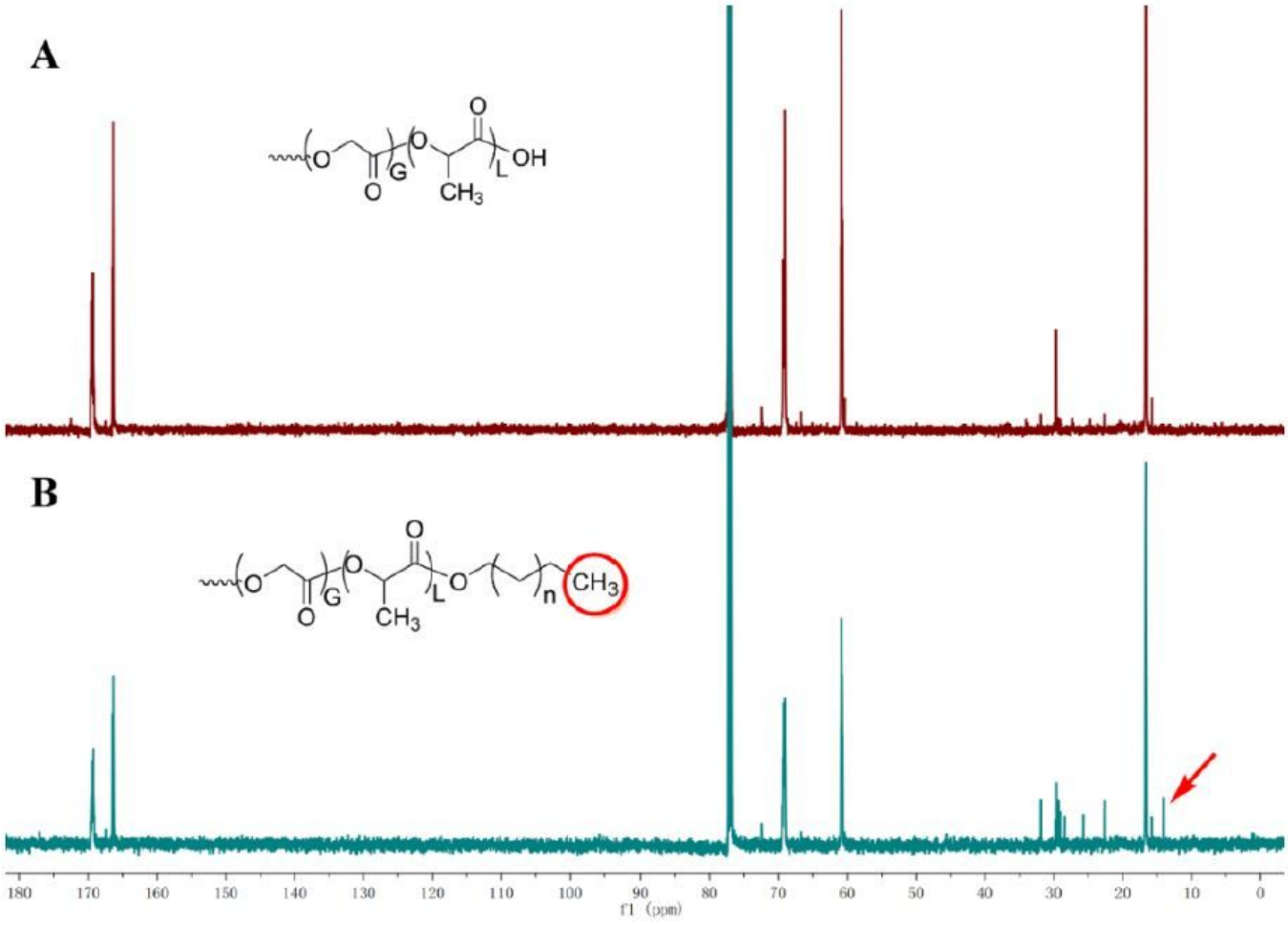

Figure 3

13CNMR (CDCl3, 500 MHz) spectra of two representative PLGAs with acid-/ester-modified end-cap. A is Expansorb® DLG 50-2A and B is Expansorb® DLG 50-2E. The red arrow directs carbon signal at upfield chemical shift (14 ppm) of the methyl group at the terminal of aliphatic ester bond.

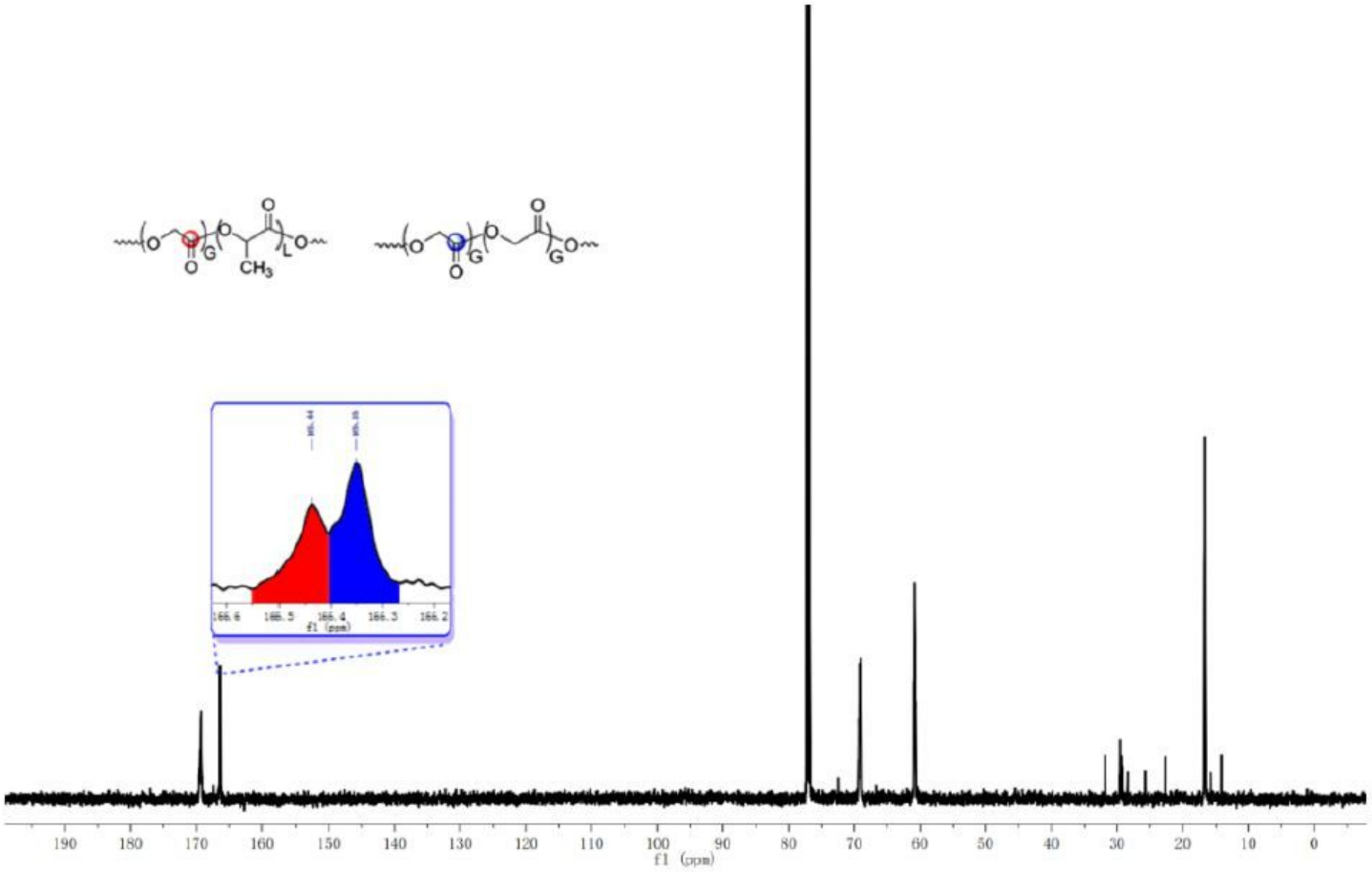

Figure 4

13CNMR spectra (CDCl3, 500 MHz) of carbonyl regions of glycolic mer units of the representative Expansorb® DLG 50-2E. The red area is intensity of the carbonyl peak of glycolic mers adjacent to lactic mers, while the blue zone represents the intensity of the carbonyl peak of a glycolic mers adjacent to another glycolic mers.

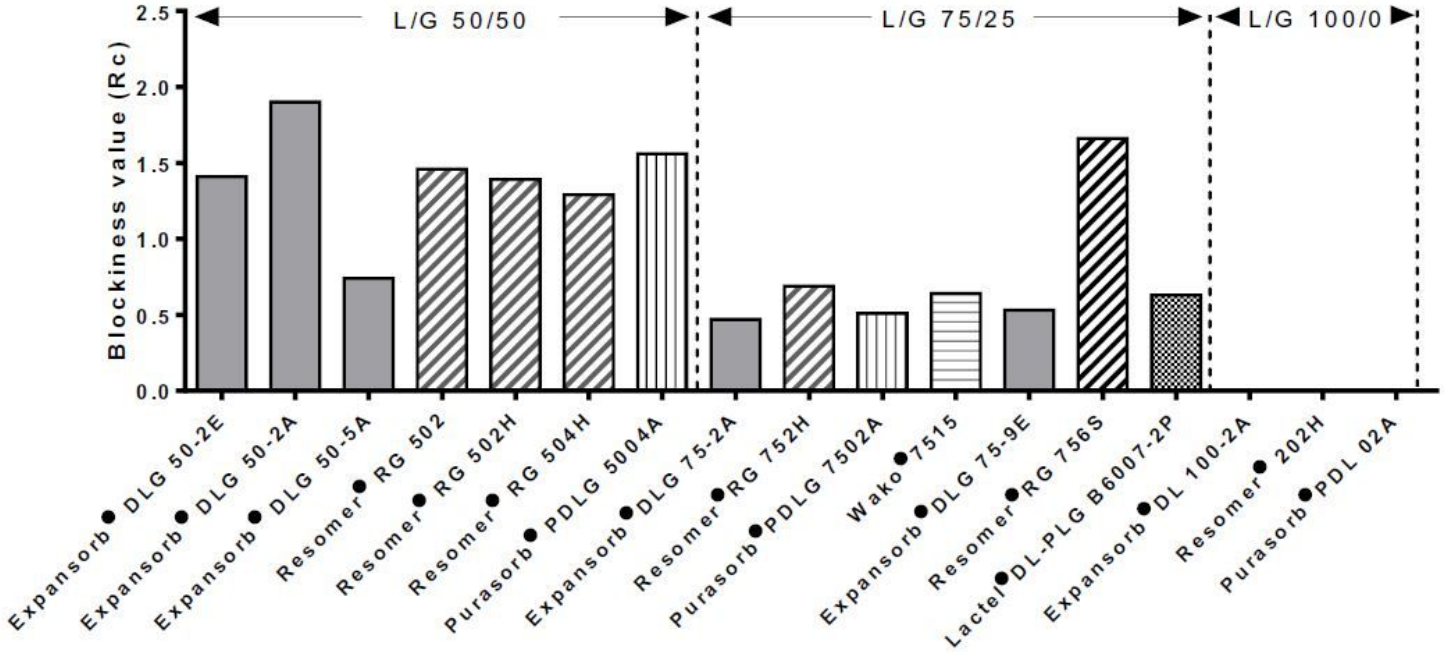

Figure 5

Blockiness values of PLGA polymers by 13CNMR spectroscopy (CDCl3, 500 MHz).

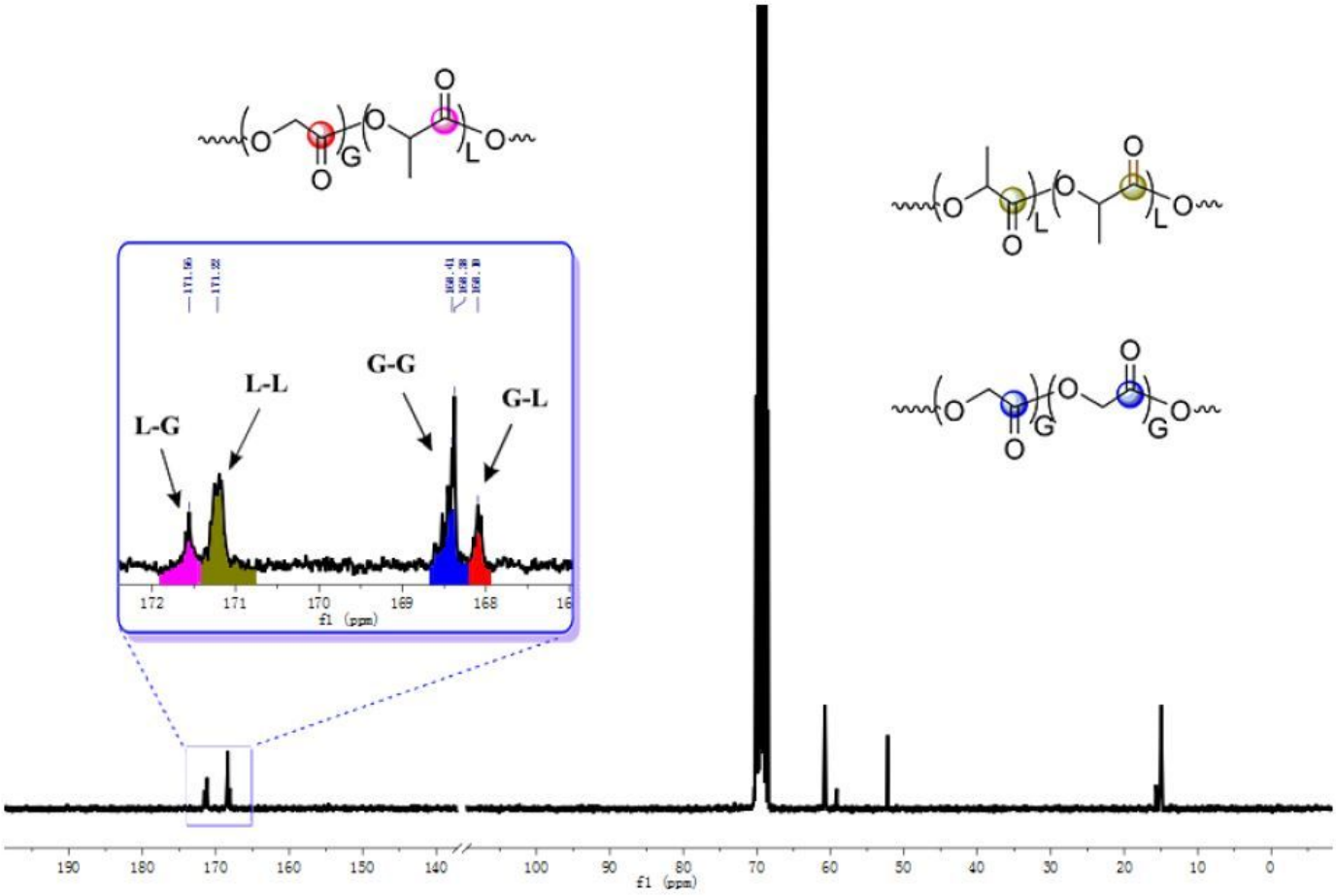

Figure 6

13CNMR spectra (hexafluoro-2-propanol/benzenze-d6, 500 MHz) of the carbonyl regions of PLGA polymer (representative is Expansorb® DLG 50-5A). Shown as the arrows, the areas of pink (L-G), brown (L-L), blue (G-G) and red (G-L) represent the intensities of the carbonyl peak of lactic mer units adjacent to glycolic mers units, lactic adjacent to lactic, glycolic adjacent to glycolic, and glycolic adjacent to lactic, respectively.

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