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A protocol for assay of poly(lactide-*co*-glycolide) in clinical products

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ABSTRACT

Poly(lactide-*co*-glycolide) (PLGA) is the key component of long acting drug products responsible for providing sustained release in a controlled manner. The objective of the current study was to develop and validate an analytical protocol to determine key properties of PLGA used in commercial long-acting drug products. Procedures to isolate PLGA from commercial products have been established and the key properties of PLGA, such as polymer molecular weight, lactide:glycolide (L:G) ratio, and nature of polymer end-cap, have been determined. Identification of the polymer end-cap was confirmed by using two PLGA polymers with acid and ester end-caps. Trelstar® and Risperdal Consta® were chosen as model products. The calculated L:G ratios of PLGA used in Trelstar® and Risperdal® are 52:48 and 78:22, respectively. PLGAs from both Trelstar® and Risperdal Consta® possess ester end-caps. Since the properties of specific PLGA in clinically used formulations are not readily available, this protocol will be useful in developing PLGA-based long acting drug products.

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1. Introduction

Poly(lactide-*co*-glycolide) (PLGA) is a well-known biodegradable polyester which degrades in the human body by random chain hydrolysis into its non-toxic monomeric components lactic acid and glycolic acid. PLGA has been widely applied to medical applications, including biodegradable sutures, e.g., Vicryl by Ethicon Inc., tissue engineering (Kastellorizios et al., 2015; Quinlan et al., 2015), and drug delivery systems, such as long-term delivery depot formulations (Lu et al., 2014; Rawat et al., 2012; Schwendeman et al., 2014; Tuladhar et al., 2015; Wang and Burgess, 2010).

PLGA is a general term used to describe a very broad group of polymers. In general, these polymers are either synthesized by ring opening polymerization of the respective lactic acid dimers (lactide rings) and glycolic acid dimers (glycolide rings), or by polycondensation of lactic acid and glycolic acid monomers. There are many limitations of polycondensation, as it does not allow for well controlled synthesis of high molecular weight polymers. For this reason, ring-opening polymerization is a more popular technique. In this technique, polymerization is typically initiated by either water or by an alkyl alcohol, such as dodecanol, to yield a PLGA which is either acid or ester end-capped, respectively (Jérôme and Lecomte, 2008; Lasprilla et al., 2012). The nature of end-group has an effect on polymer properties including degradation and drug release (Huang et al., 2013; Xu et al., 2013; Yeo and Park, 2004). Other critical parameters which define the polymer properties include the lactide:glycolide (L:G) ratio, as well as molecular weight (Anderson and Shive, 2012; Miller et al., 1977; Mittal et al., 2007). In general, PLGA with higher lactide ratio degrades more slowly than that with lower lactide ratio, as the additional side methyl unit on the chain adds hydrophobicity which creates steric hindrance and reduces water infiltration (Lu et al., 1999). Because of the impact that these properties have on drug release kinetics, it is important to identify key PLGA characteristics to ensure comparable product performance.

A protocol was developed for testing of clinical formulations comprised of PLGA microparticles to determine the three primary parameters of PLGA, the L:G ratio, polymer molecular weight distribution, and end-cap. The protocol describes a method for isolating PLGA from the microparticles by removing other excipients and active pharmaceutical ingredient (API) and analysis techniques for determining the primary parameters.

2. Materials and methods

2.1. Materials

Trelstar® 3.75 mg (Watson Pharmaceuticals) and Risperdal Consta® 25 mg (Janssen Pharmaceuticals, Inc.), delivering

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triptorelin and risperidone, respectively, were purchased. Regenerated cellulose dialysis membrane (MWCO 6000–8000 Da) was bought from Spectra/Por. Dichloromethane (DCM) and hexane was acquired from Mallinckrodt. Ethanol (200 proof) was obtained from Koptec. Lactide and glycolide monomers were purchased from Ortec Inc. Stannous Octoate, decanol, lactic acid, and anhydrous toluene were obtained from Aldrich. Prior to use, Stannous Octoate was purified by vacuum distillation. Control PLGA polymers (AP024 and AP122) were obtained from Akina Polyscitech. All other chemicals were of reagent grade and used as received.

2.2. Synthesis of known PLGA samples

Bulk synthesis of PLGA was performed to obtain samples with known properties. Into a clean and dry 500 ml single neck round bottom flask containing an oval stir bar, an alcohol bearing initiator, was first added. Either decanol or lactic acid was used as an initiator for making an ester or acid end-capped polymer, respectively. Then, a pre-determined molar quantity of lactide and glycolide was added. For ease of transfer, stannous octoate catalyst was dissolved as a 10% (w/v) solution in anhydrous toluene for pipetting in. The molar ratio of catalyst to monomers was 1:200. The flask was connected via a stopcock to a vacuum source (Welch Duoseal vacuum) and purged for 20 min to remove toluene as well as oxygen and surface moisture. The stopcock was closed and the flask was heated to 150–160 °C for 8 h with magnetic stirring. After the reaction was completed, the stopcock was removed and the flask was filled with DCM to dissolve the polymer. This solution was subsequently filtered and added slowly into a mixture of hexane:ethanol (80:20 v:v) to precipitate the polymer. The precipitate was dried under deep vacuum (−76 cm Hg) at room temperature for approximately 5 days followed by drying under deep vacuum at 40 °C for another 5 days.

The two PLGA polymers with different end-caps were made as follows. A PLGA with an ester endcap, and a number average molecular weight ranging between 35,000 and 45,000 Da, was made by combining 0.603 ml decanol, 94.0 g DL-lactide, 16.6 g glycolide, and 16.1 ml of 10% stannous octoate in toluene (AP024 Lot #40729FCK). The L:G molar ratio in the feed solution for AP024 was 82:18. A PLGA with an acid endcap, and a number average molecular weight ranging between 75,000 and 85,000 Da, was made by combining 0.07 ml lactic acid, 75.0 g DL-lactide, 25.0 g glycolide, and 14 ml of 10% stannous octoate in toluene (AP122 Lot #50123AHT). The L:G molar ratio in the feed solution of AP122 was 71:29.

2.3. Nuclear magnetic resonance (NMR)

NMR scanning was performed using a Bruker AV-III-500-HD NMR spectrometer running TopSpin software (version 3.2) equipped with 5 mm BBFO Z-gradient cryoprobe Prodigy™ (the PINMRF group, Purdue University) for both ^{1}H NMR and ^{13}C NMR.

2.4. Gel permeation chromatography (GPC)

GPC was performed using a Varian Prostar system equipped with a model 210 isocratic pump, a model 410 auto-sampler, and a model 335 photodiode array (PDA) detector. Elution was done with 1 ml/min flow of DCM across three columns in sequence starting with two phenogel columns 300 × 7.8 mm 5 μm packed with 1000 Å and 50 Å porosities, respectively. The final column is an Agilent Resipore© column. Absorbance of the sample was taken at 235 nm. Control and data export was performed using Galaxie software package.

2.5. Purification of PLGA

Clinically available PLGA formulations were purified to remove excipients (other than PLGA) and APIs. This was accomplished by dissolving the product in DCM (~20 ml) and passing through a qualitative filter (Whatman, grade 2 V) to initially remove components insoluble in DCM. The collected DCM solution was then put into a pre-wetted dialysis membrane (MWCO 6000–8000 Da) that had been rinsed in water per manufacturer's instructions and dialyzed against a dialysis solution for three days with gentle stirring to remove any low molecular weight components. The dialysis solutions used were acetonitrile and DCM for triptorelin and risperidone, respectively. The selection of the dialysis solution is based on the solubility of the drug. Subsequently, the solution was removed from the dialysis membrane and dripped slowly into approximately 2 L of stirring hexane:ethanol (80:20) mixture to precipitate out the solid PLGA. The collected precipitate was then dried under deep vacuum at room temperature for three days to remove solvents and used for analysis.

2.6. Analysis of lactide:glycolide (L:G) ratio

The L:G molar ratio of the PLGA was determined by ^{1}H NMR spectroscopy. PLGA was dissolved in deuterated chloroform ($CDCl_3$) solvent (0.8 ml) and pipetted into a 7 in × 5 mm NMR tube (Kimble Chase: 897193-7100) for collecting ^{1}H NMR spectra at 500 MHz.

Proton counting was performed by comparing the intensities at 5.2 ppm and 4.8 ppm. The integration at 5.2 ppm is ascribed to a single H of lactide while the integration at 4.8 ppm is ascribed to 2H of glycolide. For this reason the integration at 4.8 ppm was divided by two. The mole fractions of lactide (M_L) and glycolide (M_G) were calculated from peak integration (P) of each component marked with 'L' for lactide and 'G' for glycolide, respectively.

$$M_L = \frac{P_L}{P_L + (P_G/2)}$$

$$M_G = \frac{(P_G/2)}{P_L + (P_G/2)}$$

The L:G molar ratio is M_L:M_G, or simply P_L:(P_G/2).

If the L:G molar ratio is to be converted to the L:G weight ratio, each component in the above equations can be multiplied by the molecular weight of the monomeric residue which is 58.04 g/mol for glycolate and 72.06 g/mol for lactate, respectively.

2.7. End-cap analysis

The end-group of PLGA was analyzed using ^{13}C NMR after purification following the same procedure for ^{1}H NMR study. Because a long polymer chain contains only a relatively small quantity of end-cap carbons, the spectrum had to be collected in such a way to maximize the signal-to-noise ratio. For this test, a Z-restored spin-echo pulse sequence was utilized with a 30-degree observation pulse, a 3-s interpulse delay, and a 0.55-s data acquisition time. A total of 12,000 scans are acquired over 12.5 h (Xia et al., 2008). The data was processed with exponential multiplication (line-broadening factor of 3) and baseline straightening prior to plotting. The presence of an ester end-cap was determined by the existence of a peak at ~14 ppm which correlates to end-methyl unit on a long alkyl chain such as that found on dodecanol or decanol precursor.

2.8. Molecular weight analysis

For molecular weight analysis, each sample was dissolved in 0.2 μm filtered chromatography grade DCM. After dissolution,

each sample was passed through a 0.45 μm PVDF filter to remove particulates and placed directly into a septa capped 2 ml HPLC vial. Column calibration was performed using Agilent PS2 polystyrene standard series “A” and “B” per manufacturer instructions. The molecular weight indicated was as provided by the manufacturer for the standard’s peak molecular weight value (M_p). The number average molecular weight (M_n) and weight average molecular weight (M_w) were used to calculate the polydispersity index ($PDI = M_w/M_n$).

2.9. Preparation of samples with known end-cap

To validate the end-cap determination method, two control formulations were generated. For each formulation, a known PLGA with either acid or ester end-cap was utilized along with risperidone to generate a simple microparticle formulation. For this formulation, a 100 ml water bath containing 0.5% (w/v) poly (vinyl alcohol) (PVA) (Mowiol 4-88) was agitated by an overhead stirrer (Caframo). An aliquot of 1 ml of a solution of PLGA/risperidone dissolved in DCM was directly injected into the stirring water bath. Stirring continued for 5 min and then the microparticles were centrifuged and rinsed to remove excess PVA. The resultant microparticles were dried under vacuum. Subsequently, the formed microparticle formulations consisting of PLGA and risperidone were purified and assayed. The control polymers synthesized with known endcap were also characterized by the same ^{1}H NMR and GPC methods described above in neat form prior to making into microparticle formulations.

3. Results and discussion

3.1. ^{1}H NMR study

The L:G ratios in the synthesized polymers, AP024 and AP122, were examined using ^{1}H NMR. The calculated L:G molar ratios of AP024 and AP122 polymers were found to be 81:19 and 71:29, respectively. As described in the experimental section, the L:G molar ratios of the AP024 and AP122 in the feed solutions were 82:18 and 71:29, respectively. This indicates that utilization of ^{1}H NMR for determining the L:G ratio is accurate and that the L:G ratio in the synthesized polymers is controlled by the feed ratio of lactide and glycolide in the reaction.

The ^{1}H NMR spectra of commercial Trelstar® and Risperdal Consta® are shown in Fig. 1. The peaks at 5.2 ppm and 4.8 ppm indicate protons of lactide and glycolide, respectively. The peak integrations at 5.2 ppm and 4.8 ppm for Trelstar® are 31.59 and 58.90, respectively. On the other hand, the peak integrations at 5.2 ppm and 4.8 ppm for Risperdal Consta® are 37.79 and 21.69, respectively. The peak integration values were used to calculate the relative mole fractions of lactide and glycolide as described in the experimental section. The calculated L:G molar ratios of Trelstar® and Risperdal® are 52:48 and 78:22, respectively. These values suggest that the two products were prepared using PLGA polymers commonly available as PLGA 50:50 and PLGA 75:25.

3.2. GPC study

The molecular weights of PLGA used in both samples were examined by GPC, and the results are summarized in Table 1. Additionally, neat polymer controls were assayed by GPC and their results are also shown in Table 1. The amount of the initiator used in the synthesis was lower for AP122 as compared with AP024. This indicates that the molecular weight can be controlled by the initiator content.

It is noted that the data in Table 1 are for PLGA polymers isolated from the formulations which are currently in clinical use. Producing clinical products may have required certain processing steps, such as exposure of PLGA to water, drying, and sterilization, all of which may have affected the polymer molecular weights (Hausberger et al., 1995; Husmann et al., 2002; O’Hagan et al., 1994). For this reason, it would generally be the case that a higher molecular weight polymer was initially utilized for the preparation of PLGA microparticles. Despite this, having the molecular weight information of the finished formulation allows comparisons between the same products produced in different batches by the same manufacturer, and between products produced by different manufacturers.

It is important that GPC is used to obtain a spectrum of polymer molecular weights. Since commercially manufactured PLGA is usually not monodisperse, knowing the polydispersity is important in defining the polymer properties. Only GPC can generate

Table 1
M_n, M_w, and PDI as determined by GPC.

Sample	M_n	M_w	PDI
Trelstar®	25,192 Da	85,207 Da	3.38
Risperdal Consta®	44,875 Da	111,142 Da	2.48
AP024*	43,519 Da	74,870 Da	1.72
AP122*	75,704 Da	116,479 Da	1.54

* Neat polymer prior to formulation into microparticles.

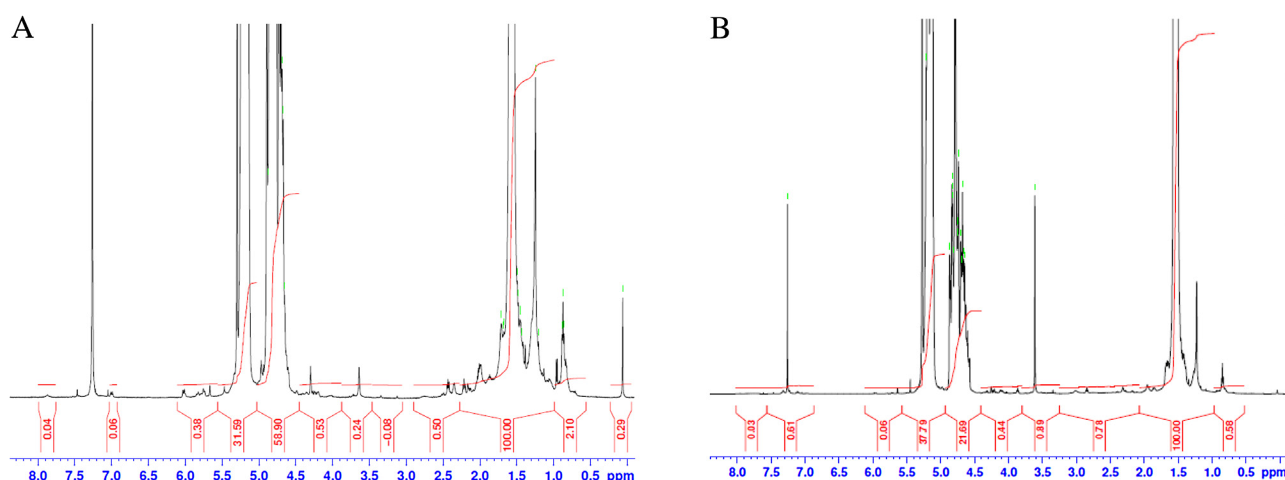

Fig. 1. ^{1}H NMR spectra of PLGA in Treslstar® (A) and in Risperdal Consta® (B).

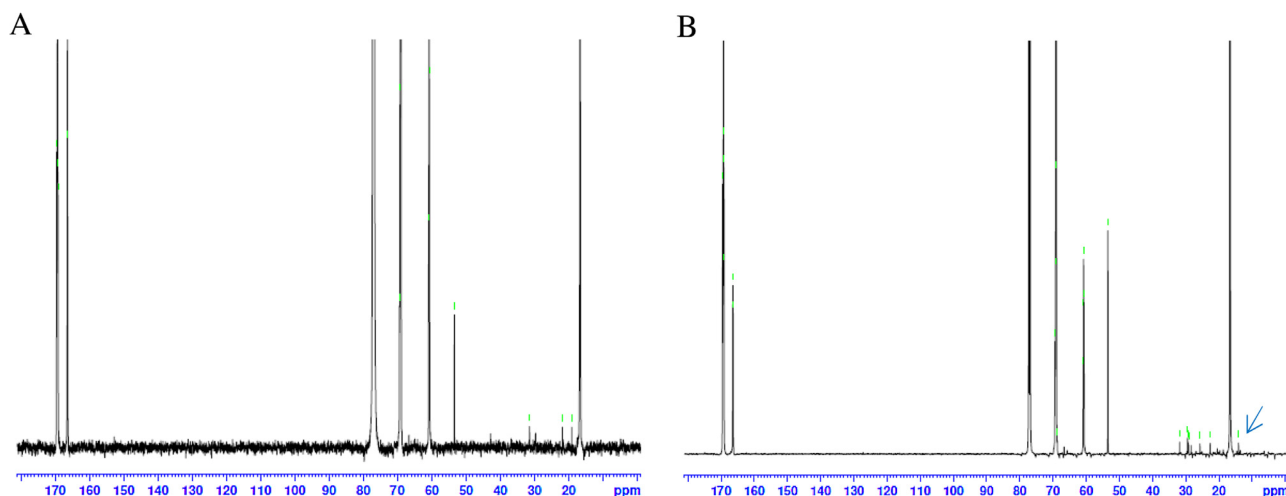

Fig. 2. ^{13}C NMR of purified PLGA from microparticles made of acid end-capped PLGA (A) and ester end-capped PLGA (B).

such data, and other techniques for measuring molecular weights, such as inherent viscosity measurements or light scattering method, do not disclose molecular weight dispersity. The molecular weight and polydispersity can influence the drug release behavior (Debye, 1947; Kenley et al., 1987; Tang and Singh, 2008).

3.3. End-cap analysis

The endcap analysis in this study was validated by using previously described AP024 and AP122 polymers generated with known endcap features. To further confirm the presence of either ester end-cap or acid end-cap, two different types of PLGA microparticles loaded with risperidone were prepared using two different PLGAs with known end-caps, i.e., either ester or acid end-cap. PLGA from each formulation was purified following the same procedure used for Trelstar® and Risperdal Consta®. The results are shown in Fig. 2. The only substantial difference between the two spectra is the presence of the methyl end-cap peak at 14 ppm (arrow in Fig. 2B) indicating the presence of ester end-cap. The results in Fig. 2 support the validity of the methyl unit at 14 ppm on ^{13}C NMR spectra as a means to determine the PLGA end-cap from clinically used PLGA formulations.

The end-cap analysis of PLGA from Trelstar® was performed by ^{13}C NMR, and the peak assignments are shown in Fig. 3. Briefly, methyl units appear very far upfield. The first one appears around 14 ppm (arrow and G in Figure 3) due to a methyl unit at the end of an alkyl chain. The peak, at 16 ppm is due to lactide side methyl units. The peaks between 20 and 30 ppm are due to alkyl region non-terminal carbons, but other carbons may appear in this region as well. The peaks between 50 and 80 ppm are ascribed to main-branch alpha carbons that are next to esters but not the ester carbon. The peaks around 160–180 ppm indicate the presence of the ester carbons. Due to its location far upfield from almost any other carbon, the presence of the methyl end at 14 ppm was purported to be indicative of an ester end-cap. In addition to Trelstar®, the PLGA from Risperdal Consta® was also examined. It also possessed a peak at 14 ppm (Fig. 4) indicating the presence of

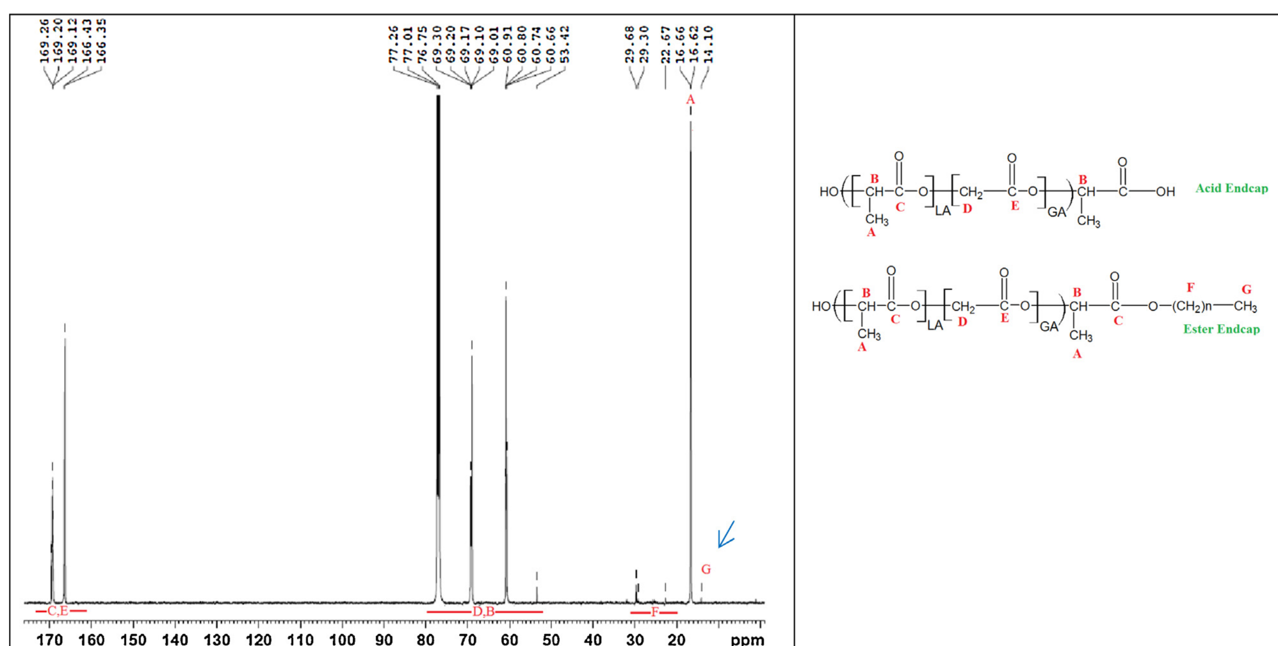

Fig. 3. ^{13}C NMR of purified PLGA from Treslstar® formulation and resultant peak assignments.

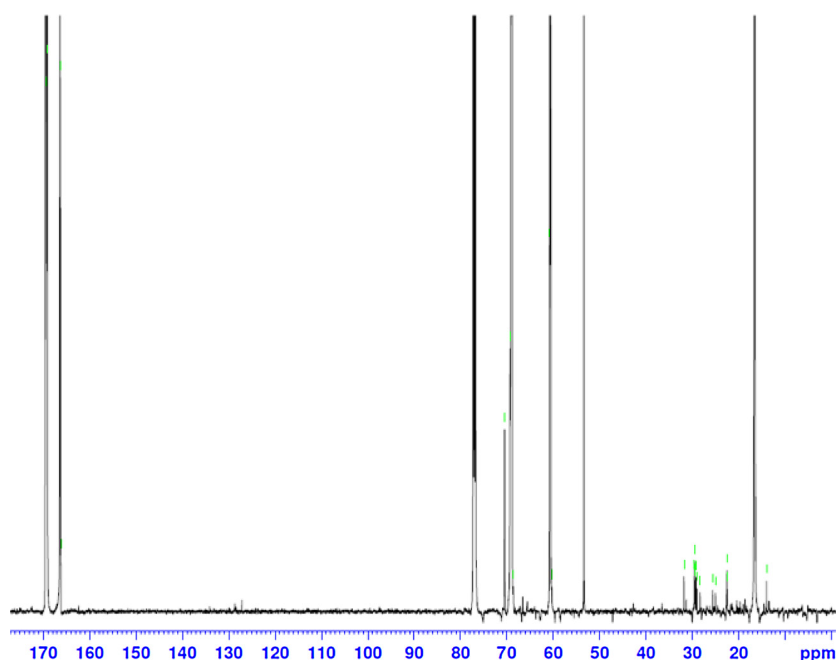

Fig. 4. ^{13}C NMR of purified PLGA from Risperdal Consta®.

an ester end-cap. Thus, PLGAs from both Trelstar® and Risperdal Consta® possess ester end-caps.

The unique nature of the NMR system utilized for this protocol allows for this testing to be performed in a timely manner. The use of a liquid nitrogen cooled cryoprobe optimized for ^{13}C nucleus has a ^{13}C sensitivity which is four times (4×) higher than a non-cryoprobe NMR. The 4X improvement in signal-to-noise results in a 16-fold reduction in signal averaging time. For the same study to be performed without this advantage would require a runtime of 192 h.

The protocol described for microparticles can be easily extended to other formulations, including solid implants and in situ forming depot formulations, because the process of isolating PLGA and subsequent characterization methods remain the same regardless of formulation types.

5. Conclusion

The protocol presented here represents methods for determining key PLGA parameters such as polymer molecular weights with polydispersity, L:G ratio, and nature of polymer end-cap. This is achieved by purifying PLGA from formulations to remove non-PLGA components and then analyzing by GPC, ^{1}H NMR, and ^{13}C NMR. The ability to determine key PLGA parameters will be useful in formulating generic products of clinically used PLGA products including microparticle, solid implants, and in situ forming depot formulations.

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