Separation and analysis of poly(lactide-co-glycolide) in Trelstar® 22.5 mg formulation

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Introduction

Poly(lactide-co-glycolide) (PLGA) has been used in most of the injectable, long-acting formulations approved by the U.S. Food and Drug Administration (FDA). Some formulations utilized complex mixtures of different types of PLGA to achieve specific release profiles. A proposed generic formulation needs to match the reference listed drug for qualitative and quantitative (Q1/Q2) sameness of the PLGA, including polymer mixtures and chirality of lactide unit. The purpose of this study is to leverage the lacto-selectivity effect of various solvents to separate and analyze the components of Trelstar® 22.5 mg formulation. In addition to the previously established parameters (L:G ratio, endcap, blockiness, molecular weight, branching) samples were also assayed by an advanced proton nuclear magnetic resonance (HNMR) technique to determine chirality of the lactide component.

Methods

Samples of Trelstar ® 22.5 mg (Allergan) were extracted to obtain PLGA as previously described [1]. Briefly, the contents of each vial were loaded into centrifuge tubes. Dichloromethane (DCM) was added to each tube and dissolved with orbital agitation at 100 RPM/30°C overnight. The supernatant was passed through a syringe filter into hexane to precipitate out the PLGA extract [1]. Afterwards, semi-solvent extraction was performed according to previously established methods for PLGA separation by lactide content [2]. Briefly, each semi-solvent was added to generate a total concentration of 25 mg/ml. The tube was incubated 30°C overnight. The solution was centrifuged and the soluble supernatant portion removed. The remaining material was dried and the process was repeated. This was done using xylenes, isopentyl-acetate, toluene, butyl-acetate, 2-pentanone, and butanone, in that order, to separate out PLGA from high lactide to low lactide fraction. Analysis of each fraction was performed as previously described [3]. Briefly, lactide content was determined by HNMR comparison of lactide peak (1H, 5.2 ppm) and glycolide peak (2H, 4.8 ppm). Blockiness (Rc) were determined by C13NMR by dividing GA-LA peak (166.4 ppm) from the GA-GA peak (166.3 ppm). Molecular weight of each fraction was determined by GPC-4D utilizing universal calibration. Prior to chirality analysis, samples were re-dissolved in DCM, passed through a 0.45 um polyvinylidene fluoride (PVDF) filter and precipitated in methanol followed by vacuum drying. Chirality analysis was performed according to previous methods [4]. Briefly, data were obtained using a Bruker Avance DRX-500 NMR spectrometer running TopSpin 1.3 software and equipped with a 5mm TBI Z-gradient probe. The sample temperature was set to ca. 299K. Data were acquired with homonuclear decoupling (Bruker pulse sequence zghd) of the methyl-group resonances using the following parameters: observe pulse width ca. 90 degrees, acquisition time 4.4 seconds, relaxation delay 1 second, 16 - 64 scans averaged following 4 dummy scans.

Methods (cont.)

The specific decoupling frequency (Bruker parameter O2) was set by inspection for each sample after obtaining the conventional (non-decoupled) spectrum. Raw data were processed with exponential multiplication (line broadening factor 0.5) prior to Fourier transformation without zero-filling. Both known samples of PLGA (DL or L) and Trelstar fractions were tested.

Results

Two Trelstar® batches (lot# 16-008386 and lot #18-01012) were assayed and the resultant data are shown in **Table 1.**

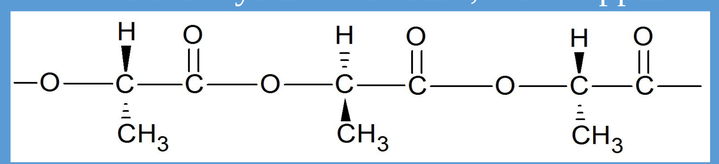
Table 1. Trelstar® Fraction Separation Analysis (Average ± STDEV, N=2 lots)

Solvent (fraction)	Percent polymer (w/w%)	Lactide content (%L, NMR)	Mw (GPC-4D)	Mn (GPC-4D)	Rc (NMR)
Original Mixture	100%	76.9 ± 0.1	41,377 ± 135	31,475 ± 81	0.78 ± 0.01
Xylenes	6.0 ± 0.1	84.0 ± 0.1	13,063 ± 2695	8755 ± 4799	0.46 ± 0.16
Isopentyl acetate	15.8 ± 0.8	82.8 ± 0.1	24,653 ± 1316	19,429 ± 811	0.48 ± 0.08
Toluene	25.5 ± 1.3	82.9 ± 0.1	47,790 ± 939	39,084± 2588	0.55 ± 0.12
Butyl acetate	12.6 ± 0.2	74.2 ± 0.2	26,592 ± 665	22,760 ± 99	0.81 ± 0.02
2-Pentanone	14.7 ± 0.2	72.5 ± 0.2	35,483 ± 264	29,658 ± 88	0.88 ± 0.001
Butanone	24.7 ± 0.7	70.8 ± 0.2	52,930 ± 640	45,267 ± 1467	0.89 ± 0.01
Butanone residual	0.6 ± 0.4	70.5 ± 0.6	NT*	NT*	NT*

* NT = Not Tested, too little quantity extracted to test.

Analysis was performed to determine if parameters relevant to the chirality nature of the polymers could be defined. In PLGA's >75L a pronounced peak based on isotactic lactide at ~ 5.17 ppm (**Figures 1-3**) could be used to assay chirality.

Atactic/syndiotactic PLA, 5.2-5.3 ppm



Isotactic PLA, 5.17 ppm

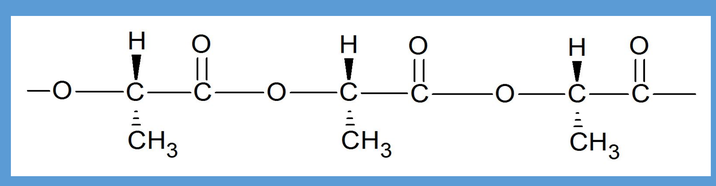


Figure 1. Schematic of lactide tacity and expected NMR locations.

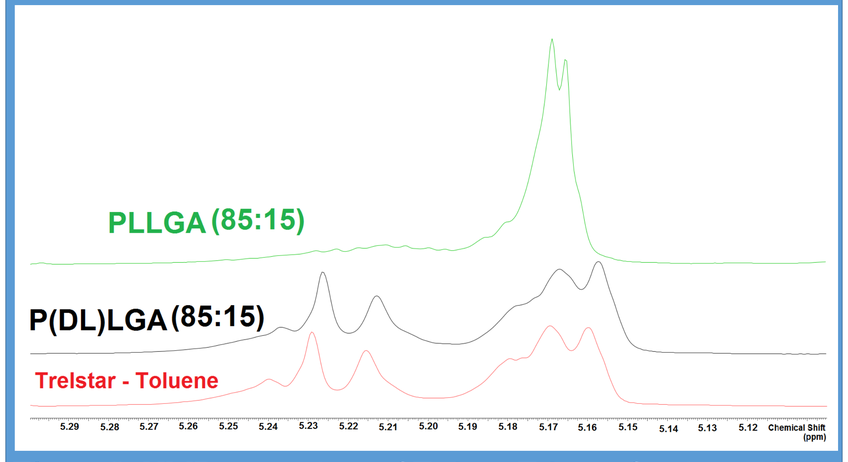


Figure 2. NMR comparison of Trelstar Toluene fraction with PLLGA and P(DL)LGA standards (85:15 LA:GA).

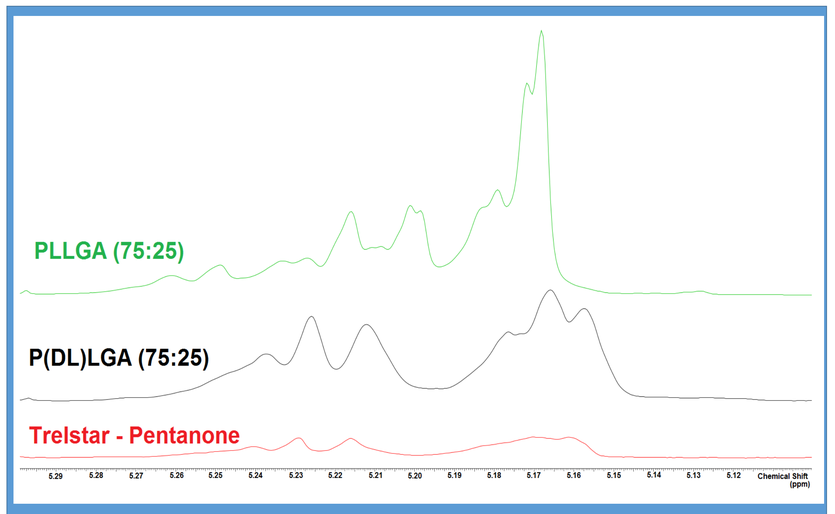


Figure 3. NMR comparison of Trelstar Pentanone fraction with PLLGA and P(DL)LGA standards (75:25 LA:GA).

Conclusion

Without fractionation, analytical techniques (such as NMR) only provide the average values of the fractions of PLGA's present in a polymer sample. Using semi-solvents, Trelstar® 22.5 mg formulation was successfully separated into fractions based on lactide content of PLGA and each component assayed individually. In addition to conventional parameters, the chirality of high-lactide content PLGA's can be obtained by decoupled NMR analysis and Trelstar fractions contain 'DL' PLGA.

References

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Acknowledgements

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