Assay of PLGA Types in Microparticle Depo Formulations

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Introduction

- Poly(lactide-co-glycolide) (PLGA) is a biodegradable polymer used in various clinical products for its biodegradation by hydrolysis into non-toxic lactic acid and glycolic acid.
- There are many different types of PLGAs varying in the lactide:glycolide (LA:GA) ratio, endcap, and molecular weight.
- Some PLGA formulations use star-shaped PLGA, e.g., glucose-PLGA, or mixture of two or more different PLGA polymers, e.g., PLGA with different LA:GA ratios, and those varibles introduce complications in assay.
- Assay methods for identifying specific PLGA polymers are necessary for ensuring that proposed generic formulations provide qualitative and quantitative (Q1/Q2) sameness in regards to reference product.
- **Purpose** of this study was to investigate methodologies for extraction and assay of the PLGAs used in clinical formulations.

Methods

- Commercially purchased PLGA depot formulations (Risperdal Consta®, Trelstar® 3.75 mg and 22.5 mg doses, and Sandostatin® LAR) were dissolved in dichloromethane (DCM) (**Fig. 1**).
- Solutions were filtered and dialyzed (MWCO 6000-8000 Da) against organic solvent for 3 days.
- Subsequently, these solutions were concentrated, precipitated in excess hexane while stirring, and dried under deep vacuum.
- The PLGA was then analyzed by gel permeation chromatography (GPC) (**Fig. 2**), H^1 nuclear magnetic resonance (NMR) and C^{13} NMR (**Fig. 3**).
- Preliminary tests, using PLGAs of known properties, have indicated that butyl acetate has good solubility for PLGAs with high lactide contents (e.g., LA:GA = 85:15) but reduced solubility for low lactide PLGA (e.g., LA:GA = 50:50).
- As an additional test, Trelstar 22.5 mg was washed with water and dissolved in butyl acetate (BA). The dissolved portion was filtered, collected, dried, and analyzed by HNMR (BA-soluble). Additionally, the solid fraction (BA-insoluble) was dried and analyzed by HNMR.

Fig. 1. Dissolution of PLGA microparticles with DCM.

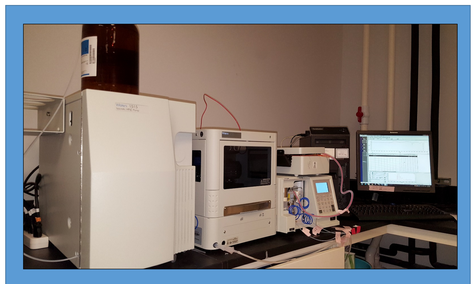

Fig. 2. Waters Breeze 2 GPC system

Fig. 3. Bruker AV-III-500HD[2] NMR system

Results

- The GPC chromatograms of three different long-acting PLGA formulations are shown in **Fig. 4**.
- Polystyrene standards were used to determine number average/weight average molecular weights (**Table 1**). Ideally, PLGA standards need to be used.

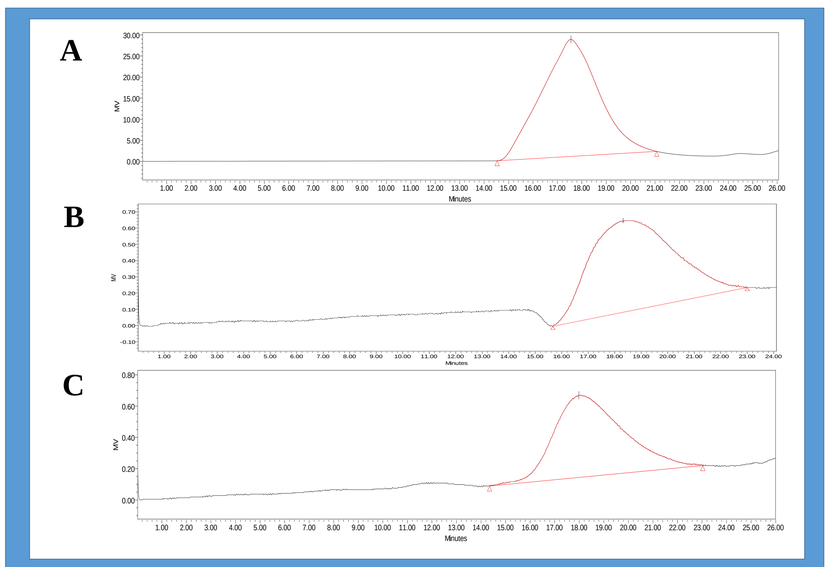

Figure 6. GPC chromatograms of PLGAs obtained from Risperidal Consta (A), Sandostatin LAR (B), and Trelstar (C)

- **Figures 5 and 6** show example NMR spectra

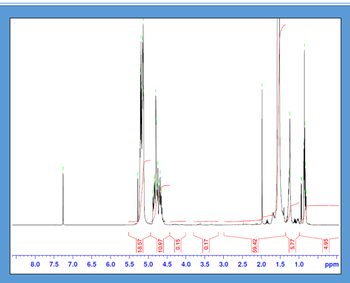

Figure 5. HNMR of Trelstar 22.5 mg.

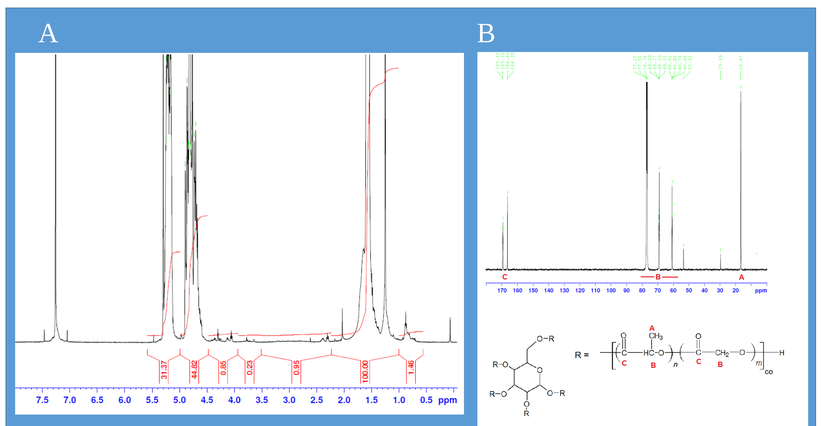

Figure 6. HNMR (A) and C13NMR (B) (peak assignments) of Sandostatin LAR .

- Lactide:glycolide (LA:GA) ratio was determined by relative peak integration at 5.2 ppm (LA, 1H) and 4.8 ppm (GA, 2H), respectively.
- Glucose could not be determined in Sandostatin from NMR methods due to overlap of peaks.
- Branching/star-shape was not readily observed from conventional GPC. Measurement was calibrated against linear standards, which have a different MW to hydrodynamic radius ratio, and thus, it may not accurately determine actual MW.
- Partially dissolving Trelstar 22.5 mg in butyl acetate (BA) allowed for separation of a portion of higher lactide content from a portion of lower lactide content, as measured by HNMR.
- All data are summarized in **Table 1**.

Table 1. Formulation PLGA parameters

Product	LA:GA ratio (Molar)	Number average (Mn)	Weight average (Mw)	End cap
Risperdal Consta	78:22	44,875	111,142	Ester
Sandostatin LAR	58:42	24,549	49,421	N/A
Trelstar (3.75mg)	52:48	25,192	85,207	Ester
Trelstar (22.5mg) (All)	77:23	46,368	74,042	N/A
Trelstar (22.5 mg) BA-soluble	81:19	ND	ND	ND
Trelstar (22.5 mg) BA-insoluble	71:29	ND	ND	ND

Conclusions

- Conventional methods (purification followed by GPC, NMR) are suitable for analysis of relatively simple formulations made of a single-type, linear PLGA .
- Conventional methods do not give any information on branching/star PLGAs. This would require more advanced analysis techniques.
- By themselves, conventional methods do not yield accurate information on mixed-polymer formulations as the results are typically the ‘average’ value for the included polymers.
- Use of separation techniques can allow for analyzing the PLGA components separately and more sophisticated separation methodologies will enable thorough characterization of different PLGA types from a single formulation.
- Future work will focus on establishing separation techniques for mixed-polymer type formulations as well as multi-detector methods for star-shaped PLGA formulations.

Acknowledgements

This work was supported by Grant U01FD05168 from the Food and Drug Administration (FDA), Center for Drug Evaluation Research (CDER). The content is solely the responsibility of the authors and does not necessarily represent the official views of the FDA. The authors would like to thank the Purdue Interdepartmental Nuclear Magnetic Resonance Facility (PINMRF) group.

References

1. Garner, John, Sarah Skidmore, Haesun Park, Kinam Park, Stephanie Choi, and Yan Wang. "A protocol for assay of poly (lactide-co-glycolide) in clinical products." International journal of pharmaceutics 495 (2015): 87-92.
2. http://www.pinmrf.purdue.edu/instruments/av500.shtml