Journal of Pharmaceutical and Biomedical Analysis 146 (2017) 273–278

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Absolute quantification of poly(DL-lactide-*co*-glycolide) in microspheres using quantitative ^{1}H NMR spectroscopy

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ARTICLE INFO

Article history:
Received 21 June 2017
Received in revised form 28 August 2017
Accepted 29 August 2017
Available online 9 September 2017

Keywords:
Poly(lactide-*co*-glycolide)
Leuprorelin acetate microsphere
Quantitative ^{1}H nuclear magnetic resonance
Size exclusion chromatography
High-performance liquid chromatography
Method validation

ABSTRACT

The complex nature and the manufacturing process of poly(DL-lactide-*co*-glycolide) (PLGA), a key component of PLGA-based microspheres, have made the quantification of this copolymer difficult. The main purpose of the current study was to investigate the potential of three different methods for the quantitative analysis of the PLGA content of clinical products. In this regard, leuprorelin acetate microspheres from different vendors were chosen as templates to validate quantitative ^{1}H nuclear magnetic resonance (qHNMR) spectroscopy, size exclusion chromatography (SEC), and high-performance liquid chromatography (HPLC) methods qHNMR proved to be an excellent and rapid PLGA quantification method compared to the other two. The recovery value was 99.12% and the linearity correlation coefficient was 0.9999. The results obtained from the qHNMR method were found to match the data provided by the vendor, suggesting that qHNMR can be utilized as a reliable quality control and inspection tool for PLGA-based clinical products.

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1. Introduction

Poly(DL-lactide-*co*-glycolide) (PLGA) is a well-known copolymer that is incorporated in a host of U.S. Food and Drug Administration (FDA)-approved therapeutic devices due to its biodegradability and biocompatibility [1–3]. PLGA is synthesized either by ring-opening polymerization of the cyclic dimers of glycolic acid and lactic acid, or by polycondensation of these monomers [4]. PLGA is a biodegradable polymer that hydrolyzes to produce its monomers in the body [1]. Notably, glycolic acid and lactic acid are by-products of various metabolic pathways in the body [5]; thus, minimal systemic toxicity is associated with PLGA.

PLGA has been widely utilized in medical applications, such as long-acting release products (LARs) [6]. One notable PLGA-based LAR is the leuprorelin microsphere (i.e., Lupron Depot [7]), used for the treatment of advanced prostate and breast cancers. Leuprorelin, the first luteinizing hormone-releasing hormone (LHRH) super agonist, is a gonadotropin-releasing hormone (GnRH) analog. Due to their unique advantages that include improved chemical castration and superior therapeutic effects, sustained-release microsphere products (e.g., leuprorelin acetate encapsulated in PLGA) are very popular for improving patient compliance by reducing dosing frequency [5,7].

Several microsphere preparation methods are reported [8]. The most common technique used for PLGA microsphere preparation is an emulsification-solvent evaporation technique [9], which has been applied to leuprorelin microspheres. This technique facilitates the encapsulation of drugs and involves dissolution of the polymer and compound together in an organic solvent. The oil-in-water emulsion is prepared by adding water and a surfactant to the polymer solution, and droplets are induced by sonication or homogenization. The solvent is then evaporated, and the microspheres are collected after centrifugation. In order to avoid aggregation and reduce initial burst release, various ingredients, such as surfactants, osmolytes, surface-active polymers, pH modifiers, and protein stabilizers can be encapsulated in PLGA-based formulations [10–12]. The PLGA content in the final product may differ from the desired content due to the complexity of the preparation process, and this difference may influence the encapsulation and loading efficiencies, thereby affecting the therapeutic benefits [13]. Since the manufacturing technique and formulation of the microsphere components are complex, it is difficult to quantitatively analyze the PLGA content in these microspheres, which is crucial for quality control and the evaluation of the manufacturing process.

Only a few studies have evaluated methods for the quantification of the polymer in PLGA-based microspheres. High-performance liquid chromatography (HPLC) [14] and HPLC-time

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http://dx.doi.org/10.1016/j.jpba.2017.08.046
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of flight/mass spectrometry [15] have been used to analyze the monomers following polymer hydrolysis. However, these methods are time-consuming and are sometimes problematic due to hydrolysis and the formation of polylactides caused by self-polymerization [16,17]. Often, only indirect observations can be made using these methods because of a lack of suitable analytical tools. To overcome such difficulties, NMR spectroscopy, which has been used to calculate the lactide/glycolide (L/G) ratio of PLGA [18], is particularly useful because single-pulse experiments give reliable integrations that can be directly related to the studied compounds. NMR spectroscopy has also been used for the quantification of PEG [19,20] and polysorbate 80 [21], with satisfactory sensitivity and excellent recovery. The aims of this study were to investigate the potential of quantitative ^{1}H nuclear magnetic resonance (qHNMR) spectroscopy, size exclusion chromatography (SEC), and high-performance liquid chromatography (HPLC) methods for the quantitative analysis of PLGA. For this purpose, leuprorelin acetate microspheres from different vendors were chosen as templates. Additionally, comparisons of the PLGA content of five commercial leuprorelin acetate microsphere samples suggest that PLGA quantification could be utilized as a reliable quality control tool for the evaluation of the manufacturing process.

2. Material and methods

2.1. Solvents and reagents

Commercial samples of leuprorelin acetate microspheres were purchased from various vendors (detailed information is available in Table S1 of the Supporting information). $CDCl_3$, mannitol, and gelatin were purchased from Sigma-Aldrich (St. Louis, MO, USA). The PLGA (L:G = 75:25) samples were purchased from Sigma-Aldrich and the Luye Pharma Group (Yantai, China). Tetrahydrofuran (THF) and acetone were purchased from Fisher Scientific (Waltham, MA, USA). The benzoic acid (BA) and sodium lactate reference standards were purchased from the National Institute of Metrology (Beijing, China), and the National Institutes for Food and Drug Control (NIFDC, Beijing, China), respectively.

2.2. NMR sample preparation

2.2.1. Samples for linearity

Approximately 2.5, 5, 7.5, 10, and 12.5 mg of PLGA, and 5 mg of BA (reference standard) were placed in separate tubes (n = 3). Approximately 1 mL of $CDCl_3$ was added, and the mixture was vortexed for 15 min at 25 °C. The clarified solutions were immediately examined by ^{1}H NMR spectroscopy.

2.2.2. Samples for recovery

10 mg of sample 1 (Table S1) with 7 mg of PLGA (from Sigma-Aldrich) and 5 mg of BA as the internal standard were placed in a tube. Approximately 1 mL of $CDCl_3$ was added, and the mixture was vortexed for 15 min at 25 °C.

2.2.3. Samples for accuracy, stability, and reproducibility

Due to the complexities of the commercial samples, two types of simulated samples (i.e., with and without gelatin) were prepared. In analogous preparations using the commercial samples, 30 mg of PLGA, 6 mg of mannitol, 4 mg of leuprorelin, and 5 mg of BA, with/without 10 mg of gelatin were placed in a tube. Approximately 1 mL of $CDCl_3$ was added, and the mixture was vortexed for 1 min at 25 °C.

2.3. NMR experiments

qHNMR spectra were recorded using an Avance III HD 500 NMR spectrometer (Bruker, Billerica, MA, USA) fitted with a 5 mm i.d. BBO probe at 500.15 MHz. The probe temperature was set to 25 °C. ^{1}H NMR spectra were acquired at a spectral width of 7500 Hz (15 ppm), with acquisition times of 4.37 s and relaxation delays (D1) of 20 s. A sufficient S/N ratio was achieved after 128 scans. The total measurement time was approximately 1 h. The ^{1}H 90° pulse widths were calculated prior to collecting the NMR spectra. The ^{1}H longitudinal relaxation time (T_1) values were determined using an inversion recovery pulse sequence available from the pulse sequence library with a D1 value of 60 s and ten different inversion times (τ) ranging from 50 ms to 50 s. The inversion profiles were analyzed using TopSpin 3.2 (Bruker, USA) to determine the T_1 values.

To process the NMR data, the line broadening was set to 1.0 using zero-filled interpolation with 2 × 64 k. After Fourier transformation, the phasing was manually adjusted to zero-order phase correction, and a baseline correction was performed using 5th-order polynomial functions from −1 to 10 ppm. All spectra were manually integrated using TopSpin 3.2. The start and end points for integration of all spectra were consistent throughout the analysis.

2.4. qHNMR analysis of PLGA with reference standards

The BA reference standard and analytical samples were accurately weighed into vials; $CDCl_3$ was added to each vial to completely dissolve the samples. Each solution was then placed into a 5 mm NMR tube and subjected to qHNMR analysis. A quantitative proton signal for BA occurs at 8.04 ppm, that of dehydrated lactic acid (LA) occurs at approximately 5.2 ppm, and that of dehydrated glycolic acid (GA) occurs at approximately 4.8 ppm. None of the signals used for the qHNMR calculations overlapped with signals from mannitol or leuprorelin (Fig. 1). The weights of each PLGA sample included the weight of LA and GA via the following equations:

$$W_{PLGA} = W_{LA} + W_{GA}$$

$$W_{LA} = \frac{W_{BA} \times P_{BA} \times I_{LA} \times H_{LA} \times M_{LA}}{I_{BA} \times H_{BA} \times M_{BA}}$$

$$W_{GA} = \frac{W_{BA} \times P_{BA} \times I_{GA} \times H_{GA} \times M_{GA}}{I_{BA} \times H_{BA} \times M_{BA}}$$

where I_{BA}, I_{LA}, and I_{GA} are the integrated values of the BA, LA, and GA signal peaks, respectively; H_{BA}, H_{LA}, and H_{GA} are the number of protons corresponding to the BA, LA, and GA signals, respectively; M_{BA}, M_{LA}, and M_{GA} are the molecular weights of BA, LA, and GA, respectively; W_{BA}, W_{LA}, and W_{GA} are the weights of BA, LA, and GA, respectively; and P_{BA} is the purity of BA. The PLGA content of the commercial sample was calculated using the following equation:

$$content_{PLGA} = \frac{W_{LA} + W_{GA}}{W_{sample}}$$

where W_{sample} is the weight of the commercial leuprorelin acetate microsphere sample.

2.5. Linearity of the qHNMR method

The ^{1}H NMR spectra of five linearity samples (see *Samples for linearity*) were recorded and processed. PLGA calibration graphs were obtained by plotting the ratio between the gravimetric mass and experimental mass derived by qHNMR spectroscopy.

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