High Performance Liquid Chromatography-Size Exclusion Chromatography (Hplc-Sec) As an Efficient Tool for The Quantification of Polymers.

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Abstract

Poly (lactide-co-glycolide acid) (PLGA) is an extraordinary well-described polymer and has excellent pharmaceutical properties like high biocompatibility and good biodegradability. Hence, it is one of the most used materials for drug delivery and biomedical systems, also being present in several US Food and Drug Administration approved carrier systems and therapeutic devices. For both applications, the quantification of polymer is important. During the development of the production process, parameters like yield or loading efficacy are essential to be determined. Although PLGA is a well-defined biomaterial, it still lacks a sensitive and convenient quantification approach for PLGA-based systems. Thus, we present a new method for fast and precise quantification of PLGA by HPLC-SEC. The method includes a shorter run time of 20 minutes with a size exclusion column of 300mm x 8.0mm diameter, tetrahydrofuran as mobile phase and diluent, the detection was carried out using the refractive index detector. The developed method has a detection limit of 0.1 ppm, enabling the quantification of low amounts of PLGA. Compared to existing approaches, like gravimetric or nuclear magnetic resonance measurements, which are tedious or expensive, the developed method is fast, ideal for routine screening and it is selective since no interference. The developed method is validated in terms of selectivity, precision, linearity, accuracy and solution stability. Due to the high sensitivity and rapidity of the method, it is suitable for both, laboratory and industrial use.

Keywords: Poly (Lactide-Co-Glycolide Acid); PLGA; Quantification; HPLC-SEC, Refractive Index.

Introduction

Since the introduction of Poly (lactic-co-glycolic acid) (PLGA) as bioresorbable fiber material in 1960, PLGA became one of the most used polyesters for the development of drug delivery and biomedical systems [1-3]. PLGA is a copolymer of lactic acid (LA) and glycolic acid (GA), with varying ratios, and belongs to a group of highly biodegradable and biocompatible polymers. The US Food and Drug Administration (FDA) approved PLA/PLGA based drug products that are currently available, and due to its enormous popularity, it is one of the best-defined biomaterials available for drug delivery. In aqueous medium the ester backbone of PLGA undergoes slow hydrolysis. The polymer degrades into the monomers LA and GA both being entirely eliminated from the body. Lactic acid converts to pyruvate, which degrades into water and carbon dioxide via the Krebs cycle [4]. Glycolic acid will be either excreted directly via urine or will be oxidized into glyoxylate, which will be converted to glycine, serine, and pyruvate [5]. For particle engineering, the tenability of the material is essential. Therefore, characteristics like surface modifications or adjustment of physicochemical properties, and the biodegradation rate of the material are crucial. Several protocols for the preparation and modification of micro and nanoscale PLGA systems have been established [6-10]. Depending on the manufacturing method and the operating parameters, the yield of the method and the loading efficiency of nano and microparticles can vary drastically, influencing the therapeutic use [11-12]. Despite the high interest in PLGA, a convenient method for the quantification of the polymer matrix is still lacking. Usually, the PLGA system is estimated by gravimetric methods after sample lyophilization or, for increasing the sensitivity, by more complex methods like mass spectrometry, nuclear magnetic resonance or other analysis methods [13-17]. However, these approaches suffer

partly from sensitivity, as the pure polymer matrix cannot be quantified selectively or the required equipment for improving the analysis sensitivity is expensive and the quantification time consuming. In general, PLGA based drug delivery or biomedical systems are a complex mixture, composed of several molecules like loaded drugs and excipients. For estimating the yield of the manufacturing techniques, adjusting the concentration of assays, determining the loading efficiency and therapeutic dose of a drug or for fast quality control of the product, knowledge about the exact PLGA amount in the formulation is essential. This study presents a rapid and precise method for the selective quantification of PLGA. For the development, the technological requirements were kept as low as possible without losing selectivity and sensitivity. PLGA soaked and dissolved in tetrahydrofuran solvent. This PLGA solution is quantified against the different molecular weight polystyrene standards in a high-performance liquid chromatography-size exclusion chromatography (HPLC-SEC). The chromatographic conditions mainly content the organic size exclusion column with a dimension of 300mm x 8.0mm, tetrahydrofuran as a diluent and mobile phase, column temperature of 40°C, sample temperature of 10°C with a run time of 20 minutes. The detection was carried out on a refractive index detector with sampling rate 1.0, sensitivity 16 and detector temperature of 35°C. This method even allows the quantification of low amounts of PLGA. Hence the developed method is suited for various production phases.

Materials and Methods

Poly (lactic-co-glycolic acid) with a glycolic/lactic acid ratio of 50:50 and 75:25 was purchased from Evonik Nutrition, Expansorb, and Picas. The reference standards with different molecular weights are purchased from Waters Corporation. The solvents acetonitrile, methanol purchased from Merck limited. Stabilized tetrahydrofuran was purchased from J.T. Baker.

Preparation of standards for determination of molecular weight

Accurate weight of each Polystyrene standard taken in a dry volumetric flask to obtain a 1000ppm of concentration. The polystyrene standards in this study having theoretical molecular weights of 10700, 19900, 42400,116000 and 264000. All weighed standards are kept for soaking for 30 minutes of time. Each individual polymer was filled in the HPLC vials for analysis.

Quantitative analysis of Polystyrene standards and PLGA

For the quantification of PLGA, an HPLC system (Waters Corporation), equipped with a quaternary pump and a refractive index detector used. Furthermore, the system used an autosampler with 10°C, a column oven 40°C, and a column Shodex HT 804 (300mm x8.0 mm and 13 µm). Stabilized Tetrahydrofuran solvent is used as a mobile phase and diluent. The degasser mode was kept off in HPLC. The column was saturating with tetrahydrofuran for overnight with a flow of 0.2mL per minutes. The 0.7 mL per minute flow is given in method with the injection volume of 10µL. For data analysis, the chromatography software Empower 3.0 was used. The retention time and observed molecular weights with a correlation of more than 0.99 were considered as authentic. With the help of this calibration curve standards and retention time of particular PLGA obtained in the sample, the Molecular weight of that particular sample is determined. This optimized method is validated for specificity, linearity, precision, accuracy and solution stability.

Results and discussion

Various parameters were analyzed and optimized for the sensitive quantification of the method.

Specificity

The specificity of the method is carried out by injecting the blank tetrahydrofuran in the set method and observed that no interfering peak is observed from the blank at the retention of Polystyrene standard and PLGA sample retention.

Linearity

The linearity of the method is proposed by injecting five different molecular weight standards and observing its correlation coefficient. Table 1 shows the actual molecular standard weight, observed retention time, observed molecular weight and correlation coefficient obtained through software. Similarly Figure 1 shows the calibration plot of time versus molecular weight.

Figure 1: Standard calibration plot

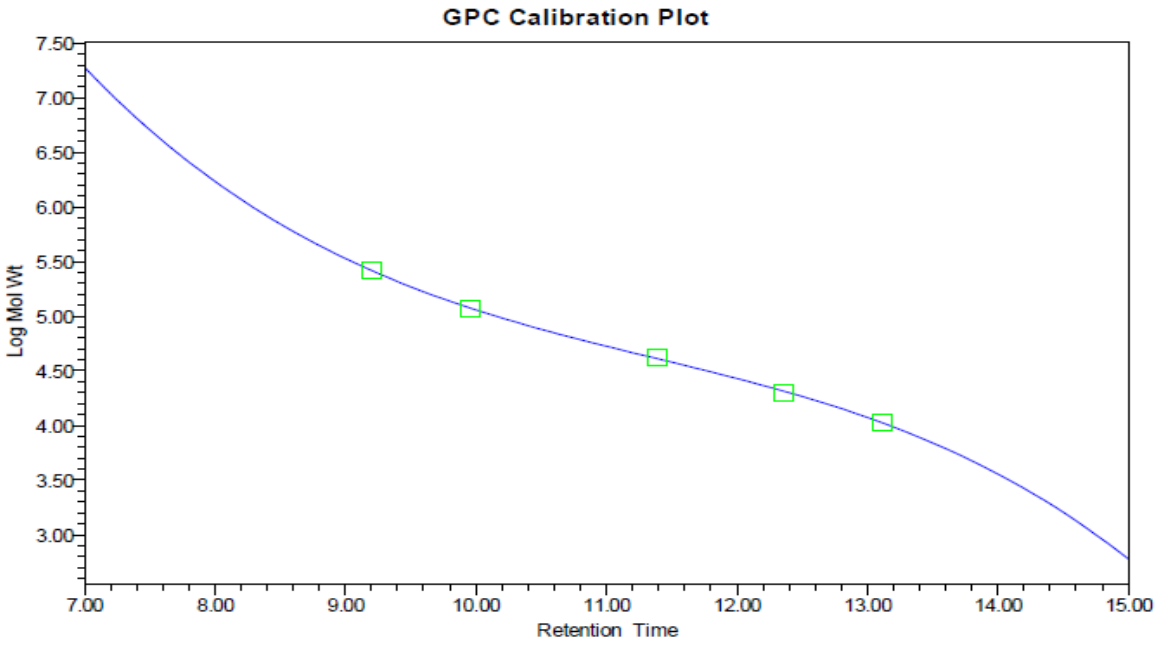

Table 1: Molecular weight calibration table

Standard details	Molecular weight (Daltons)	Retention Time (min)	Calculated weight (Daltons)	R 2
1	264000	9.200	261666	
2	116000	9.958	118600	
3	42400	11.385	40961	0.999561
4	19900	12.363	20559	
5	10700	13.114	10579	

Precision

System suitability and method precision carried out by injecting known molecular weight standards and PLGA containing samples six times. Below mentioned figures 2-6 shows the suitability of the system. The results mentioned in table 2 show the preciseness of the method.

Figure 2: Molecular weight calibrant 10700

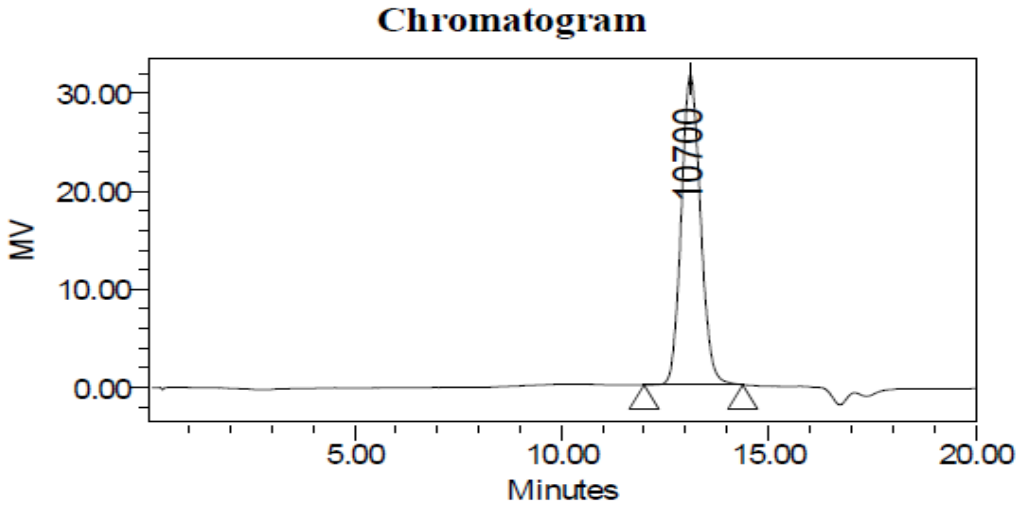

Figure 3: Molecular weight calibrant 19900

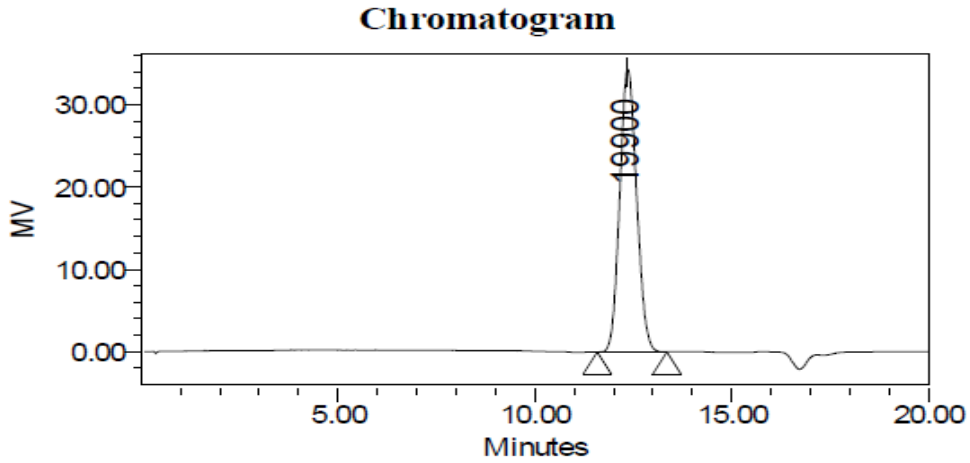

Figure 4: Molecular weight calibrant 42400

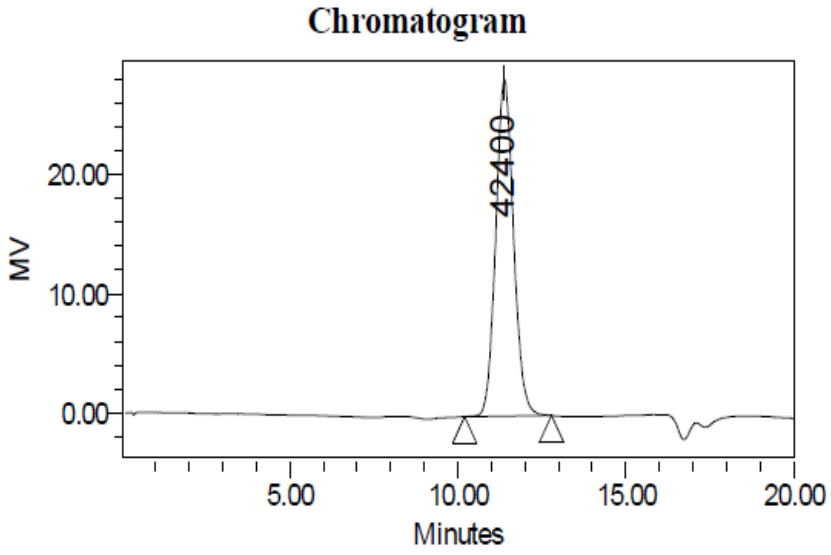

Figure 5: Molecular weight calibrant 116000

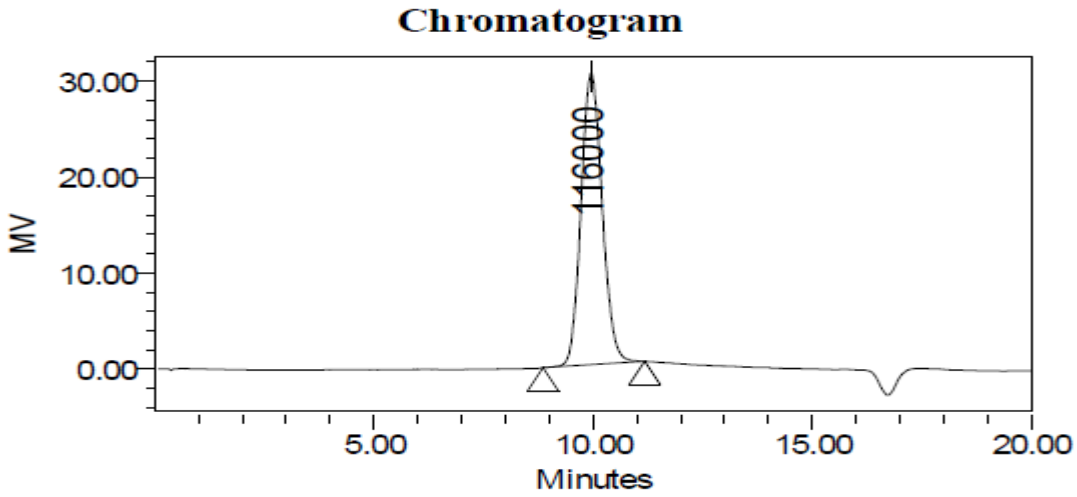

Figure 6: Molecular weight calibrant 264000

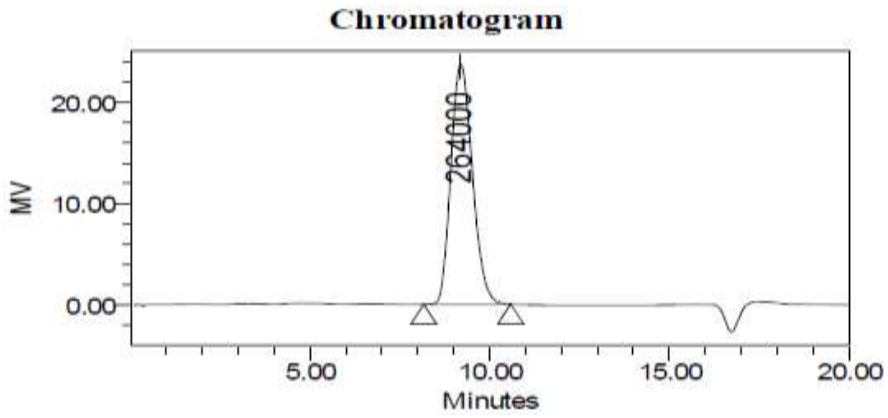

Table 2: Method precision results

Sample no.	Mn	Mp	Mz	Polydispersity	Mw
1	65630	102184	163276	1.59	104643
2	67642	105932	167257	1.58	106942
3	63994	102958	195173	1.74	111457
4	66545	104565	175225	1.65	108850
5	67098	103960	180850	1.58	117810
6	66110	104668	185590	1.67	108758
				Mean	109743
				SD	4552.7
				%RSD	4.15

Accuracy

Accuracy of the method is determined by the percentage relative difference between the theoretical molecular weight of standard and calculated molecular weight of standard at the initial 24 Hrs. of the time interval.

Observations for known standard solution (Initial) as broad unknown:

Table 3: Standard solution details at initial

Standard name	Theoretical molecular weight of standard	Calculated molecular weight of standard	% Relative difference
1.07×10^4	10700	10695	0.05
1.99×10^4	19900	20646	-3.75
4.24×10^4	42400	41312	2.57
1.16×10^5	116000	118937	-2.53
2.64×10^5	264000	262571	0.54

Observations for known standard solution (24 Hrs) as broad unknown:

Table 4: Standard solution details after 24 Hrs.

Standard name	Theoretical molecular weight of standard	Calculated molecular weight of standard	% Relative difference
1.07×10^4	10700	10772	-0.67
1.99×10^4	19900	20704	-4.04
4.24×10^4	42400	41414	2.33
1.16×10^5	116000	116054	-0.05
2.64×10^5	264000	254862	3.46

Conclusion

Although PLGA is a well-described and one of the most used polymers in many research fields, it still lacks a simple, fast and reliable quantification method. Therefore, a new HPLC-SEC method for the quantification of PLGA was developed. The presented study is first fully validated; the described method combines low instrumental requirements with short preparation time and high precision and selectivity for polymers. The validated parameters show a good range for polymer determination with high precision. Therefore, the present method can be considered as simple, fast, and easy to apply, making it very suitable for routine analysis in quality control of PLGA based systems. The presented method can be used for a dual quantification of the polymer matrix and the encapsulated drug within a single run. The reported method was very suitable for routine analysis in quality control of PLGA based systems with promising future application features.

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