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Original Article

Development of a fast and precise method for simultaneous quantification of the PLGA monomers lactic and glycolic acid by HPLC

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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 the polymer is inalienable. During the development of a 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 novel method for the fast and precise quantification of PLGA by RP-HPLC. The polymer is hydrolyzed into its monomers, glycolic acid and lactic acid. Afterwards, the monomers are derivatized with the absorption-enhancing molecule 2,4′-dibromoacetophenone. Furthermore, the wavelength of the derivatized monomers is shifted to higher wavelengths, where the used solvents show a lower absorption, increasing the sensitivity and detectability. The developed method has a detection limit of 0.1 μg/mL, enabling the quantification of low amounts of PLGA. By quantifying both monomers separately, information about the PLGA monomer ratio can be also directly obtained, being relevant for degradation behavior. 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 stabilizer or excipient is interfering. Due to the high sensitivity and rapidity of the method, it is suitable for both laboratory and industrial uses.

1. Introduction

Since the introduction of poly(lactic-*co*-glycolic acid) (PLGA) as bioresorbable fiber material in the 1960s, PLGA has become 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 15 PLA/PLGA-based drug products which are currently available, and due to its enormous popularity, it is one of the best-defined biomaterials available for drug delivery. In aqueous environments, the ester backbone of PLGA undergoes slow hydrolysis. The polymer degrades into the monomers LA and GA both being entirely eliminated from the body. LA converts to pyruvate, which degrades into water and carbon dioxide via the Krebs cycle [4]. GA is either excreted directly via urine or oxidized into glyoxylate, which is converted to glycine, serine, and pyruvate [5]. For particle engineering, the tunability 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 nano-scale 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 sensitivity is expensive and the quantification is time consuming. In general, PLGA-based drug delivery or biomedical systems are a complex mixture, composed of several molecules like loaded

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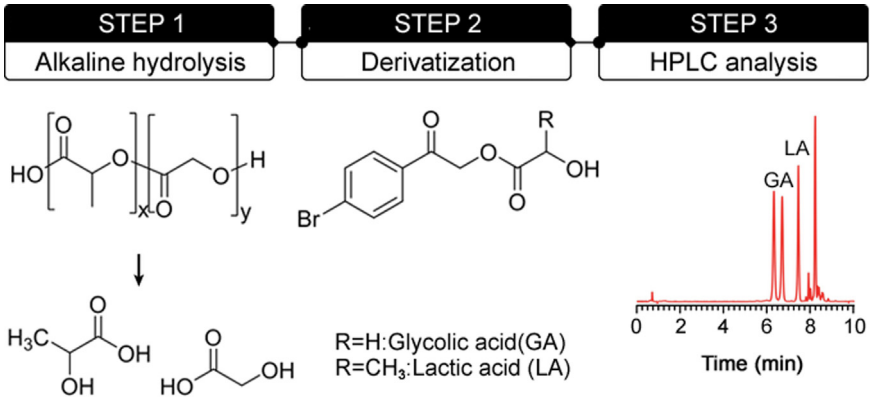


Fig. 1. Sample preparation for PLGA quantification by RP-HPLC. PLGA degrades into the monomers LA and GA by alkaline hydrolysis. Then, the monomers are derivatized with DBAP and analyzed by RP-HPLC.

drugs and excipients. For estimating the yield of the manufacturing technique, adjusting the concentration for assays, determining the loading efficiency and therapeutic dose of drug or for a fast quality control of the final 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. Therefore, a coupling reaction of esters with an UV-enhancer was adopted [18]. For the development, the technological requirements were kept as low as possible without losing selectivity or sensitivity. PLGA is hydrolyzed under alkaline conditions, and the polymer degrades into its monomers LA and GA. The monomers are then separated and quantified by reversed-phase high-performance liquid chromatography (RP-HPLC). The two low molecular α-hydroxy acids absorbe at a wavelength close to the cut-off range of most commonly used mobile phases. Hence, the baseline noise of the chromatogram will increase, and the sensitivity and detectability of the molecules will decrease. Therefore, LA and GA is derivatized with the UV-enhancing molecule 2,4′-dibromoacetophenone (DBAP) by esterification before analysis. The derivatized monomers are detected at a wavelength of 254 nm, allowing to increase the signal-to-noise ratio and thus the sensitivity and detectability of the quantification method [19,20]. This even allows the quantification of low amounts of PLGA. Hence, the developed method is suited for various product phases: development, requiring a fast and sensitive quantification, up to production, and requiring a reproducible and precise measurement.

2. Materials and methods

2.1. Materials

Poly(lactic-*co*-glycolic acid) with a GA/LA ratio of 50:50 (RG 503H) and 75:25 (RG 752H) was purchased from Evonik Nutrition & Care GmbH (Darmstadt, Germany). The reference standards for GA (99%) and LA (≥ 95%) were purchased from Sigma Aldrich (Darmstadt, Germany). Poly(D,L-lactic acid) (≥ 95%) was purchased from Polysciences, Inc. (Hirschberg an der Bergstraße, Germany). For the derivatization, 2,4′-dibromoacetophenone (≥ 98%) and triethanolamine (TEA) (≥ 98%) were purchased from Sigma Aldrich (Darmstadt, Germany). The solvents acetonitrile, acetone, tetrahydrofuran, ethyl acetate, dimethylformamide, and chloroform were all purchased from Sigma Aldrich (Darmstadt, Germany). For the hydrolysis and pH adjustment, potassium hydroxide (KOH) and aqueous hydrobromic acid (HBr) were purchased from Sigma Aldrich (Darmstadt, Germany). The pH indicator bromocresol green was purchased from Honeywell (Seelze, Germany).

2.2. Preparation of HPLC references of LA and GA

The reference samples of GA and LA were prepared by dissolving or diluting the samples in acetonitrile. The derivatization reagents DBAP and TEA were added as described below.

2.3. Standard conditions for the sample preparation

To compare the influence of different parameters, specific conditions were set as default and one variable was changed within each experiment. Each optimization is described with default conditions and the changed parameters are highlighted in the results. The sample preparation is based on the alkaline hydrolysis of PLGA into the monomers LA and GA. Subsequently, the monomers were modified with the UV-enhancing molecule DBAP. Finally, the sample was analyzed by RP-HPLC. The PLGA amount can be calculated with the masses of LA and GA. For an overview, the sample preparation is illustrated in Fig. 1.

2.3.1. Alkaline hydrolysis of PLGA

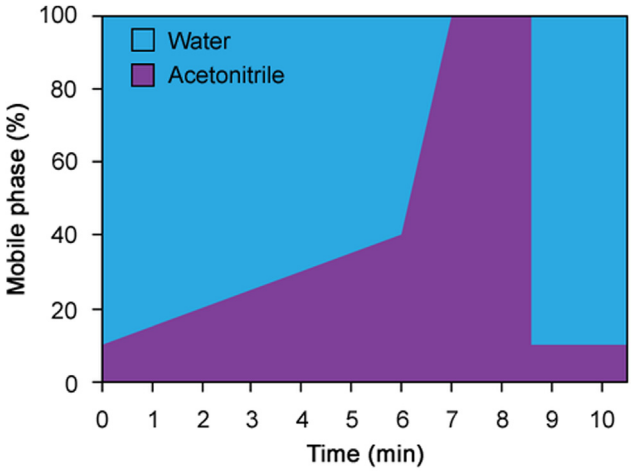
A 0.5 mg/mL PLGA solution was prepared by dissolving the polymer in acetonitrile under continuous stirring. For the hydrolysis, 800 μL of the PLGA solution was mixed with 200 μL of a 0.5 mol/L aqueous KOH solution. The hydrolyzation was supported by heating under continuous shaking in a thermal shaker for 90 min at 100 °C and 600 rpm (MHR 13, Hettich Benelux, Geldermalsen, the Netherlands). After hydrolyzation, the sample was cooled to room temperature. Optionally, storing over-night at 4 °C was possible and did not affect the yield.

2.3.2. Derivatization of GA and LA

For the esterification of the monomers with DBAP, the pH value was adjusted between 4 and 5. Therefore, 50 μL of an aqueous bromocresol green solution was added and the pH was adjusted with 0.5 mol/L hydrobromic acid. Subsequently, the derivatization reagents DBAP and TEA were dissolved in acetonitrile. Each time, 400 μL of 50 μmol/mL DBAP and 400 μL of 50 μmol/mL TEA, both in acetonitrile, were added to the sample. The reaction was conducted under continuous shaking in a thermal shaker for 90 min at 100 °C and 600 rpm. After derivatization, the sample was cooled down to room temperature and analyzed with RP-HPLC.

2.3.3. Quantitative analysis of derivatized GA and LA by RP-HPLC

For the separation and quantification of derivatized GA and LA, an HPLC system (Ultimate 3000 series, Rapid Speed, Thermo Fisher Scientific, Waltham, MA, USA), equipped with a quaternary pump and a variable wavelength, was used. Furthermore, the system



	Retention time (min)					
	0	6	7	8.5	8.5	10.5
Water	90%	60%	0%	0%	90%	90%
Acetonitrile	10%	40%	100%	100%	10%	10%

Fig. 2. HPLC gradient used for the quantification of LA and GA. The mobile phase consists of water (blue) and acetonitrile (purple).

used an autosampler (ASI-100, Dionex, Sunnyvale, CA, USA), a column oven (STH 585, Dionex, Sunnyvale, CA, USA) and a LiChrosphere 100 RP-18e column (5 μm material, 4 mm × 125 mm; Merck KGaA, Darmstadt, Germany). The column oven was heated up to 30 °C. The mobile phase was prepared with degassed and filtered acetonitrile and deionized water. The gradient, illustrated in Fig. 2, started with 10% acetonitrile and increased linearly up to 40% in 6 min. Within 1 min, acetonitrile raised up to 100% and the concentration was then kept for 1.5 min. Afterwards, the column was equilibrated with 10% acetonitrile and 90% water for 2 min. The flow rate was 1.5 mL/min, and both monomers were detected at a wavelength of 254 nm. The sample injection volume was set to 20 μL.

The sample peaks were identified by comparing the retention times of the related peaks with reference standards of GA and LA. The retention time of the derivatized GA was 6.95 min and for the derivatized LA was 7.65 min. For data analysis, the chromatography software Chromeleon 6.8 Chromatography Data System (Thermo Fisher Scientific, Waltham, MA, USA) was used, and the peaks were quantified by determining the area under the peak. The peak area of both monomers was set in relation to the highest measured signal for each experiment, simplifying the comparison of all evaluated conditions.

2.3.4. Preparation of calibration standards

To consider the hydrolysis of PLGA, poly(lactic acid) (PLA) and GA, instead of poly(glycolic acid) (PGA) due to its insolubility in most organic solvents, were used for the preparation of LA and GA standards. Standards with concentrations of 0.0001, 0.001, 0.01 and 0.1 mg/mL were dissolved in acetonitrile. For the hydrolysis, 200 μL of 0.05 M KOH was added and heated at 80 °C for 60 min. For the derivatization, 400 μL of 75 μmol/mL DBAP and 75 μmol/mL TEA were added and the pH was adjusted between 8 and 9. Then, the samples were heated again at 80 °C for 60 min.

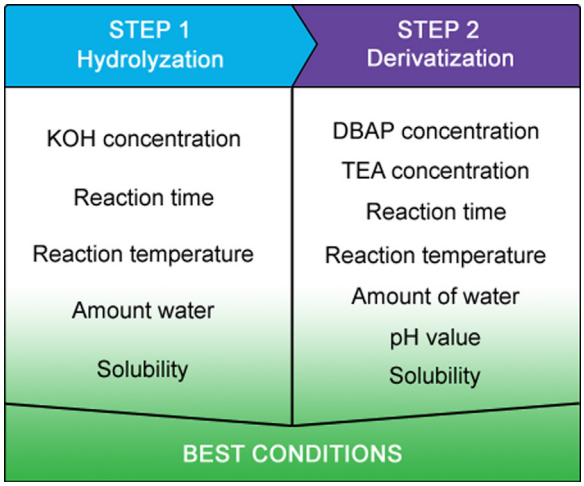


Fig. 3. Method optimization. For the hydrolyzation of PLGA and the derivatization of both monomers, various parameters needed to be optimized. This overview summarizes each parameter for both steps.

3. Results and discussion

3.1. Optimization of sample preparation

Various parameters were analyzed and optimized for the development of a sensitive quantification method. Fig. 3 gives an overview of all investigated parameters. PLGA degraded to LA and GA and both monomers were quantified by HPLC analysis. The hydrolyzation step is influenced by the concentration of KOH, the amount of water, the duration and temperature of the reaction. Before analysis, both monomers were coupled with an UV-enhancer. The yield of this step depends on the concentration of the chemical reagents, the pH value, the amount of water, and the duration and temperature of the reaction. Furthermore, the solubility of the polymer and the reagents in the used organic solvent is indispensable. Therefore, different solvents were tested. For each parameter, various conditions were analyzed and the yield was compared to the standard procedure.

3.1.1. Comparison of various solvents

By using different commonly used organic solvents for the sample preparation, the influence of the organic phase on the quantification yield was investigated. The results of LA and GA are displayed in Fig. 4. For both monomers, using acetonitrile as a solvent, the highest signal was measured. In comparison, using a different solvent the peak area reduced drastically. Consequently, it is not advisable to change to another organic solvent. Furthermore, the vapor pressure of acetonitrile is, compared to other solvents such as acetone, lower, which is helpful in carrying out the analysis due to the lower evaporation which makes it easier to keep the volume constant.

3.1.2. Hydrolysis of PLGA into GA and LA

An efficient and reproducible degradation of PLGA to the monomers LA and GA is essential for the quantification of low amounts of PLGA. For the alkaline hydrolysis of PLGA, different concentrations of KOH, varying amounts of water, and the duration and temperature of the reaction were investigated and compared. In Fig. 5 the results of all conditions are displayed. The duration of the hydrolysis ranged from 10 up to 150 min. For both monomers, the signal slightly increased with an extended hydrolyzation time until it reached a plateau (Fig. 5A). After 60 min, the peak of GA reached the highest signal and showed also a low difference to the LA peak. Compared to the first 10 min the signal

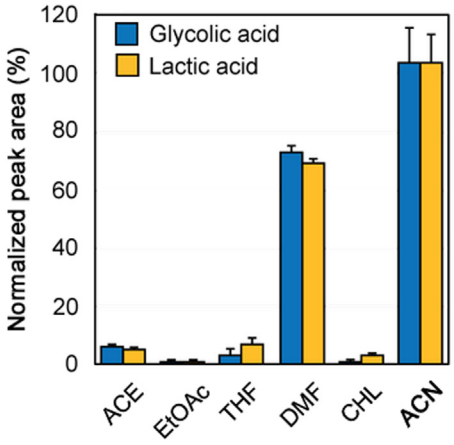


Fig. 4. Influence of the organic solvent on the signal. Samples have been prepared in various organic solvents and the impact was measured by RP-HPLC. ACE: Acetone, EtOAc: Ethyl acetate, THF: Tetrahydrofuran, DMF: Dimethylformamide, CHL: Chloroform, ACN: Acetonitrile. The peak areas were normalized by the highest signal (F/F_{max}). The initial standard condition is marked in bold. The experiment was performed as a triplicate ($n = 3$). For each condition, the standard deviation is marked with error bars.

increased by 32%. LA reached the highest signal after 90 min. The peak area increased by 35% compared to the 10 min value. The different temperatures for the hydrolysis ranged from 60 to 135°C. For both monomers, the highest peak area was measured at 80 °C (Fig. 5B). GA and LA raised by ~91% compared to 60 °C. KOH was used in a concentration range from 0.01 to 2 mol/L (Fig. 5C). Increasing the concentration from 0.01 to 0.05 mol/L raised the signal for LA by 83% and for GA by 60%. With a further increase in the concentration, the signal started decreasing. At a concentration of 2 mol/L, the signal reduced by 94% for LA and 97% for GA with respect to the maximum value. The following derivatization of the monomers is based on an ester formation. Unconsumed KOH could interfere with the derivatization reaction, resulting in underivatized monomers and thus in a lower signal. Since alkaline hydrolysis is a water-based reaction, the available amount of water is a limiting factor. For determining the minimum amount of water, the volume was varied from 200 to 600 μL (Fig. 5D). No distinct difference between the different volumes was detectable, indicating that 200 μL is enough for the tested amounts of PLGA.

It could be demonstrated that KOH is suitable for the degradation of PLGA and that the concentration has a strong effect on the detectability being optimal at 0.05 mol/L. The hydrolysis was supported by an increased temperature and a longer reaction time, with 80 °C and 60 min as the best parameters. At 80 °C, both peaks reached high values, without a strong variation between the two. Furthermore, the time requirement was an important aspect for the quantification, as we wanted to develop a fast quantification method. Besides, longer hydrolyzation times and higher temperatures may increase the risk of evaporation. This made it, on the one hand, necessary to repeat the experiment, in case the volume evaporated was high enough to be visible; on the other hand, if the volume was comparably small and thus not visible, it would be an explanation for higher standard deviations which can be seen in Fig. 5A. Thus, the shortest possible time with the highest signal seems to be best suited. Furthermore, the initial

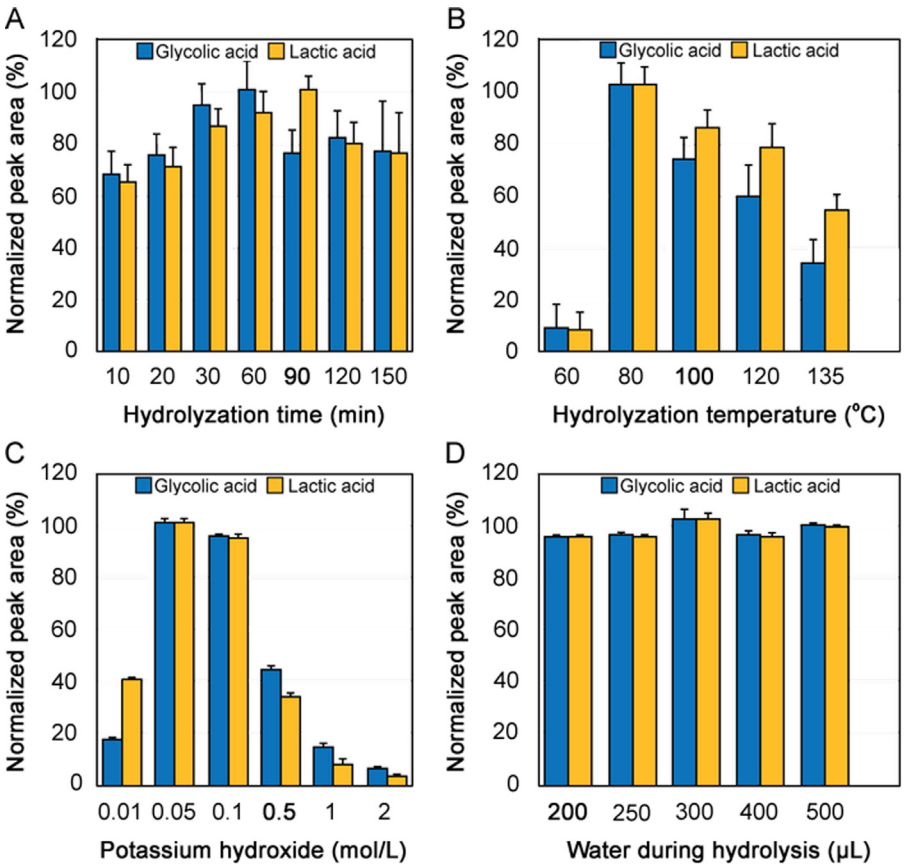


Fig. 5. Optimization of the hydrolyzation step. For the decomposition of PLGA into GA (blue) and LA (yellow), different conditions for several parameters were compared. (A) Different reaction times for the hydrolyzation, ranging from 10 to 150 min were used. (B) Varying temperature conditions for the reaction, ranging from 60° to 135°C. (C) The concentration of KOH varied from 0.01 to 2 mol/L. (D) The impact of the water volume on the reaction was determined by using different volumes, ranging from 200 to 500 μL. The peak areas for each graph were normalized by the highest signal (F/F_{max}). The initial standard conditions are marked in bold. The experiments were performed as a triplicate ($n = 3$). For each condition, the standard deviation is marked with error bars.

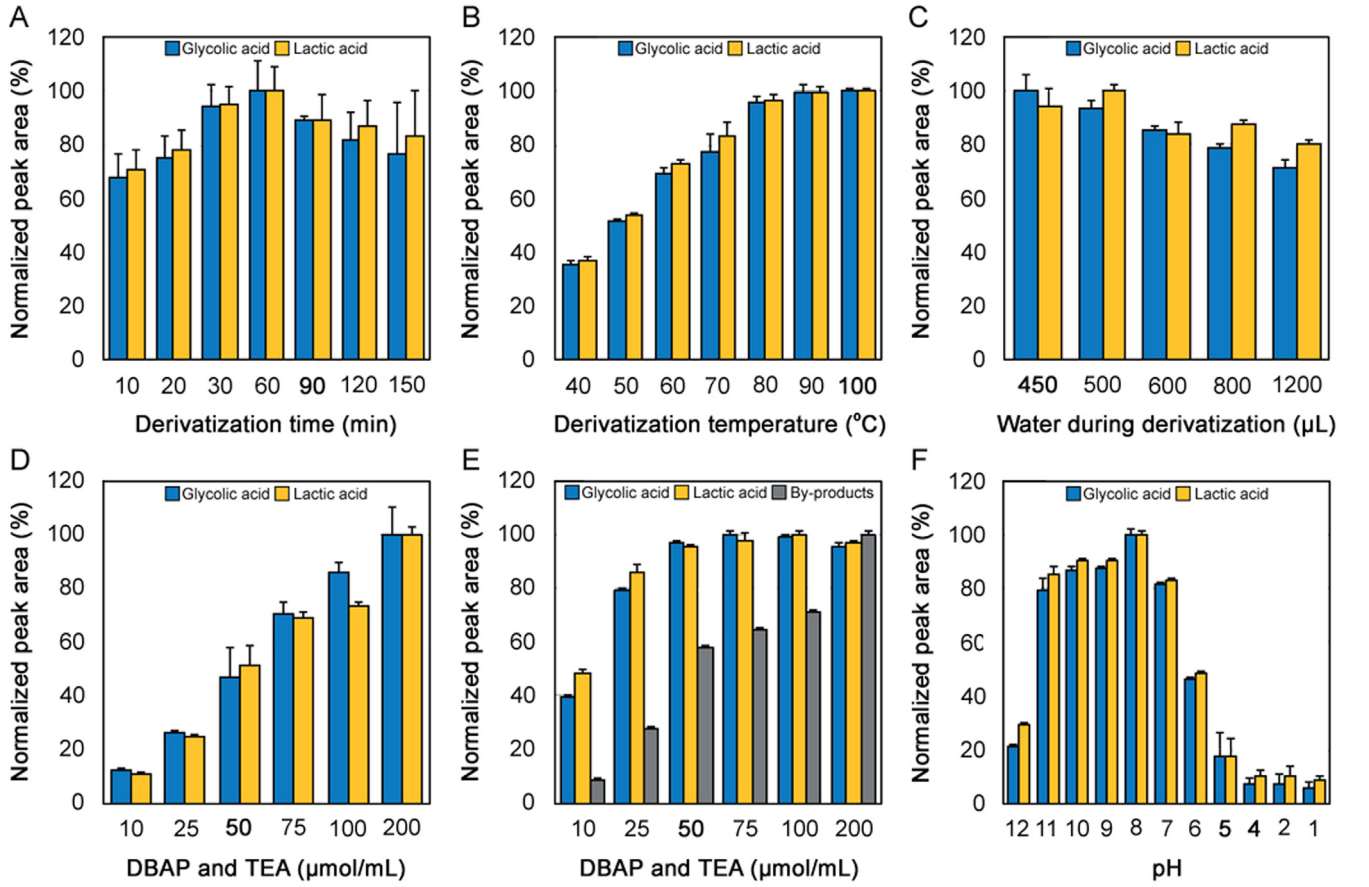


Fig. 6. Optimization of the derivatization step. For the derivatization of GA (blue) and LA (yellow), different conditions for several parameters were compared. (A) Different reaction times for the derivatization, ranging from 10 to 150 min were used. (B) Influence of the temperature on the derivatization reaction was investigated. The temperature ranged from 40° to 100°C. (C) Determining the influence of water on the derivatization reaction. Various water volumes were tested, ranging from 450 up to 1200 µL. (D) The concentration of the derivatization reagents DBAP and TEA were varied, from 10 to 200 µmol/mL. The concentration of KOH was 0.5 mol/L. (E) Repeated experiment for determining the influence of the concentration of DBAP and TEA with a changed concentration of KOH of 0.1 mol/L. To highlight the problem of increasing concentration of DBAP and TEA, the normalized peak area of all by-products is included (grey). (F) The derivatization reaction was run at various pH values, determining the optimal pH range. The peak areas for each graph were normalized by the highest signal (F/Fmax). The initial standard conditions are marked in bold. The experiments were performed as a triplicate ($n = 3$). For each condition, the standard deviation is marked with error bars.

200 µL water was enough for the alkaline hydrolysis. A further increase of the parameters showed no impact on the yield. Therefore, a concentration of 0.05 mol/L potassium hydroxide, a temperature of 80 °C and a hydrolysis time of 60 min are chosen as the final parameters for the degradation of PLGA.

3.1.3. Derivatization of GA and LA

The monomers were derivatized with the UV-enhancer DBAP to increase sensitivity and detectability. For optimizing the derivatization, the influence of the temperature and the duration of the reaction, the concentration of the UV-enhancer and the non-nucleophilic base, the amount of water, and the pH during derivatization were analyzed. The results are displayed in Fig. 6. For efficient derivatization, different reaction times were tested, starting at 10 up to 150 min (Fig. 6A). Both monomers, LA and GA, reached a maximum after 60 min. A further increase of the derivatization time did not change the yield drastically, indicating that most of the monomers were derivatized. The different temperatures for the derivatization ranged from 40° to 100 °C (Fig. 6B). By increasing the temperature, the yield of the reaction improved. At 80 °C, a plateau was reached for both monomers, and a further increase of the temperature did not change the signal. Since a complete removal of water is a time-consuming step, the influence of different volumes of water on the derivatization reaction was determined. Volumes ranging from 450 to 1200 µL have been added to different samples. The starting value of 450 µL is due to the contained water necessary for hydrolysis and the following neutralization step. Decreasing the value would limit the concentration range of PLGA. Keeping the sample volume constant, the difference was filled up with acetonitrile. The results are displayed in Fig. 6C. An increased water content reduced the signal of both peaks slightly. It is advisable to keep the amount of water as low as possible and, even more important, constant between comparative samples within one experiment. Both derivatization reagents, DBAP and TEA, were tested at different concentrations, ranging from 10 to 200 µmol/mL (Fig. 6D). No plateau was reached, and the signal of both monomers raised continuously for the tested concentrations. Since KOH influenced the signal, we hypothesize that a high concentration of KOH cleaves the ester bond of the coupled UV-enhancer, shifting the plateau to higher derivatization concentrations. Therefore, the experiment was repeated with a reduced KOH concentration of 0.05 mol/L (Fig. 6E). The lower KOH concentration changed the trend of the results clearly. A plateau for GA and LA was reached after a DBAP and TEA concentration of 75 µmol/mL. As expected, the signal intensity increased, comparing to the experiment with 0.5 mol/L KOH (Fig. S1). If the hypothesis that KOH cleaves the product is correct, we would expect a plateau at much higher concentrations of DBAP with 0.5 mol/L KOH because DBAP is consumed. The solubility of the derivatization reagent was the limiting factor to proof this hypothesis. Increasing the concentration of DBAP and TEA further, the peaks of the derivatization reagents got wider, overlapping the LA peak. To

indicate this problem, we added the signal area of all by-products in this graph. It can be seen that the constantly increasing signal worsen the chromatogram. A high concentration of the apolar derivatization agents can clog the column, increasing the necessary time of washing steps (data not shown). Therefore, a concentration of 75 μmol/mL for DBAP and TEA is recommended and should be only increased if a higher PLGA concentration is analyzed. Fig. 6F displays the influence of the pH on the coupling reaction of DBAP. The derivatization step was performed at different pH values, ranging from 1 to 12. A strong influence was detectable. The reaction is not influenced considerably in the pH range from 7 to 11. Therefore, this pH range is a suitable working space for sample preparation. Becoming more acidic, the signal decreased drastically. Increasing the pH above 11, the signal also decreased.

Summarizing the results, the best signals were obtained at a temperature of 80 °C, for a reaction time of 60 min and in a pH range of 7–11. The concentration of DBAP and TEA should not be higher than 75 μmol/mL; otherwise the peak area of the reaction agents increased drastically overlapping with the relevant signals. For improving the sensitivity of the method, the amount of water should be kept as low as possible.

3.2. Calibration curve and validation of the HPLC analysis under optimized conditions

Based on the best settings for the hydrolyzation of PLGA and the derivatization of LA and GA, a calibration curve was prepared. Standards for PLA and GA, since PGA is not soluble in most organic solvents, haven been independently prepared by hydrolyzing both standards at 80 °C for 60 min with a KOH concentration of 0.05 mol/L. Then, the monomers were derivatized at 80 °C for 60 min with each 400 μL of 75 μmol/mL DBAP and TEA. For the derivatization, the pH was adjusted to 8–9 with HBr. PLA and GA concentrations ranged from 0.0001 to 0.1 mg/mL and the results are displayed in Fig. 7A. The measurement shows a linearity in a range of 0.1 μg/mL to 100 μg/mL, with a regression equation of $y = 1079.0x + 0.25$ for LA and $y = 1098.6x + 0.81$ for GA. For both monomers, the equations show a good coefficient of determination of 1 for LA and 0.9999 for GA.

For the validation of the method, the methodology of the ICH Guideline Q2(R1) was used [21]. The repeatability was measured with 6 determinations of a test concentration of 0.05 mg/mL. For GA and LA, the standard deviation is 0.5%. The intermediate precision was determined with 3 concentrations (0.025, 0.05, 0.075 mg/mL), each as a triplicate and prepared by 2 operators. For LA 0.7%, 0.3% and 0.4% and for GA 0.6%, 0.4% and 0.4% were calculated. Additionally, the limit of detection (LOD) and the limit of quantification (LOQ) were calculated for LA and GA regarding the ICH guidelines with following equations.

$$LOD = 3.3 * \sigma/S$$

$$LOQ = 10 * \sigma/S$$

Where σ is the standard deviation of the blank and S is the slope of the calibration curve. For LA and GA, LOD is below 0.1 μg/mL and the LOQ is at 0.2 μg/mL.

For the analytical application, PLGA with varying GA and LA ratios and different PLGA concentrations have been investigated (Fig. 7B and C). Regarding the PLGA ratio, for both samples (50:50 and 75:25), good results were obtained. The first PLGA sample, Resomer RG 503 H (50:50), showed for LA a value of 51% with a standard deviation of 0.5% and for GA 49% with a standard deviation of 0.2%. The second PLGA sample, Resomer RG 753 H (75:25), showed for LA a value of 75% with a standard deviation of 0.4% and for GA 25% with a standard deviation of 0.1%. For the varying concentrations, PLGA (50:50) with 0.1 and 0.2 mg/mL was analyzed. With 0.1 mg/mL, a concentration of 0.054 mg/mL and a standard deviation of 0.0002 mg/mL were determined for LA. For GA, a concentration of 0.052 mg/mL and a standard deviation of 0.0002 mg/mL were measured. Combining the values of LA and GA, a calculated concentration of 0.1058 mg/mL for PLGA was found. As expected, increasing the PLGA concentration the values for LA and GA changed too. For LA and GA, a concentration of 103.0 μg/mL and 104.4 μg/mL and a standard deviation of 2.0 μg/mL and 3.3 μg/mL were measured yielding a PLGA concentration of 207.4 μg/mL.

The results show that the RP-HPLC system and the gradient elution are suitable for the separation and quantification of LA and GA to values as small as 0.1 μg/mL. Furthermore, good

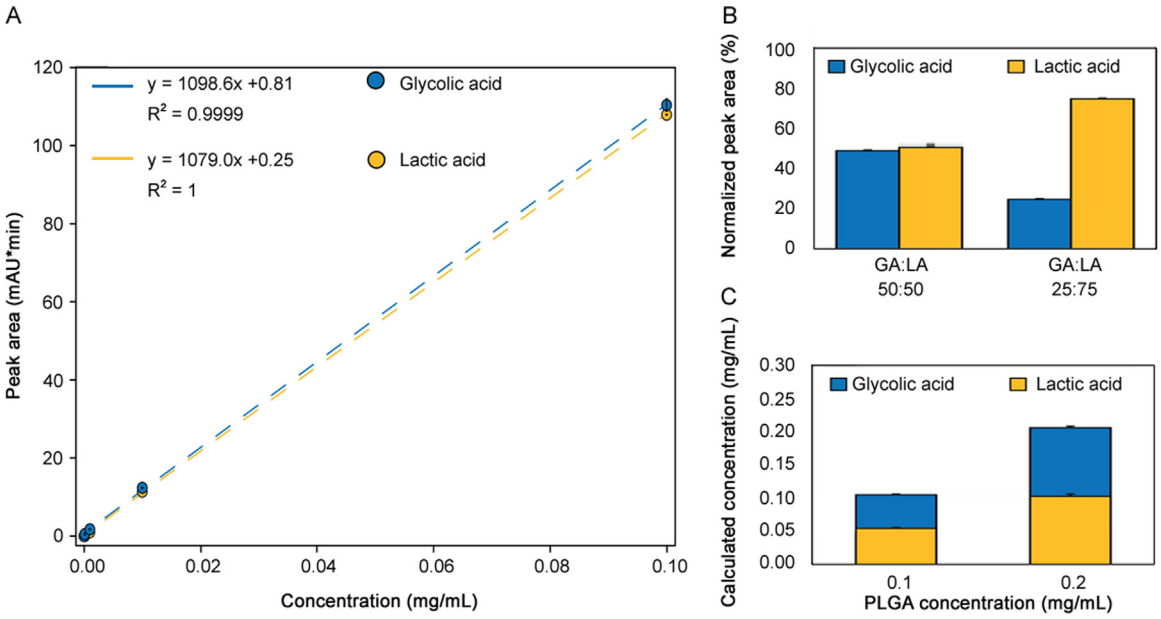


Fig. 7. Calibration curve and analytical application. (A) Calibration curve for poly (lactic acid) (yellow) and glycolic acid (blue). PLGA concentrations in the linear range from 0.0001 to 0.1 mg/mL are displayed. (B) Quantification of two PLGA types with different LA:GA ratios. (C) Quantification of two PLGA samples with different concentrations and a GA:LA ratio of 50:50. The experiments were performed as a triplicate ($n = 3$). For each condition, the standard deviation is marked with error bars.

Table 1
Overview of the initial standard and final optimized parameters for the analytical method.

Parameters	Initial, standard	Final, optimized
Solubility		
Organic solvent	Acetonitrile	Acetonitrile
Step 1: Hydrolyzation		
Duration	90 min	60 min
Temperature	100 °C	80 °C
KOH concentration	0.5 mol/L	0.05 mol/L
Water volume	200 μL	200 μL
Step 2: Derivatization		
Duration	90 min	60 min
Temperature	100 °C	80 °C
DBAP/TEA concentration	50 μmol/mL	75 μmol/mL
pH value	4–5	8–9

reproducibility and repeatability were obtained for the method. With a first analytical application, the possibilities of the developed method could be shown, highlighting the strength of detecting the PLGA monomers independently.

4. 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 novel chromatographic method for the quantification of PLGA was developed. To our knowledge, the presented study is the first fully validated, chromatographic method for the parallel quantification of LA and GA. Compared to previously published PLGA quantification methods, the described method combines low instrumental requirements with a short preparation time and a high precision and selectivity for the monomers [17,18,22]. The polymer degrades into both monomers, LA and GA, by alkaline hydrolysis. By optimizing the conditions for the hydrolysis, the required time for the complete degradation of PLGA was reduced to 60 min, whereas other methods need several hours or degrade PLGA not completely. Subsequently, the monomers are derivatized with an UV-enhancing molecule. The derivatization method was adopted from a previous study, but the reaction was investigated in more detail and thus could be improved. Our study shows that a low water fraction does not drastically interfer with the derivatization, avoiding a time-consuming freeze-drying step and reconstitution of the sample. Furthermore, the ratio of monomers and DBAP was analyzed and the strong influence of the pH value on the yield could be demonstrated. More importantly, a strong interaction of the alkaline hydrolysis and the derivatization was revealed. For the quantification, a RP-HPLC system with a water and acetonitrile-based gradient was chosen. By analyzing several crucial parameters, a deep insight of the method capacities is reported.

Summarizing the results, for the hydrolyzation, keeping the sample for 60 min at 80 °C with a concentration of KOH of 0.05 mol/L was found to be well-suited. For the derivatization, a concentration of 75 μmol/mL for DBAP and TEA, as well as a temperature of 80 °C and a reaction duration of 60 min obtained the best values. All changed parameters of the analytical method are grouped in Table 1. The method validation shows a good range for the determination of LA and GA, combined with a 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.

A promising future application would be the transfer of the method to particulate carrier systems. Since the monomers of PLGA are quantified separately, the method is suited for in-depth analysis on the degradation behavior of polymeric carrier systems. For drug carrier systems, the drug loading and the loading efficacy are of high importance; thus, a lot of methods for the quantification of encapsulated drugs in polymer systems have been published [23,24]. A pre-study shows that the presented method can be used for a dual quantification of the polymer matrix and the encapsulated drug within a single run. By this, all relevant data needed for characterizing such a carrier system can be determined with one single method, making the reported method very suitable for routine analysis in quality control of PLGA-based systems with promising future application features.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.jpha.2019.01.004.

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