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ANALYTICAL CONTROL OF LACTIDE SYNTHESIS

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Abstract

The technique of identification and quantification of D,L-lactide, lactic acid and its oligomers by the method of high performance liquid chromatography (HPLC) was developed. The separation was done on two columns connected consecutively using an aqueous solution of orthophosphoric acid and acetonitrile in the ratio 88:12 vol. % at a flow rate of 1.2 ml min^{-1} as a mobile phase. The developed method is simple and linear. Research of specificity, accuracy, repeatability and reproducibility shows that the technique is suitable for analytical control of all stages of the lactide synthesis from lactic acid.

Keywords: lactide; lactic acid; oligomer; analysis lactide; chromatography.

1. Introduction

Polylactide or poly(lactic acid) is a polymer of lactic (2-hydroxypropanoic) acid. It is biodegradable and biocompatible like other polyesters based on α-hydroxy acids [1]. Due to a combination of these properties polylactide causes interest in the field of packaging [2-3], medicine and pharmaceutics [4-5].

The main method of the polylactide synthesis is ring-opening polymerization of L-lactide. The preparation of L-lactide includes the following stages: polycondensation of the L-lactic acid for obtaining oligo(L-lactic acid); depolymerization of the oligo(L-lactic acid) to produce crude lactide; purification of the crude lactide. The impurities in L-lactide significantly affect the synthesis and properties of polylactide. The presence of the lactic acid and its oligomers is particularly undesirable because they decrease a polymerization rate and reduce an achievable degree of lactide polymerization [1].

Analytical control of the lactide synthesis allows solving the following problems:

1. Monitoring of intermediate and product purity;
2. Investigation of the reaction kinetics;
3. Material balance calculations;
4. Choice of the method for crude lactide purification.

High performance liquid chromatography is a simple and accurate method for quantitative analysis of lactic acid and lactide. The chiral columns are used to study the content of lactide isomers of D- and L-forms [6-8], in other cases the total content of D- and L-lactide (D, L-lactide) is determined quantitatively. It provides the method for HPLC analysis of the lactic acid oligomer, where lactic acid, lactide, dimer, trimer and other lactic acid oligomers were separated [9-11]. This method allows quantifying the content of lactic acid and D,L-lactide, however, the analysis time lasts from 60 to 150 minutes at a high flow rate of acetonitrile that is a significant drawback of the proposed method. Besides, the method for quantitative determination of lactic and glycolic acid, lactide, glycolide and ethyl acetate by HPLC is described [12], but it does not include analysis of the lactic acid oligomers.

The purpose of the study is to develop a simple, accurate and rapid method of determining D,L-lactide, lactic acid and its oligomers for analytical control of the lactide synthesis.

2. Experimental

2.1. Materials

HPLC grade acetonitrile (Cryochrom, Russia), bi-distilled water and orthophosphoric acid (Reachem, Russia) were used for mobile phase preparation. 85 % aqueous L-lactic acid solution (Sigma-Aldrich, USA) and lactide were used as standards. Lactide of 99.9% purity was obtained from 85% aqueous L-lactic acid solution in a laboratory.

2.2. Chromatographic conditions

The reversed-phase high performance liquid chromatography (RP-HPLC) was carried out using a liquid chromatograph YL9100 (YoungLin Clarity, South Korea) with a UV spectrophotometric detector YL9120 UV/Vis. The chromatographic columns Tracer Excel 120 ODSA (250 mm × 4.6 mm × 5 μm) and Zorbax Eclipse XDB-C18 (250 mm × 4.6 mm × 5 μm) were connected consecutively. The chromatographic separation was achieved by isocratic elution using a mobile phase consisting of acetonitrile and an aqueous solution of orthophosphoric acid containing 1 g L^{-1} (12:88, v/v) at a flow rate 1.2 mL min^{-1}. Determinations were performed at a column oven temperature of 40±2 °C. The analytical wavelength of the UV detector was 210 nm; the injection volume was 20 μL. Each test needed 20 min.

2.3. Chromatographic system suitability

The system suitability was tested by the following parameters: retention time (t_R, min); peak width at half height ($w_{1/2}$, min) compared with peak height (h, mV); peak asymmetry factor (K_a); resolution (R_s); theoretical plate number (N); relative standard deviation for area of chromatographic peaks (Sr(Area), %) and for retention time (Sr(t_R), %). All parameters were calculated by YL Clarity software (version 3.0.4.444).

2.4. Validation parameters

The validation parameters included specificity of each compound; linearity of the calibration curves (R^2); precision and accuracy. To evaluate linearity, calibration curves were obtained from six solutions with different concentrations of lactic acid and lactide. The calibration solutions were analyzed six times.

The data were fitted to least-squares linear regression of the peak area versus concentration for the range of measured concentrations 104-3286 μg mL^{-1}.

Intra- and inter-day precisions were determined by measuring six replicates of two concentration levels of lactic acid and lactide solution on two consecutive days. The accuracy of the analytical method or recovery was assessed by calculating the percentage recoveries of a known amount of the analyte at two different concentrations. The percentage recovery was determined by comparing the mean concentration value received for each level with the theoretical value.

2.5. Sample preparation

Standard solution of lactic acid and lactide with different concentration were prepared in HPLC grade acetonitrile and bi-distilled water. Samples (about 100 mg) were dissolved in 0.6 mL of HPLC grade acetonitrile and 4.4 mL of bi-distilled water.

3. Results and Discussion

HPLC conditions were investigated by a combination of chromatographic columns, mobile phase, flow rate and column oven temperature in order to obtain narrow and symmetrical peaks and acceptable retention times. In that case, Tracer Excel 120 ODSA (250 mm × 4.6 mm × 5 μm) and Zorbax Eclipse XDB-C18 (250 mm × 4.6 mm × 5 μm) were chosen due to their high efficiency and improved peak shape.

3.1. System suitability

The obtained characteristics of chromatographic system suitability are presented in tab.1.

Table 1 Assessment of system suitability

Compounds	t_R, min	$w_{1/2}$, min	h, mV	K_a	R_s	N	Sr(Area), %	Sr(t_R), %
Lactic acid	6.8	0.13	6.95	1.1	22	18709	1.6	0.6
D,L-lactide	15.9	0.27	12.187	1.3		3129	0.7	0.6

According to the data from the tab. 1, the value of resolution R_s of two adjacent peaks indicates the good separation of respective substances. The asymmetry factor K_a is close in value to 1. The theoretical plate number N is more than 2000 that shows performance and effectiveness of columns. The relative standard deviation for both the area of chromatographic peaks (Sr(Area), %) and the retention time (Sr(t_R), %) was satisfactory as it were less than 2 %.

3.2. Analytical method validation

In order to evaluate the specificity of the method, the chromatograms of lactic acid and lactide were compared. The obtained data provide evidence that the method is considered to be specific because no peaks are overlapping. The linearity of the method was identified between 104 and 3286 μg mL^{-1} with correlation coefficients more than 0.995 %. Response equation and correlation coefficients are presented in the tab. 2.

Table 2 Linearity of HPLC method

Compounds	Concentration range, μg/mL	Response equation y=ax+b	Correlation coefficient, R^2
Lactic acid	104 – 1498	y = 0.381x + 2.1456	0.9989
D,L-lactide	247 – 3286	y = 1.0145x – 86.914	0.9968

The correlation coefficients for the lactic acid is 0.9989, and for the D,L-lactide is 0.9968, indicating an excellent linearity in this range of concentrations (fig. 1).

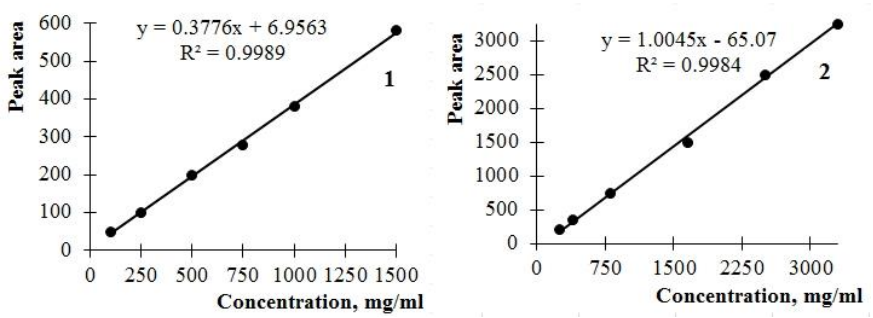

Figure 1. Calibration curves of the peak area versus concentration: 1 – lactic acid; 2 – D,L-lactide

The obtained results of precision are summarized in the tab. 3. For the lactic acid and D,L-lactide the relative standard deviation (RSD) values range from 0.67 to 2.0 and from 0.65 to 1.48, respectively. In both cases, RSD values are less than 2.0 % that indicates the satisfactory precision of the analytical method.

Table 3 Precision of HPLC method

Compound	Intra-day, %	Inter-day, %
Lactic acid	0.67	2.0
D,L-lactide	0.65	1.48

The statistical analysis was expressed as percentage recoveries of known amounts of the analytes, mean values and standard deviations as shown in the tab. 4. It is obvious that the accuracy is within acceptable limits 98.2-99.9 %.

Table 4. Accuracy of HPLC method

Compounds	Amount took, μg/mL	Amount found ± SD, μg/mL	Accuracy, %	RSD, %
Level 1				
Lactic acid	1833	1848±15	99.2	0.9
D,L-lactide	10017	11917±24	99.8	0.1
Level 2				
Lactic acid	917	961±17	98.2	1.3
D,L-lactide	5008	5075±5	99.9	0.2

3.3. Quantitative determination of lactic acid and D,L-lactide

The developed method is universal for analytical control of all stages of the lactide synthesis:

- the lactic acid concentration and oligomerization stage results in lactic acid oligomers, aqueous distillate, and aqueous organic distillate;
- the lactic acid oligomers depolymerization stage results in crude lactide;
- the crude lactide purification stage results in purified (recrystallized) lactide and mother liquor.

In this case, analysis of the purified lactide is performed by the method described above. However, the elution mode changes from isocratic to gradient for other objects of the synthesis. It is necessary in order to wash completely such compounds as lactic acid oligomers from the columns. The conditions of gradient elution are shown in tab.5.

Table 5. Conditions of gradient elution

Retention time, min	Aqueous solution of ortho phosphoric acid,%	Acetonitrile, %
0	88.0	12.0
15.00	88.0	12.0
15.20	0	100.0
20.00	0	100.0
20.20	88.0	12.0

The developed method allows quantifying lactic acid and D,L-lactide and qualifying the lactic acid oligomers that come out of the column as unseparated peaks (fig. 2).

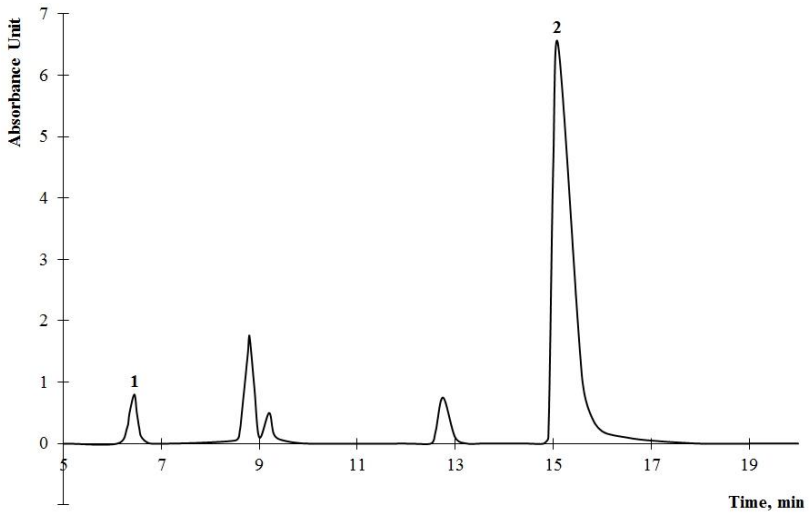

Figure 2. HPLC chromatogram of recrystallized lactide: 1 – lactic acid; 2 – D,L-lactide

The analytical control of the lactide synthesis and its purification was carried out using this method. The objects of the HPLC analysis were the following: four samples of the lactic acid polycondensation stage (LA1-LA4); aqueous distillate obtained from the lactic acid polycondensation stage; a crude lactide; lactide after recrystallization from ethyl acetate; mother liquor. The HPLC analysis results are presented in the tab. 6.

Table 6. Results of HPLC analysis

No.	Sample	Content, % mass.		Presence of oligomers
		Lactic acid	D,L-lactide	
1	LA1	40.8 ± 0.73	-	Present
2	LA2	17.4 ± 0.31	-	Present
3	LA3	3.8 ± 0.07	-	Present
4	LA4	3.8 ± 0.04	-	Present
5	Aqueous distillate	23.3 ± 0.42	-	Present
6	Crude lactide	23.9 ± 0.43	41.0 ± 0.08	Present
7	Recrystallized lactide	6.7 ± 0.12	80.3 ± 0.16	Absent
8	Mother liquor	13.2 ± 0.24	12.1 ± 0.02	Present

These data illustrate the possibility to control the reaction for obtaining the lactic acid oligomer when lactic acid concentration (LA1-LA4) decreasing. It is also observed that the substantial amount of lactic acid (up to 20% of the initial lactic acid) goes out with distillate that reduces the yield of lactide. The determination of the crude lactide composition allows choosing an appropriate way of purification. The results of the HPLC analysis of lactide before and after purification indicate that using recrystallization only allows reducing the lactic acid content by 3 times and increasing the lactide content up to 2 times.

4. Conclusions

The method for the quantitative determination of lactic acid and D,L-lactide by RP-HPLC method was developed for the analytical control of all stages of the lactide synthesis. This method is simple and has specificity and linearity. In addition, all investigations confirm its reproducibility and accuracy. The developed method has been used to control the reaction of obtaining the lactic acid oligomer, lactide, and its purification.

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