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Monitoring of the poly(D,L-lactic acid) degradation by-products by capillary zone electrophoresis

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Abstract

Capillary zone electrophoresis (CZE) is presented as a new tool to resolve and analyze the ultimate degradation products of poly(D,L-lactic acid) aliphatic polyesters, namely water-soluble oligomers with a degree of polymerization lower than 8. The investigated oligomers were those present in a concentrated commercial D,L-lactic acid solution and those obtained by concentration of the same solution under vacuum. The repeatability of peak migration time was increased by introducing a relative migration factor aimed at minimizing the run-to-run peak shifts which depend on the experimental conditions, especially on the capillary and even on the instrument. Ring-opening of D,L-lactide was used to assign CZE peaks due to lactoyllactate and lactate. The rate constant and the activation energy of lactoyllactate hydrolysis were determined by monitoring the formation of lactate by CZE.

1. Introduction

Degradation of degradable and biodegradable polymers is usually monitored through indirect phenomena like oxygen consumption and CO_2 production, in the case of outdoor degradation, and by water absorption, mass loss and molecular mass decreases for both in vivo and outdoor degradations. To obtaining information at the molecular level, size exclusion chromatography (SEC) or high-performance liquid chromatography (HPLC), both in organic solvents or in mixtures of water with organic solvents, are very well suited. Hydrolytic degradation of polymeric biomaterials typically occurs in aqueous media and thus analytical tools compatible with water or polar solvents are required. Generally, hydrolysable polymers, such as aliphatic polyesters, lead to degradation by-products which bear ionic groups at one chain end, at least. Ultimate degradation by-products are thus water-soluble or water-dispersable. This makes high-performance capillary electrophoresis (HPCE) of interest for monitoring the final stage of hydrolytic degradation, and of biomediated degradation as well.

Capillary zone electrophoresis (CZE), which is the simplest HPCE technique, has been successfully used to separate oligomers of natural [1–4] and synthetic [5] origin.

Poly(β-malic acid), a water-soluble degradable polyester of the polycarboxylic type is now well-known as a hydrolytically degradable polymer [6,7]. Oligomer production during in vitro ageing was monitored by aqueous SEC using Biorad P2 gel. However, this technique, which is

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able to separate only the five smallest oligomers is rather time-consuming since it requires several hours. CZE appeared much more efficient as it allowed easy separation of the fifteen smallest oligomers within 15 min [6].

In the case of poly(lactic acids), which are of increasing surgical and pharmacological interest [8], it has been demonstrated that slow release of lactic acid [9] and of lactoyllactic acid [10] reflects the last states of the hydrolytic degradation processes. However, the frontier between water-insoluble and water-soluble oligomers is still unknown. This lack of information originates from analytical difficulties to detect low-molecular-mass compounds especially in aqueous environments, and in the presence of high-molecular-mass polymers.

In the present paper, we report the results of a CZE monitoring of poly (D,L-lactic acid) oligomers which are formed in lactic acid concentrated solutions or during concentration of commercial D,L-lactic acid aqueous solutions under vacuum. Assignment of the ultimate oligomers was done on the basis of D,L-lactide hydrolysis.

2. Experimental

2.1. Materials

Commercial D,L-lactic acid (85% w/w aqueous solutions) and D,L-lactide were obtained from Sigma and Purac respectively and were used without further purification. All other chemicals were of analytical grade.

2.2. Synthesis

Oligo(D,L-lactic acid)

Commercial D,L-lactic acid solution (200 g) was introduced in a round-bottom flask (1000 ml) and heated at 85°C under vacuum for 2 h and then heated at 120°C for 9 h. The reaction medium was kept under argon atmosphere during the experiment.

Sodium D,L-lactoyllactate

D,L-lactide (500 mg) was dispersed in 50 m*M* sodium phosphate buffer adjusted to pH 6.8 (100 ml). As the reaction proceeded, the initial pH value was maintained by adding 1 *M* NaOH. After 3 h at room temperature, no lactide crystals remained in the medium. The buffered solution was then filtered on a 0.22-μm Millipore filter for sterilization. The resulting sodium D,L-lactic-lactoyllactate was stored in solution.

2.3. Methods

CZE was carried out using either 3D-CE (Hewlett-Packard) or a P/ACE 5000 (Beckman) instrument equipped with UV detection at 205 nm and 200 nm, respectively. Fused-silica capillaries of different sizes were filled with running buffer (0.1 *M* sodium borate adjusted to pH 8.9 with 1 *M* NaOH). Before each run, the capillary was rinsed, first for 2 min with 0.1 *M* HCl, second for 2 min with 0.1 *M* NaOH, and third for 1 min with water. Separation was performed at 25°C with an applied voltage of 30 kV. All the lactic acid derivatives ($C \approx 0.3\%$) which were analyzed by CZE, were initially dissolved in 0.05 *M* sodium phosphate buffer adjusted to pH 6.8. About 20 nl were injected in the capillary by the hydrostatic pressure method. Experiments were also performed by using 0.05 *M* sodium phosphate buffer at pH 6.8 and at a voltage of 20 kV with similar rinsing procedure.

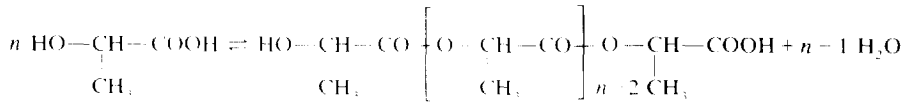
SEC was carried out on 1% tetrahydrofuran (THF) solutions, using a chromatograph (Spectra-Physics SP 8810) equipped with two PL gel columns (50 Å + 100 Å; 5 μm gel beads; 300 × 7.5 mm I.D.). Separation was performed at a flow-rate of 0.6 ml min^{-1}. Refractometric detection was monitored by a Spectra-Physics SP 430 detector.

Potentiometric titration was performed on acid solution [500 mg of lactic acid oligomers dissolved (or dispersed) in 50 ml of pure water] which was neutralized with 1 *M* NaOH using a 665 Dosimat (Metrohm). pH values were recorded at 25.0°C with a TC-III combined electrode (Taccusel) connected to an ionoprocessor II (Taccusel) in order to determine the free acidity in the sample. Back titration was performed with 1 *M* HCl after addition of an excess alkali.

3. Results and discussion

3.1. D,L-lactic acid condensation

It is well-known that concentrated aqueous solutions with more than 80% (w/w) of lactic acid are composed of mixtures of monomer, dimer and higher oligomer species [11]. By heating such a solution under vacuum, it is possible to obtain polycondensates [low-molecular-mass polylactic acid (LMWPLA)] with molecular mass ranging up to a few thousands [12] which exhibit solubilities similar to those of high-molecular-mass poly(D,L-lactide). The most common solvents are then chlorinated hydrocarbons dioxane, THF, acetone. In contrast, water and alcohols are non-solvents.



In order to obtain the very first oligomers, mild polycondensation has been performed. First, the commercial D,L-lactic acid solution was heated for 2 h at a relatively low temperature (85°C) to slowly remove water. Then, the mixture was maintained at 120°C for 9 h. The resulting oligomeric mixture was referred to as oligo(D,L-lactic acid).

The value of the number-average degree of polymerization of oligomers present in the commercial lactic acid solution and of oligo(D,L-lactic acid) were respectively 1.16 and 2.10 as determined by potentiometric titration. This result showed that oligo(D,L-lactic acid) was composed of a very small oligomers.

An aliquot of the lactic acid commercial solution was dissolved in THF and analyzed by SEC in THF. Four peaks were observed (Fig. 1a). However, this chromatographic separation was carried out in an organic medium, the carboxyl groups being non-ionized. Aqueous SEC with a Biorad P2 gel was successfully used for the separation of the sodium salt form of poly(β-malic acid) oligomers [4]. In the case of commercial lactic acid solution, resolution was too poor for proper separation. In contrast, CZE showed four well separated peaks (O1, O2, O3 and O4 in Fig. 2a) within 6 min in 0.1 *M* borate buffer (pH 8.9).

The SEC traces of oligo(D,L-lactic acid) (Fig. 1b) revealed the formation of new oligomers within the heated lactic acid commercial solution, as shown by comparing with traces of Fig. 1a. Oligo(D,L-lactic acid) was soluble in methanol. It was also soluble in water provided the –COOH end groups were ionized by adding sodium hydroxide in such a way that pH values remained under 6.8. Above this value, the oligomers progressively degraded to yield sodium lactate.

Fig. 2b shows the electrophoregram of oligo(D,L-lactic acid). Comparison of Figs. 2a and 2b

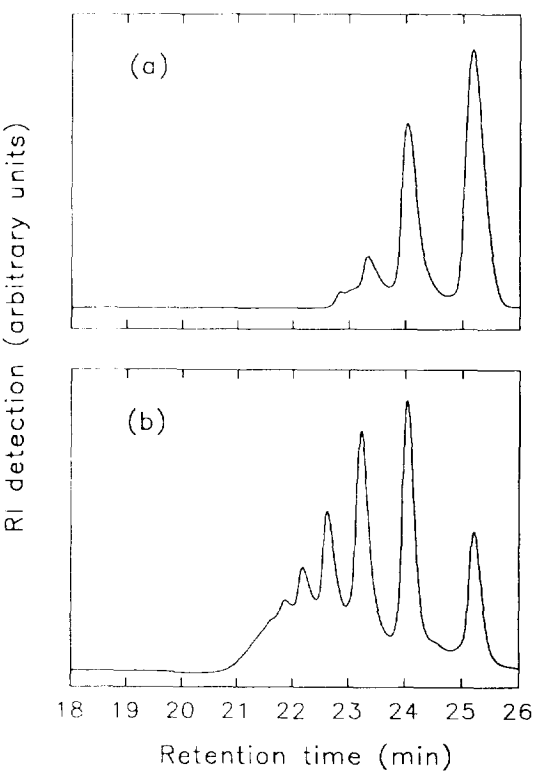


Fig. 1. SEC traces in DMF of commercial D,L-lactic acid solution (a) and of oligo(D,L-lactic acid (b).

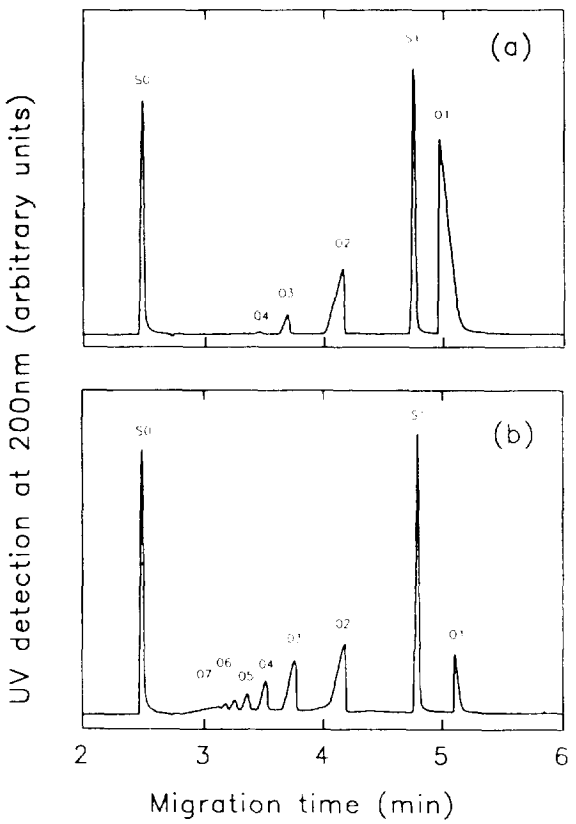


Fig. 2. Electropherograms of the sodium salt forms of commercial D,L-lactic acid solution (a) and of oligo(D,L-lactic acid) (b) [recorded on Beckman instrument with a fused-silica capillary (ϕ_{int} = 75 μm, L_t = 57.0 cm); 0.1 *M* sodium borate buffer at pH 8.9; 25°C; for peak assignment see the text].

showed that O3 and O4 peaks were enlarged. Moreover, O5, O6, O7 peaks were now detected. These peaks can be reasonably assigned to tri-, tetra-, penta-, hexa- and heptamers, as polycondensation leads normally to a continuous distribution of degrees of polymerization.

The relative high pH value of the borate buffer did not affect the stability of oligo(D,L-lactic acid) since the electropherograms recorded by using 0.05 *M* phosphate buffer at pH 6.8 showed also seven peaks comparable to those of Fig. 2b. It must be noted that the running time was higher in the phosphate buffer (~20 min) than in the borate buffer. This longer time was due to the lower voltage (20 kV) imposed by the higher conductivity of the phosphate ions. Therefore, all further analyses were performed in borate buffer.

3.2. Electrophoretic characteristics of CZE peaks

In order to avoid the effects of a progressive deviation of peak migration time after several consecutive runs, two internal standards (S0, benzyl alcohol which reveals the electroosmotic flow [13] and SI, benzoic acid, a negatively charged standard molecule) were added to the tested solutions. The peak of an oligomer i was thus defined by the relative migration factor:

$$RMF_i = (T_i - T_{S0})/(T_{S1} - T_{S0})$$

where T_i, T_{S0} and T_{S1} are the migration time of the oligomer i and those of the added standards, respectively. T_i values were deduced from the middle of the width of the peak at half-height.

The reproducibility of RMF_i values (Table 1) was checked by comparing data obtained from the same instrument equipped with different capillaries and from different instruments whose characteristics are listed in Table 2. When RMF_i values are plotted versus i (Fig. 3), it clearly appears that the capillary electrophoretic separation was limited to oligomers smaller than $i = 8$

Table 1
Electrophoretic characteristics of O_i peaks observed in Fig. 2b

i	RMF_i
1	1.13 ± 0.02
2	0.71 ± 0.02
3	0.54 ± 0.01
4	0.44 ± 0.01
5	0.38 ± 0.01
6	0.34 ± 0.01
7	0.31 ± 0.01

Table 2
Physical characteristics of the differents capillaries used to determine intrinsic parameters of the two internal standards with fresh buffer solutions

Origin	ϕ_{int} (μm)	L_t (cm)	L_d (cm)	I (μA)	t_{EO} (min)	t_{benz} (min)	$\mu_{benz} \cdot 10^4$ (cm^2 V^{-1} s^{-1})
Hewlett-Packard	50	65.0	57.0	29	3.23	6.49	−3.20
Beckman	50	49.0	42.4	38	1.72	3.24	−3.15
Beckman	75	57.0	50.4	74	2.49	4.79	−3.08

ϕ: internal diameter; L_t: total length, L_d: distance between the inlet and the detector; EO: benzyl alcohol; benz: benzoic acid, applied voltage, 30 kV giving intensity I.

since RMF$_i$ values levelled off at 0.3 when the oligomer length increased.

3.3. Kinetics of lactoyllactate hydrolysis

Peaks O1 and O2 in Figs. 2a and 2b were unambiguously assigned to lactate and lactoyllactate respectively from D,L-lactide (I) hydrolysis. The D,L-lactide ring opened in sodium phosphate buffer at pH 6.8. Under this condition, only sodium lactoyllactate (II) was formed as shown by the unique peak observed in Fig. 4a and characterized by a RMF value of 0.71. When the resulting solution was allowed to age, the dimer was progressively transformed into sodium lactate (III).

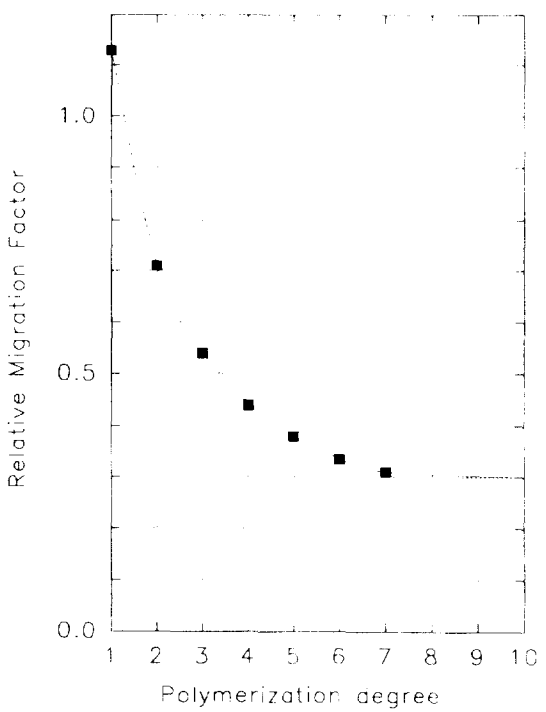


Fig. 3. Plot of relative migration factor (RMF) as a function of the degree of polymerization from data of Table 1.

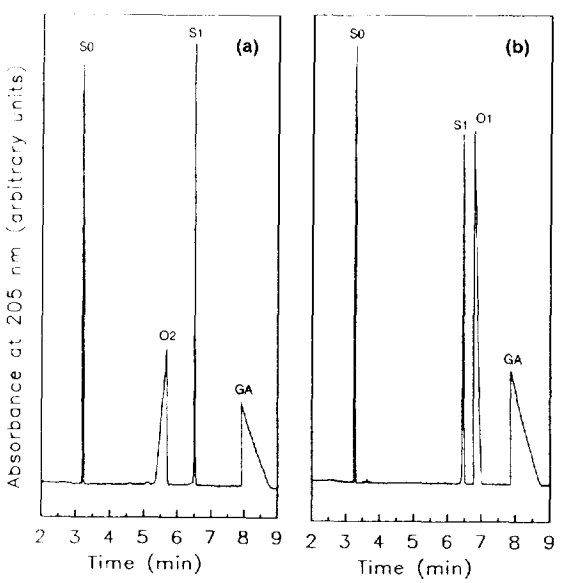
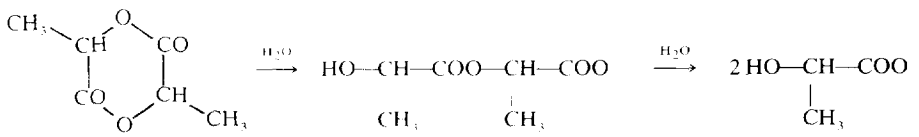


Fig. 4. Electropherograms of sodium D,L-lactoyllactate (a) and of sodium D,L-lactate (b) in the presence of 0.066 M glycolic acid used as internal reference [recorded on Hewlett-Packard instrument with a fused-silica capillary ($\phi_{int} = 50$ μm, $L_t = 65.0$ cm); 0.1 M sodium borate buffer at pH 8.9; 25°C; for peak assignment see the text].



(I) (II) (III)

The peak at RMF = 0.71 progressively disappeared and a second peak (RMF = 1.13) appeared which was assigned to lactate (Fig. 4b).

The relative concentrations of the two species were deduced from the electropherograms recorded for different reaction times in the presence of an internal standard, glycolic acid. This allowed the determination of the kinetics parameters of the reaction:

$$\text{Dimer} + H_2O \rightarrow 2\ \text{Monomer} \quad (1)$$

Let us define the molar concentration $[X]^t$ and the measured area $(A_X)^t$ where X is either the dimer (Di), the monomer (Mono) or the internal standard (GA) and t is the time. These parameters are linked by the relation:

$$(A_X)^t = K\varepsilon_X[X]^t \quad (2)$$

where K is a constant depending on the detector and ε_X the molar extinction coefficient of species X.

According to the conservation law:

$$2[Di]^0 = 2[Di]^t + [Mono]^t = [Mono]^\infty \quad (3)$$

The degree of conversion, $p = [Mono]^t/[Mono]^\infty$ is then:

$$p = [Mono]^t/(2[Di]^t + [Mono]^t) = 1/(1 + 2[Di]^t/[Mono]^t) \quad (4)$$

Experimentally, (A_{Di}), (A_{Mono}) and (A_{GA}) were measured as a function of t. Data allowed calculation of:

$$(\%A_{Mono})^t = 100(A_{Mono})^t/[(A_{Mono})^t + (A_{Di})^t] = 100/[1 + (A_{Di})^t/(A_{Mono})^t] \quad (5)$$

Glycolic acid being an internal standard, one has:

$$(A_{Gly})^0 = (A_{Gly})^\infty \quad (6)$$

If we define:

$$R_{Mono} = (A_{Mono})^\infty/(A_{GA})^\infty \quad (7)$$

$$R_{Di} = (A_{Di})^0/(A_{GA})^0 \quad (8)$$

Eqs. 6, 7, 8 and 3 lead to:

$$R_{Mono}/R_{Di} = (A_{Mono})^\infty/(A_{Di})^0 = (\varepsilon_{Mono}[Mono]^\infty)/(\varepsilon_{Di}[Di]^0) = 2\,\varepsilon_{Mono}/\varepsilon_{Di} \quad (9)$$

and combination of Eqs. 5 and 2 leads to:

$$(A_{Di})^t/(A_{Mono})^t = 100/(\%A_{Mono})^t - 1 = \varepsilon_{Di}[Di]^t/(\varepsilon_{Mono}[Mono]^t)$$

and

$$[Di]^t/[Mono]^t = \varepsilon_{Mono}/\varepsilon_{Di}\ \ (100/(\%A_{Mono})^t - 1) \quad (10)$$

Using Eqs. 9 and 10, Eq. 4 can be written:

$$p = 1/[1 + (R_{Mono}/R_{Di})\ \ (100\%A_{Mono})^t - 1)] \quad (11)$$

Figs. 4a and 4b show respectively the electropherograms which were obtained for $p = 0$ and $p = 1$. From these figures, $R_{Di} = 0.68$ and $R_{Mono} = 0.85$ can be determined. Introducing these values in Eq. 11, one could plot experimental values of the degree of conversion vs. time (Fig. 5). Experimental data obeyed Eq. 12 which is typical of a pseudo first order reaction, water being in large excess:

$$1 - p = e^{-K't} \quad (12)$$

as shown by the good fit between experimental and calculated data (Fig. 5).

Furthermore, data in Fig. 5 were used to show that constant K' obeyed the Arrhenius law $K' = A\,e^{-E/RT}$ with $A = 6.8 \cdot 10^{11}\ h^{-1}$ and $E = 87.4$ kJ.

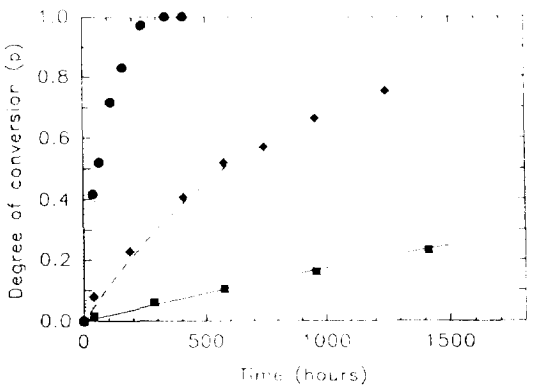


Fig. 5. Degree of conversion of ester bond scission of sodium D,L-lactoyllactate in 0.05 *M* sodium phosphate buffer (pH 6.8) at different temperatures (●: 60°C; ◆: 37°C; ■: 21°C). Data were fitted with $K_{60} = 14.10^{-3}$ h^{-1} (-----), $K_{37} = 12 \cdot 10^{-4}$ h^{-1} (– – –), and $K_{21} = 19 \cdot 10^{-5}$ h^{-1} (———) by using Eq. 12.

4. Conclusions

This study showed that HPCE can easily detect oligomers of D,L-lactic acid in the salt form in aqueous systems. Monomer and dimer peaks were unambiguously assigned. In aqueous media at neutral pH, i.e. when –COOH groups are ionized, oligomers with a degree of polymerization smaller than 8 were detected. Therefore, CZE can be considered as a tool for monitoring the late stages of the hydrolytic degradation of HMWPLA. It is of interest to note that CZE appears to be a helpful complement of SEC in organic media which is used to monitor the early states of HMWPLA degradation.

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