ELSEVIER

Journal of Chromatography A, 688 (1994) 251–259

JOURNAL OF
CHROMATOGRAPHY A

Identification by headspace gas chromatography–mass spectrometry of in vitro degradation products of homo- and copolymers of L- and D,L-lactide and 1,5-dioxepan-2-one

Sigbritt Karlsson, Minna Hakkarainen, Ann-Christine Albertsson*

Department of Polymer Technology, Royal Institute of Technology (KTH), S-100 44 Stockholm, Sweden

First received 1 November 1993; revised manuscript received 2 September 1994

Abstract

Poly(L-lactide) (PLLA), poly(D,L-lactide) (PDLLA), poly(1,5-dioxepan-2-one) (PDXO) and six different types of copolymers of L-LA–DXO and D,L-LA–DXO (80:20, 50:50 and 20:80) were hydrolysed in vitro in phosphate buffer (pH 7.4) at 37°C for periods of up to 20 months and the degradation products were identified by headspace gas chromatography–ion trap mass spectrometry. A suitable method to identify the decarboxylic acids and hydroxy acids formed was developed using derivatization with N-*tert.*-butyldimethylsilyl-N-methyltrifluoroacetamide reagent. The derivatives gave a single chromatographic peak with no tailing in the mass spectra. The main products identified in the in vitro buffers are lactic acid and 2-hydroxyethoxypropionic acid, which are the linear forms of the monomers D,L- or L-lactide and 1,5-dioxepan-2-one. The amount of degradation products formed depends on the copolymer composition and the degradation time.

1. Introduction

The detection and identification of low-molecular-mass compounds formed in aged inert and degradable polymers are means of determining the interaction of polymers with the environment. By headspace gas chromatography–mass spectrometric Hs-GC–MS, GC–MS and LC techniques, different natural and synthetic polymers have been studied. Natural polyhydroxyalkanoates are interesting polymeric materials for application in packaging and in biomedical materials and considerable research efforts are being made to explore their characteristics and long-term properties mainly due to their source, a renewable carbon source in contrast to the traditional oil-based bulk polymers. Alkaline hydrolysis products of poly(β-hydroxybutyrate) (PHB) and poly(β-hydroxybutyrate–co-β-hydroxyvalerate) (PHB/PHV) have been detected using LC [1]. Monomers, oligomers and some of their derivatives, mainly produced by dehydration at the OH terminus, were present in the hydrolysed samples. In thermally oxidized PHB samples the amount of crotonic acid formed increases as the molecular mass decreases [2].

In another study of thermally oxidized inert low-density polyethylene (LDPE), we identified hydrocarbons, ketones, aldehydes and alcohols in various amounts and patterns [3]. Degradable polymers using PE as the matrix and different additives promoting degradation are also materi-

* Corresponding author.

0021-9673/94/$07.00 © 1994 Elsevier Science B.V. All rights reserved
SSDI 0021-9673(94)00859-O

als under study in our department [4]. LDPE with corn starch and a pro-oxidant is one formulation which gives an increased susceptibility to photo-oxidation and thermolysis and also to biodegradation. Using GC–MS we have analysed the subsequent degradation products formed after photo-oxidation, thermolysis and biodegradation of the samples [5–7]. Inert LDPE was degraded in an accelerated environment (water at 95°C), resulting in the formation of 2-butanol, propionic acid, 1-pentanol, butyric acid, valeric acid, caproic acid, *n*-octane, *n*-nonane, *n*-decane, *n*-dodecane, *n*-tridecane and *n*-tetradecane as monitored by GC–MS [8]. Similar types of compounds (butane, ethanol, pentane, formic acid, butanal, 2-butanone, 1-butanol, 3-hexanone and higher alkanes) were also identified in LDPE containing starch filler [5].

Environmental interaction was also demonstrated in connection with the "sick building" syndrome, where we discovered a problem experienced in connection with the use of self-levelling floor-covering material [9]. The bad odour was related to emission of volatile mono-, di- and polyamines formed during putrefactive degradation of casein, a natural polymer present in floor covering material [10]. GC and LC techniques were adapted to this special application, resulting in detection of ppm–ppb levels of amines and organic acids in the degraded casein-containing concrete [9–11].

Many synthetic polymers used inside the body are subject to biodegradation (generally a simple hydrolysis reaction). In many instances these polymers are deliberately designed to be degradable to meet specific end-uses. Aliphatic polyesters are the largest group of biodegradable polymers, and poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) are the most widely studied of these synthetic biodegradable polymers. The PLAs have been investigated for controlled-release devices [12], degradable sutures [13], absorbable fibres [13] and implants for bone fixation [14]. There is also increasing interest in using PLA for disposable packages and table-ware and techniques are under development to produce PLA from renewable resources.

High-molecular-mass PLA is normally obtained by ring-opening polymerization of the lactide monomer. The polymer stereochemistry is controlled by the original lactide stereochemistry. The resulting stereopolymer has widely different physical characteristics, e.g. poly(L-lactide) (PLLA) is a semicrystalline polymer with a glass transition temperature (T_g) of 67°C, while poly (DL-lactide) (PDLLA) is amorphous (T_g = 58°C). High-molecular-mass copolymers of glycolic and lactic acid have T_g values above body temperature [15] and usually a crystalline character with little elasticity.

Copolymerization of lactide with monomers with different values of T_g and crystallinity creates opportunities for designing polymers with widely different properties. One interesting monomer is 1,5-dioxepan-2-one, synthesized by Albertsson and co-workers [16,17] in ongoing studies focused round the design of new monomers used in ring-opening polymerization for creating degradable biomedical materials. 1,5-Dioxepan-2-one (DXO) copolymerized with lactide yields materials with an amorphous character and interesting properties for new degradable biomedical polymers [18].

The aliphatic polyesters all belong to a group with hydrolytically unstable linear aliphatic ester bonds which is very useful for in vivo degradation in the body. From the literature it is difficult to determine the precise mechanism of degradation of polyesters in the body. It has been proposed that chain scission occurs through simple hydrolysis, although the presence of cations or anions and also enzymes may influence the kinetics [19]. The kinetics of chain scission are indicative of an autocatalytic process in which the carboxylic acid and groups generated by ester hydrolysis participate in the transition state. Water preferentially penetrates the amorphous parts, but crystalline domains are also affected [20].

The increased use of polyesters in a variety of implant devices and drug-release systems has resulted in the need to develop analytical methods to identify the degradation products. All implants cause a foreign body reaction due to the surgical trauma and most often also due to differences in mechanical properties in comparison with the tissue surrounding the implant. For

degradable polymers, especially those with short service times, leakage of degradation products may be significant and contribute to the local tissue response at the implantation site.

The objective of this work was to find suitable methods to identify low-molecular-mass compounds formed by hydrolysis of new degradable biomedical polymers. Degradable homopolymers of 1,5-dioxepan-2-one (DXO), L-dilactide, D,L-dilactide and DXO–LLA and DXO–DLLA copolymers were made by ring-opening polymerization and subsequently hydrolysed in vitro. The degradation products were identified by Hs-GC with detection by ion trap mass spectrometry (ITD-MS). Molecular mass changes were monitored by size-exclusion chromatography (SEC).

2. Experimental

2.1. Polymerization and copolymer compositions

Simplified reactions for the formation and subsequent hydrolysis of (a) PDXO, (b) PLA and (c) the hydrolysis of LA–DXO copolymer are shown in Fig. 1. PLLA, PDLLA, PDXO and three different compositions of both L-LA–DXO and D,L-LA–DXO copolymers were hydrolysed in vitro. The copolymer compositions (molar ratios) were 80:20, 50:50 and 20:80 LA:DXO in both instances. All the polymers were synthesized by ring-opening polymerization. The detailed synthesis and characterization of these polymers have been described previously [21,22].

2.2. In vitro degradation

In vitro degradation was performed in phosphate buffer at 37°C and pH 7.4 using melt-pressed films of 0.5 mm thickness. Circular discs with a diameter of 13 mm were punched from the films. The discs were immersed in serum bottles with 20 ml of phosphate buffer consisting of 7.57 g of Na_2HPO_4, 1.61 g of NaH_2PO_4 and 4.4 g of NaCl in 1000 ml of water. The buffer was filtered (0.46 μm) before use. Aliquots were withdrawn after different periods of time and subsequently analysed.

2.3. Solvent extraction and TBDMS derivatization

MTBSTFA (N - *tert.* - butyldimethylsilyl - N-methyltrifluoroacetamide) (98%; Aldrich), isooctane (99%; Aldrich), diethyl ether (99.9%;

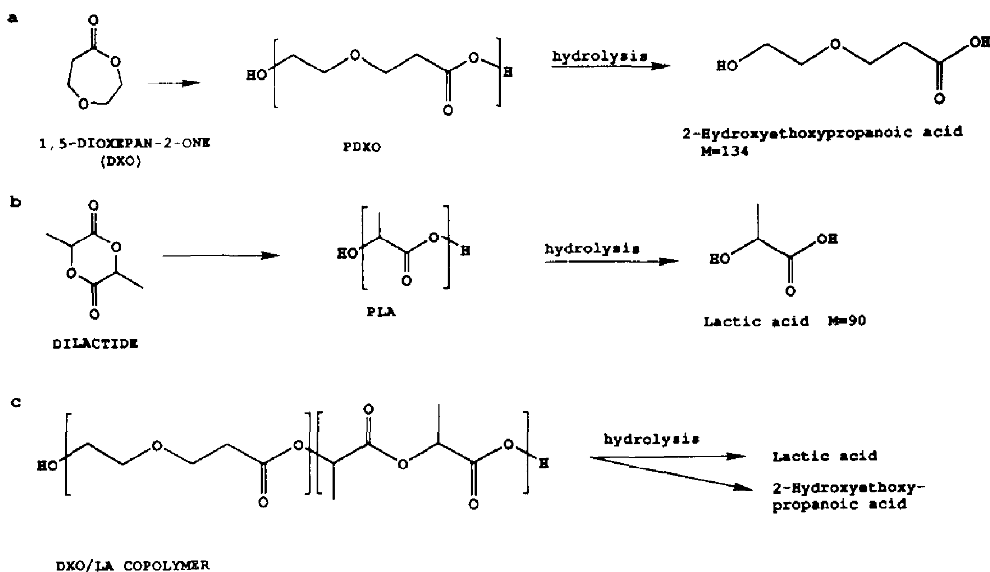

Fig. 1. Reactions for formation of aliphatic polyesters and tentative hydrolysis. (a) PLA; (b) PDXO; (c) PDXO–PLA.

Merck) and chloroform (99.0–99.4%, Riedel-de Haën) were used as received. Acidified (pH 2–3) in vitro buffer solutions were extracted three times with diethyl ether (analytical-reagent grade). The extracts were combined and evaporated with a stream of nitrogen. The derivatization reactions were carried out in 4-ml glass vials with PTFE-lined plastic screw-caps. MTBSTFA was added to the dry residue from solvent extraction and the solution was diluted with 0.1 ml of isooctane. When too little reagent was used, one of the reagent peaks was missing in the chromatograms and the unreacted acid was retained in the column. By injecting mixture of isooctane and MTBSTFA it was possible to elute the retained acids. Therefore, the influence of the amount of reagent and the reaction temperature and time were studied and optimum conditions were found to be 20 μl of MTBSTFA with reaction at 60°C for 30 min. After derivatization the samples were stored in a refrigerator or injected directly into the GC system. Fig. 2 shows the formation of *tert.*-butyldimethylsilyl (TBDMS) derivatives of acids and alcohols with MTBSTFA reagent.

2.4. Gas chromatography–mass spectrometry

The gas chromatograph used was a Perkin-Elmer Model 8500 with a split–splitless injector. It was connected to a Perkin-Elmer ITD mass spectrometer with the ITD 4.10 data handling program (Finnegan). The gas chromatograph was equipped with a WCOT fused-silica CP-Sil-43 CB or CP-Sil-19 CB capillary column from Chrompack (25 m × 0.32 mm I.D.). Helium was used as the carrier gas. Samples were introduced in the splitless injection mode at 250°C. The oven temperature was initially held at 40°C for 1 min, programmed to 200°C at 10°C/min and then held at 200°C for 8 min. The operating conditions for ITD are given in Table 1. All TBDMS derivatives were separated in the CP-Sil-19 CB column.

Table 1
Operating conditions for ITD

Mass range	20–500
Seconds per scan	1.0
Multiplier voltage	1600 V
Transfer temperature	250°C
Peak threshold	1
Mass defect	30 mmu/100 amu
Scan mode	Full

2.5. Headspace gas chromatography–mass spectrometry

The above equipment was connected to a Perkin-Elmer HS 101 headspace autosampler and a WCOT fused-silica CP-Sil-19 CB column from Chrompack (25 m × 0.32 mm I.D.). Samples were thermostated at 90°C for 12 min. The oven temperature was held at 40°C for 1 min, then programmed to 200°C at 10°C/min and held

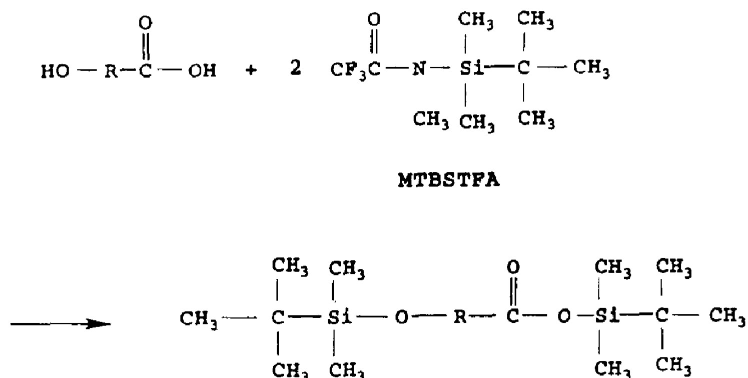

Fig. 2. Derivatization of acids and alcohols with MTBSTFA.

there for 8 min. The operating conditions for ITD are given in Table 1.

2.6. SEC analysis

SEC was used for monitoring molecular mass changes in the residual polymer. The measurements were made at 30°C with five μStyragel columns (10^5, 10^4, 5000, 1000, 100 Å). A Waters Model 510 instrument Waters Model 410 with a refractive index detector and tetrahydrofuran (THF) as solvent at a flow rate of 1.0 ml/min was used. Polystyrene standards were used for calibration.

3. Results and discussion

3.1. Development of analysis

Two different type of columns were tested (CP-Sil-43 CB and CP-Sil-19 CB) in the Hs-GC–MS analysis. The capacity to separate polar compounds was higher with the latter column. Using the CP-Sil-43 CB column no degradation products were detected in the extract from the in vitro solutions. CP-Sil-19 CB was better but part of the polar compounds were retained in the columns and peak tailing was observed for the remaining compounds. It can also be expected that underivatized compounds of low concentrations would be absorbed in the injection port line or in the column. Aliquots from the in vitro solutions were therefore derivatized in order to improve the separation and to avoid retention in the GC system.

N-*tert.*-Butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) was chosen because it can be applied to all components with proton donor capacity (e.g., decarboxylic acids and hydroxy acids) and the subsequent *tert.*-butyldimethylsilyl (TBDMS) derivatives have high hydrolytic stability and superior GC and MS properties [23–25].

A single chromatographic peak with no tailing was usually obtained for TBDMS derivatives separated in the CP-Sil-19 CB column. It is typical of mass spectra of TBDMS derivatives that the molecular ions and $[M\text{-}15]^+$ ions are absent or of low intensity. The characteristic $[M\text{-}57]^+$ ions, due to the loss of a *tert.*-butyl group, are, however, very intense, and thus permitted the easy identification of acids. The ion at m/z 73 corresponding to $(CH3)_3Si^+$ is of high intensity, as is expected for the bis-TBDMS derivatives of dicarboxylic acids and hydroxy acids. Other prominent ions were observed at m/z 75, 115, 147 and 189, corresponding to $HO^+=Si(CH_3)_2$, $[(CH_3)_3C](CH_3)_2Si^+$, $(CH_3)_3SiO^+{=}Si(CH_3)_2$ and $[(CH_3)_3C](CH_3)_2SiO^+=Si(CH_3)_2$, respectively. All the derivatives are stable for at least 3 months, as shown when freshly derivatized standards were compared with those kept in a refrigerator for that time. GC and MS data are summarized in Table 2.

3.2. In vitro degradation

Immediately after placing the amorphous PLA–PDXO polymer in the in vitro phosphate buffer, absorption of water could be observed as the transparent appearance of the samples changed to opaque. PLA–PDXO (20:80) also changed its shape, whereas PLA–PDXO (80:20) showed a smaller water uptake and very small changes in dimension, which can be attributed to the higher crystallinity of this copolymer.

Fig. 3 shows the Hs-GC–MS of derivatized degradation products of PDXO hydrolysed for three different periods of time. Prolonged degradation leads to a continuous increase in the amount 2-hydroxyethoxypropanoic acid formed. The monomer DXO was also identified in the in vitro solutions of PDXO. The amount of DXO is, however, the same irrespective of the degradation time and hence this DXO is a residual monomer originating from the polymerization. For biomedical application the amount of residual compounds must be minimized by careful purification steps, otherwise the in vivo behaviour will be affected. In the chromatograms unidentified peaks also appear, and the amounts of these products tend to increase with the exposure time, which is an indication that these are degradation products of the polymer, or

Table 2
Retention times and prominent ions from the mass spectra of *tert*.-butyldimethyl (TDMS) derivatives of lactic acid, dimer of lactic acid and 2-hydroxyethoxypropanoic acid.

Compound	Retention time (min)	m/z	Relative intensity (%)	Fragment
Lactic acid	12.4	318		M
	(43 CB)[a]	303	5	M - 15
		261	72	M - 57
		233	71	
		189	38	
		147	99	
		133	24	
		75	22	
		73	100	
Dimer of lactic acid	17.5	380		M
	(43 CB)[a]	375	10	M - 15
		333	100	M - 57
		285	51	
		261	69	
		159	83	
		103	68	
		75	43	
		73	83	
2-Hydroxy-ethoxy-propanoic acid	18.0	362		M
	(43 CB)[a]	347	8	M - 15
		305	92	M - 57
	17.4	187	60	
	(19 CB)[a]	159	40	
		103	48	
		99	41	
		89	78	
		75	21	
		73	100	

[a] CP-Sil-43 CB or CP-Sil-19 CB column.

products of further reaction of the initial degradation products.

Fig. 4 shows GC-MS analyses of PLLA and LLA-DXO copolymers hydrolysed for 20 months. Lactic acid and 2-hydroxyethoxypropanoic acid were identified. In the 50:50 composition the dilactide was also identified, which is the dimer of the lactic acid.

The molecular mass decrease of the samples starts as soon as they are incubated in the in vitro solution [26]. Table 3 gives the initial and final molecular masses (M_r) after 20 months for

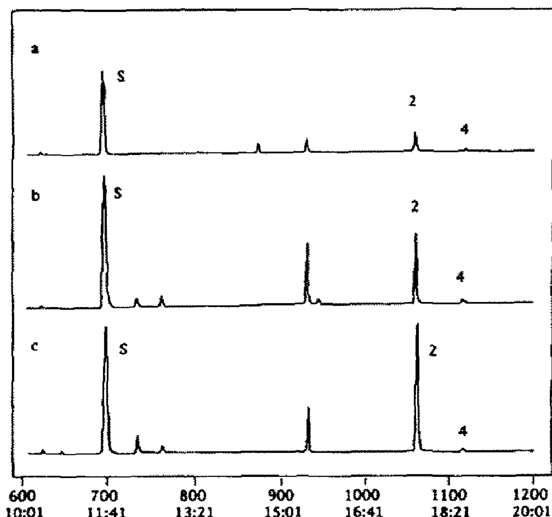

Fig. 3. Hs-GC-MS analysis of degradation products of PDXO. Polymers were hydrolysed in phosphate buffer (pH 7.4) at 37°C for (a) 7, (b) 13 and (c) 27 weeks. S = system peak; TBDMS derivatives of (2) 2-hydroxyethoxypropanoic acid and (4) DXO. Upper scale: scan; lower scale: time in min:s.

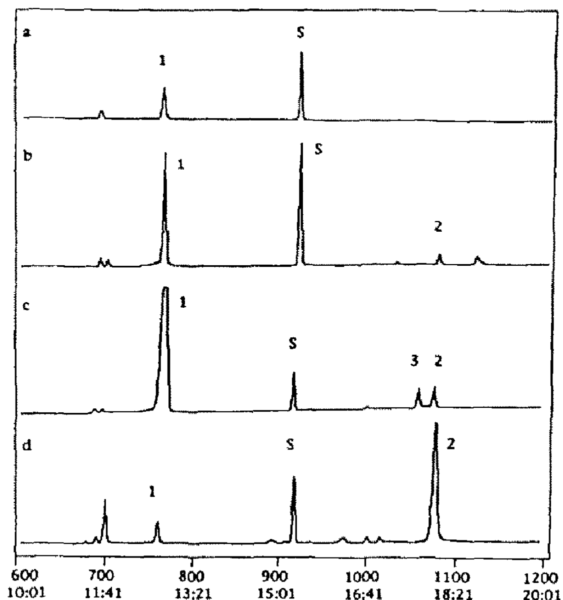

Fig. 4. GC-MS of degradation products of (a) PLLA and (b)-(d) L-LA-DXO copolymers with molar ratios (b) 80:20, (c) 50:50 and (d) 20:80. Samples were hydrolysed in phosphate buffer (pH 7.4) at 37°C for 20 months. S = system peak; TBDMS derivatives of (1) lactic acid, (2) 2-hydroxyethoxypropanoic acid and (3) dilactide. Upper scale: scan; lower scale: time in min:s.

Table 3
Molecular masses (M_r) of copolymers of (D), L-LA and DXO before and after 20 months of hydrolysis in vitro

Copolymer	Composition	M_r (g/mol)	
		Initial	Final
PLLA–PDXO	80:20	68 000	7 000
	50:50	70 500	13 000
	20:80	76 000	23 000
PDLLA–PDXO	80:20	68 100	7 000
	50:50	66 700	11 000
	20:80	70 300	20 000

the PLLA–PDXO and PDLLA–PDXO copolymers as obtained by SEC analysis. The amount of L-LA influences the molecular mass changes; thus the molecular mass of the 80:20 PLLA–PDXO decreased from 68 000 to 7000 after exposure for 20 months whereas that of the 20:80 copolymer decreased from 76 000 to 23 000 during the same period. Many aliphatic polyester show a phenomenon where the initial degradation is slow, indicating a bulk hydrolysis where the induction period is associated with a lack of significant mass loss. This induction period has been observed to range from 5 to 9 weeks depending on the composition of the copolymer [18]. This mass loss behaviour occurs simultaneously with a broadening of the molecular mass distribution (MMD). This can be explained by an initial chain cleavage after water absorption, which produces shorter chains and broadens the MMD. When the molecular masses of the chain are sufficiently small, the oligomers can diffuse through the bulk and dissolve, causing mass loss.

Fig. 5 shows GC–MS of hydrolysed PDLLA and the copolymer of DLLA with DXO. The same type of products could be identified as in Fig. 4, although the absence of a dilactide (the dimer) for the 50:50 DLLA–DXO copolymer must be noted.

The same type of behaviour with maximum lactic acid formed in the 50:50 copolymer can be observed for both PLLA–PDXO and PDLLA–PDXO (Figs. 4c and 5c). The molecular mass changes in the PDLLA–PDXO are generally

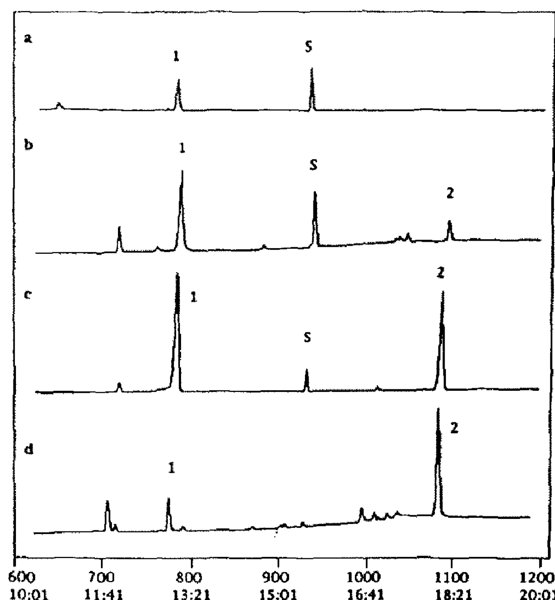

Fig. 5. GC–MS of degradation products of (a) PDLLA and (b)–(d) D,L-LA–DXO with molar ratios of (b) 80:20, (c) 50:50 and (d) 20:80. Samples were hydrolysed in phosphate buffer (pH 7.4) at 37°C for 20 months. S = system peak; TBDMS derivatives of (1) lactic acid and (2) 2-hydroxyethoxypropanoic acid. Upper scale: scan; lower scale: time in min:s.

larger than those observed in the PLLA–PDXO samples, although the same induction period was experienced [26]. Mass spectra for the lactic acid, dilactic acid and 2-hydroxyethoxypropanoic acid are shown in Fig. 6.

The pH of the buffer solutions was monitored continuously and a constant pH was observed during the first 2 weeks in all samples. The rapidly degrading lactide-rich copolymers produced acidic degradation products, in excess of the buffer capacity, and lowered the pH to 6.4 for the 80:20 PDLLA–PDXO, which is the largest pH change observed. The pH change correlates well with the molecular mass changes and the total amount of degradation products in the most degraded D,L-LA-rich copolymer.

The higher the content of lactic acid units in the copolymer, the faster is the degradation. Pitt and Gu [27] investigated the kinetics of the hydrolysis and derived two equations based on uncatalysed and autocatalysed hydrolysis. Albertsson and Löfgren [18] showed that the co-

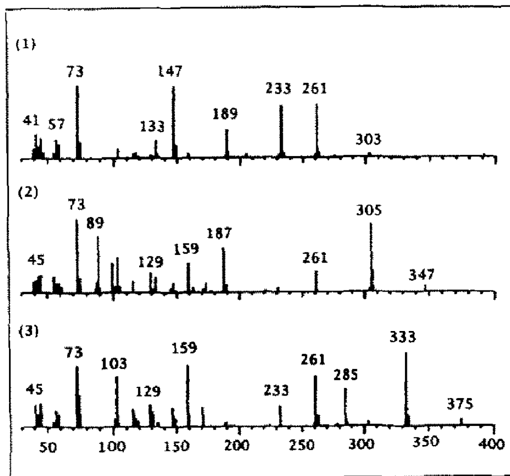

Fig. 6. Mass spectra of TBDMS derivatives of (1) lactic acid, (2) 2-hydroxyethoxypropanoic acid and (3) dilactic acid. Scale: *m/z*.

polymers with a high content of L-LA have almost identical fits to both models, whereas for the 50:50 and 20:80 PLLA-PDXO and PDLLA-PDXO the uncatalysed mechanism fits slightly better. Fig. 7 shows the hydrolysis mechanism for the two copolymers. The degradation products of the copolymers as identified by GC–MS are consistent with the ester hydrolysis mechanism, although deviations were observed in the chromatograms.

The in vivo reaction to biomedical implants is a major issue determining the usability of the materials. A significant difference in tissue response has been demonstrated between the semicrystalline L-LA–DXO copolymer and the amorphous D,L-LA–DXO copolymer. The amorphous D,L-LA–DXO (77:23) degraded at a faster rate, causing a less pronounced foreign body reaction than the corresponding L-LA–DXO (80:20) [28]. This investigation provides a means to predict the long-term properties of the degradable biomedical homo- and copolymers as manifested by the type of degradation products formed and a starting point for the discussion of in vivo reactions to new biomedical degradable polyesters.

4. Conclusions

Hs-GC–MS and GC–MS techniques were developed that allow the identification of hydrolysis products of new degradable biomedical aliphatic polyesters. By Hs-GC–MS, N-*tert*.-

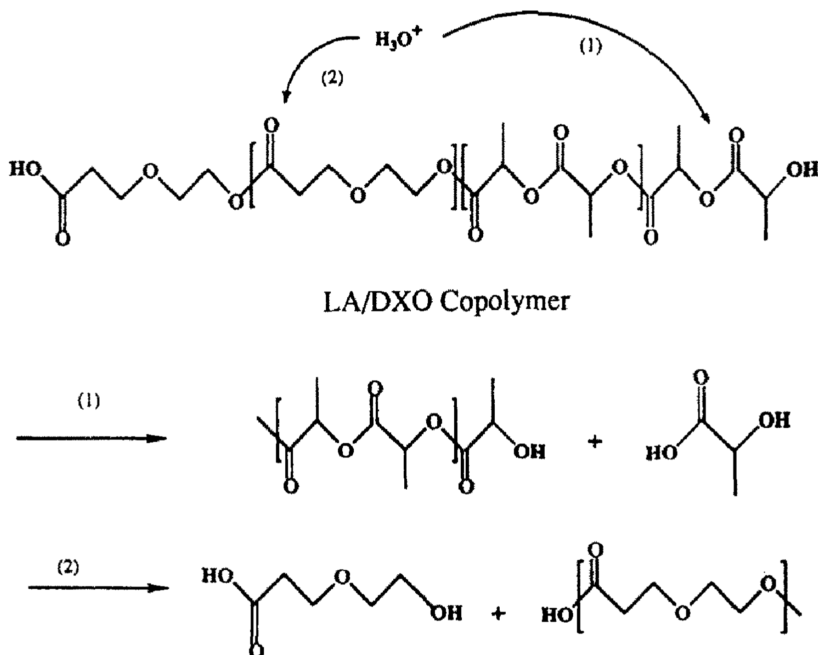

Fig. 7. Hydrolysis mechanism of PLLA–PDXO copolymer.

butyldimethylsilyl-N-methyltrifluoroacetamide derivatives were demonstrated to give a single chromatographic peak with no tailing when separated in a CP-Sil-19 CB column. This method proved to be suitable for the separation and identification of the highly polar degradation products of homo- and copolymers of L- or D,L-lactide and 1,5-dioxepan-2-one. The main products formed in amounts depending on composition and degradation time were lactic acid and 2-hydroxyethoxypropionic acid. We could also demonstrate a higher amount of lactic acid formed in the 50:50 copolymers of PLLA–PDXO and PDLLA–PDXO than in the 80:20 copolymers.

Acknowledgement

Financial support by NorFa is gratefully acknowledged.

References

[1] S. Karlsson, C. Sares and A.-C. Albertsson, in H.G. Schlegel and A. Steinbüchel (Editors), *International Symposiun on Bacterial Polyhydroxyalkanoates—ISBB '92*, Goltze-Druck, Göttingen, 1993, p. 455.
[2] S. Karlsson, C. Sares, R. Renstad and A.-C. Albertsson, *J. Chromatogr. A*, 669 (1994) 97.
[3] A.-C. Albertsson and S. Karlsson, in G. Glass and G. Swift (Editors), *Agricultural and Synthetic Polymers* (*ACS Symposium Series*, No. 433), American Chemical Society, Washington, DC, 1990, p. 60.
[4] A.-C. Albertsson and S. Karlsson, *Makromol. Chem., Makromol. Symp.*, 48/49 (1991) 395.
[5] A.-C. Albertsson, C. Barenstedt and S. Karlsson, *Polym. Degrad. Stab.*, 37 (1992) 163.
[6] A.-C. Albertsson, C. Barenstedt and S. Karlsson, *J. Appl. Polym. Sci.*, 51 (1994) 1097.
[7] A.-C. Albertsson, C. Barenstedt and S. Karlsson, *Acta Polym.*, 45 (1994) 97.
[8] A.-C. Albertsson and S. Karlsson, *Polym. Mater. Sci. Eng.*, 62 (1990) 976.
[9] S. Karlsson and A.-C. Albertsson, *Mater. Struct.*, 23 (1990) 352.
[10] S. Karlsson, Z.G. Banhidi and A.-C. Albertsson, *J. Chromatogr.*, 442 (1988) 267.
[11] S. Karlsson, Z.G. Banhidi and A.-C. Albertsson, *Mater. Struct.*, 22 (1989) 163.
[12] J. Heller, *Biomaterials*, 1 (1980) 51.
[13] J.P. Pennings, H. Dijkstra and A. Pennings, *Polymer*, 34 (1993) 942.
[14] M. Vert, P. Christel, F. Chabot and J. Leray, in G.W. Hastings and P. Ducheyne (Editors), *Macromolecular Biomaterials*, CRC Press, Boca Raton, FL, 1984, p. 120.
[15] H. Fukuzaki, M. Yoshida, M. Asano and M. Kumakura, *Eur. Polym. J.*, 25 (1989) 1019.
[16] T. Mathisen, K. Masus and A.-C. Albertsson, *Macromolecules*, 22 (1989) 3842.
[17] A.-C. Albertsson and R. Palmgren, *J. Macromol. Sci., Pure Appl. Chem.*, A30 (1993) 919.
[18] A.-C. Albertsson and A. Löfgren, *J. Appl. Polym. Sci.*, 52 (1994) 1327.
[19] T.G. Park, S. Cohen and R. Langer, *Macromolecules*, 25 (1992) 116.
[20] T. Mathisen and A.-C. Albertsson, *J. Appl. Polym. Sci.*, 39 (1990) 591.
[21] S. Lundmark, M. Sjöling and A.-C. Albertsson, *J. Macromol. Sci. Chem.*, A28 (1991) 15.
[22] A.-C. Albertsson and A. Löfgren, *Makromol. Chem., Makromol. Symp.*, 53 (1986) 221.
[23] K.R. Kim, M.K. Hahn, A. Zlatkis, E.C. Horning and B.S. Middleditch, *J. Chromatogr.*, 468 (1989) 289.
[24] Y. Ghoos, B. Geypens, M. Hiele, P. Rutgeerts and G. Vantrappen, *Anal. Chim. Acta*, 247 (1991) 223.
[25] S. Steffenrud, P. Borgeat, H. Salari, M.J. Evans and M.J. Bertrand, *J. Chromatogr.*, 416 (1987) 219.
[26] A.-C. Albertsson and A. Löfgren, *J. Macromol. Sci., Pure Appl. Chem.*, A32 (1995) 41.
[27] C.G. Pitt and Z. Gu, *J. Controlled Release*, 4 (1987) 283.
[28] A. Löfgren, A.-C. Albertsson, Y. Zhang and L.-M. Bjursten, *J. Biomater. Sci., Polym. Ed.*, 6 (1994) 411.