Downloaded from UvA-DARE, the institutional repository of the University of Amsterdam (UvA)
http://dare.uva.nl/document/122451

File ID	122451
Filename	4: Hydrolytic degradation of poly(D,L-lactide-co-glycolide 50/50)-diacrylate network as studied by liquid chromatography–mass spectrometry
Version	Final published version (publisher's pdf)

SOURCE (OR PART OF THE FOLLOWING SOURCE):

Type	Dissertation
Title	Characterisation of polymeric network structures
Author	R.A.H. Peters
Faculty	Faculty of Science
Year	2009
Pages	214
ISBN	9789052787831

FULL BIBLIOGRAPHIC DETAILS:
http://dare.uva.nl/record/292197

Copyright

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use.

UvA-DARE is a service provided by the library of the University of Amsterdam (http://dare.uva.nl)

4 Hydrolytic degradation of poly(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate network as studied by liquid chromatography–mass spectrometry

Abstract

The soluble products of the hydrolytic degradation of photo-chemically cross-linked poly-(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate film are analysed at different stages to obtain insight into the complex (bio)degradation processes. Liquid chromatography–mass spectrometry analyses have been employed to identify and quantify the various oligomeric and polymeric degradation products from the soluble fraction. The products are analysed directly after release and also after complete hydrolysis of the soluble fraction. The study shows a rapid release of residual photo-initiator followed by a gradual release of lactide/di-ethyleneglycol/glycolide oligomers with varying composition and chain length. The final stage of the sigmoidal weight loss profile reflects the release of polyacrylate chains with lactide/glycolide side chains. The molecular weights of the polyacrylate chains released increase with degradation time, which indicates that the release of these polyacrylate chains is determined by the number and type of ester-groups that must be degraded hydrolytically to dissolve these chains. The analysis of the soluble degradation products provides detailed insights in the chemical changes at the different stages of degradation; extraction, network attack, network penetration, bulk degradation, and finally release of persistent network fragments. Chromatographic and mass spectrometric techniques prove to be powerful tools to enhance the understanding of the hydrolytic degradation process of chemically cross-linked acrylates.

R. Peters, J. Lebouille, B. Plum, V. Litvinov, A.A. Dias, P. Schoenmakers, Sj. van der Wal, to be submitted.

4.1. Introduction

Biodegradable polymers are increasingly exploited as medical materials, for applications including controlled drug delivery and tissue engineering [1–3]. These biodegradable polymers consist mostly of polyglycolic acid, polylactic acid, poly-2-hydroxy butyrate, polyhyaluronic acid, polycaprolactone, and their copolymers, since polymers with these building blocks have acceptable toxicological profiles [4,5]. Aliphatic polyesters are hydrophobic, which is the main drawback of the use of these polymers as carriers for hydrophilic drugs such as proteins [6,7]. Therefore, these biodegradable aliphatic polyesters are modified with amides, anhydrides, urethanes, imides, ethers or other functional groups, which offer opportunities to tune the biomechanical properties of these polymers within a broad range of desired properties [8,9]. The use of biocompatible polyethylene glycol (PEG) as hydrophilic building block in polylactide (PLA) or poly(lactide-*co*-glycolide) polymers enhances their biocompatibility. The resulting materials are good candidates for controlled drug-delivery systems [10–13].

The biodegradable PLA-type materials that are used for controlled release of bioactive agents and for the encapsulation of cells or biomolecules are mainly linear polymers, which are physically cross-linked by solvent or salt casting. An emerging type of synthetic biomaterials for medical applications is based on photo-cured chemically cross-linked PLA and PEG containing systems [14]. These chemically cross-linked materials have promising applications, ranging from tissue engineering materials, drugs delivery systems to (in situ) orthopaedic implant materials [15–18]. Drugs-delivery is mainly based on the systematic degradation of the ester units in aqueous media [19]. However, the (stereoregularity) composition, the molecular weight, the block length, the shape of the specimen, the morphology, the presence of additives, and the medium all influence the degradation [20,21]. The main advantage of chemically cross-linked systems over physically cross-linked systems is the possible controlled of the degradation rate through the type, ratio and organisation of the building blocks, as well as through the cross-link density. To tailor these chemically cross-linked systems to a given biomedical application so as to obtain better control of the degradation characteristics as well the understanding of the biological influence of the intermediates and the final end products, an in-depth understanding of the biological influence of the intermediates and the final end products and of the chemical changes during the biodegradation process is required.

Several approaches can be followed to study the biodegradation of PLA-type

polymers and the influence of various factors on the degradation process. The degradation of both chemically and physically cross-linked networks can be followed easily by average macroscopic changes, such as the pH of the medium, the weight loss, and the water content (swelling) of the gel. The physical changes (morphology) as a function of the degradation of cross-linked materials can be studied using different techniques, *i.e.* optical microscopy, optical rotation [22], mercury-intrusion porosimetry [23], dynamic light scattering [24], X-ray scattering [22], scanning electron microscopy [25], and differential scanning calorimetry [26,27]. Release studies of drugs as a result of degradation are typically performed using chromatographic methods after simulating the appropriate degradation medium and conditions [25,28], while drug mobility and drug-polymer interaction can be determined by nuclear-magnetic resonance (NMR) spectroscopic methods [29].

The chemical changes during degradation of physically cross-linked linear PLA-type materials can be studied by analysising of the molecular weight of the PLA-type polymers and by determining the concentrations of released glycolic acid and lactic acid by liquid-chromatographic (LC) methods [26,27]. Enzymatic methods have been described to determine the release of monomers [25]. To monitor the chemical composition of the low-molecular-weight degradation products of these physically cross-linked PLA-type systems, different techniques can be used, such as LC coupled to mass spectrometry (LC–MS) [21,30], infrared spectroscopy, NMR spectroscopy [31,32], pyrolysis-gas chromatography [33] and time-of-flight secondary-ion mass spectrometry [34]. The approaches described above have provided many valuable insights into the degradation of physically cross-linked PLA-type systems. However, knowledge about the degradation process of chemically cross-linked PLA-type polymers is rather limited and is based mostly on average macroscopic changes, such as water uptake and morphology [14,18,35,36]. Based on these macroscopic changes, a statistical kinetic (theoretical) model for bulk degradation of PLA-type hydrogel was developed, which indicates that the degradation profile was greatly influenced by the network structure [14,19,37]. Recently, low-resolution NMR relaxation experiments have been applied for the study of in vitro degradation of a biodegradable photo-cured poly(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate system [38]. Changes in molecular mobility of the network were followed as a function of degradation time with a flow-NMR experiment, which revealed large molecular-scale heterogeneity and large changes in molecular mobility of the matrix material (gel fraction) during different stages of degradation.

Knowledge of the complex degradation process of chemically cross-linked

PLA-type systems (*e.g.* degradation rate, erosion profile, release of toxic compounds, *etc.*) is important for effectively designing materials suitable for specific applications. The present study was performed to gain more insight in the chemical changes during the in vitro degradation of a chemically cross-linked poly(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate coating (see *Fig.* 4.1 for a schematic representation of the network structure). Degradation of this network results in sol fractions (soluble degradation products) and (non-dissolved) gel fractions. The present study focuses on the chemical characterization (*e.g.* composition, molecular weights, concentrations) of the soluble degradation products as a function of the degradation time. The chemical characterisation of the gel fraction during degradation by high-resolution solid-state NMR spectroscopy will be described in a separate paper.

The sol fraction consists of different products, which chemical composition varies during the course of the degradation due to the continuously changing network structure. Hydrolysis of the ester-bonds releases oligomers with lactide, glycolide and/or di-ethyleneglycol building blocks, which originate from the chains between cross-links junctions. As the network has multiple degradation sites along the chains between cross-link junctions, the compositions and lengths of the released oligomers can vary. Typically, the concentration and the composition of these lactide/diethyleneglycol/glycolide oligomers can be obtained by LC–MS, which has proved to be a versatile tool for the analysis of different polyester oligomers and polymers [39–41]. Despite the importance of the degradation process of these chemically cross-linked PLA-type systems, no detailed LC–MS analysis of the released oligomers has yet been reported to our knowledge. Apart from oligomers, polyacrylic acid (PAA) chains which represent the polymeric backbone chains can be released during degradation. If partial hydrolysis takes place, polyacrylate chains with lactide/diethyleneglycol/ glycolide oligomeric side-chains can be released as well. Direct analysis of these polyacrylate backbone structures would be preferred, but this is almost impossible, since to date no suitable analytical methods are available. To obtain insight in the composition of these polymeric backbone chains, the sol fraction can be analysed after complete hydrolysis of the degradation products released, which results in lactic acid, glycolic acid and polyacrylic acid chains. The lactic acid and glycolic acid monomers can be quantified by LC analysis, while the concentration and the average molecular weight of the polyacrylic acid backbone chains in these fully hydrolysed degradation products can be determined using aqueous-phase size-exclusion chromatography (SEC) [42].

Here we demonstrate the feasibility of LC-MS and SEC analysis for the study of the degradation of a chemically cross-linked poly(*D,L*-lactide-*co*-glycolide

50/50)-di-acrylate coating using a phosphate saline buffer solution (pH 7.4) at 37°C. The identification and quantification of the different oligomeric and polymeric degradation products is performed directly after release and also after complete hydrolysis of the degradation products released. The different quantified degradation products are classified according to the different stages of degradation to obtain valuable insight in the complex degradation process of chemically cross-linked poly(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate films.

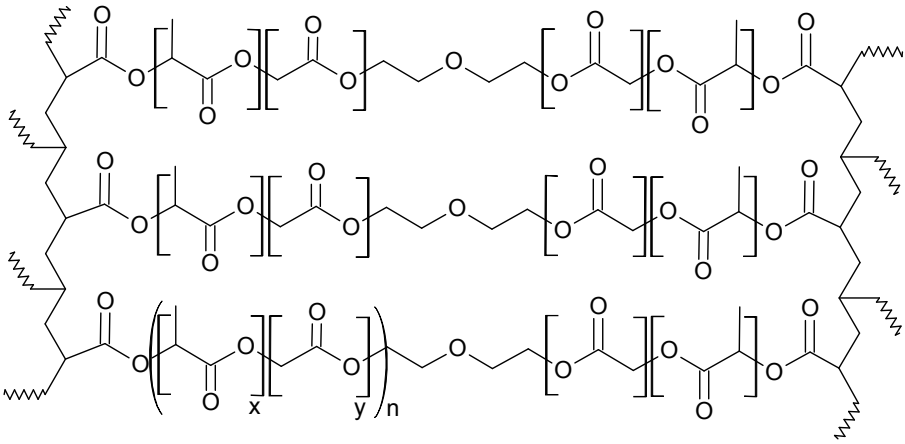

Fig. 4.1. Schematic representation of the resulting ideal network structure of cross-linked poly(D,L-lactide-co-glycolide 50/50)-di-acrylate.

4.2. Experimental

The poly-(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate is an experimental batch, prepared in the laboratory of DSM Research. First, poly-(*D,L*-lactide-*co*-glycolide 50/50)-diol was synthesized. Therefore, 0.358 mol *D,L*-lactide, 0.358 mol glycolide, 64.5 mmol diethyleneglycol and 29 mg catalyst tin(II)-ethylhexanoate was mixed. To obtain poly-(*D,L*-lactide-*co*-glycolide 50/50)-diol the reaction mixture was cooled to room temperature, after 18 h at 150°C. Both *D,L*-lactide and glycolide were purchased from Purac (CSM Biochemicals, Gorinchem, The Netherlands), while diethyleneglycol and tin(II)-ethylhexanoate were purchased from Sigma-Aldrich (St-Louis, MO, USA).
Poly-(*D,L*-lactide-*co*-glycolide 50/50)-diol (100 gr) and 14.36 gr triethyl-amine was dissolved in 100 mL water-free tetrahydrofuran (THF). Acryloylchloride

(12.8 gr) dissolved in 50 mL water-free THF was added dropwise to the solution at controlled temperature (<5°C). After 18 h of stirring at room temperature, the THF was evaporated and the reaction mixture was quenched in 2.5 L ethyl-acetate. The precipitated triethylamine.HCl salt was removed via filtration. The ethyl-acetate layer was washed twice with 150 mL water, saturated with NaCl, and twice with 150 mL water. The resulting solution was dried with $NaSO_4$, filtered, and evaporated to dryness. Poly-(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate was obtained as a slightly yellow-colored oil. The structure of the formed product (*e.g.* endgroups) was investigated by ^{1}H-NMR analysis (Varian Inova 600 MHz NMR Spectrometer (Palo Alto, US), $CDCl_3$, 16 scans, 60 seconds relaxation delay, 22°C, TMS).

A clear formulation was prepared by mixing 7.98 gr of poly-(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate with 78 mg photo-initiator 4-(2-hydroxyethoxy)-phenyl-(2-hydroxy2-methylpropyl) ketone (1%, *w/w*, Irgacure 2959, Ciba Geigy, Basel, Switzerland). The formulation was heated to 40°C and applied onto a thin float glass plate with a coating doctor blade designed to give a 200 µm thick wet coating. This wet film was photo-cured on a conveyor belt (speed 20 m/s) at 22°C, fitted with a Fusion F600 (6000 W, Fusion UV Systems, Inc., Gaithersbrug, USA) electrodeless H-bulb. A UV-dose of 1 J/cm^2 was measured using an UV Power Puck Light meter (EIT, Inc., Virginia, USA). The coatings were dried for 4 h at 60°C in the vacuum oven (200 mbar), resulting in a dry and cured film with a thickness of 150-160 µm. The resulting cured films were analysed with ATR-FT-IR. The cross-linked film showed no residual C=C at 1637 cm^{-1}, which suggest a conversion of >98% (limit of detection), considering that the depth of the IR signals is approximately 1.5 µm.

Cured films (~200 mg, ~150 µm film thicknesses) were placed in sieves with a mesh size of 350–370 µm. Subsequently, the coatings were degraded at 37°C in 15 mL aqueous phosphate saline buffer solution (PBS: pH 7.4 via dissolving 0.2 gr KCl, 0.2 gr KH_2PO_4, 8 gr NaCl and 1.15 gr Na_2HPO_4 in 1 L ultra-pure water). Every 2 or 3 days the buffer was exchanged for fresh buffer. Before adding the fresh buffer the sieves were washed twice with 15 mL ultra-pure water, dried overnight at 50°C and weighed. The hydrolytic degradation was followed in time by monitoring the weight loss and the pH change of the medium. The soluble degradation products were collected and subsequently characterised by chromatographic – mass spectrometric methods.

The residual photo-initiator Irgacure 2959 and oligomeric degradation products were analysed using LC-DAD-MS. This system (Agilent 1100) consist of a quaternary pump, degasser, autosampler, column oven, diode-array detector (DAD, 10-mm cell) and a single-quadrupole MS (Agilent, Waldbronn,

Germany). The UV-signals at 195, 200, 220, 250, and 280 nm were collected. The ionisation was performed by electrospray ionisation (ESI) in positive mode with the following conditions: *m/z* 100-1500, 70 V fragmentor, 0.1 *m/z* step size, 350°C drying gas temperature, 10 L N_2/min drying gas, 45 psig nebuliser pressure and 4 kV capillary voltage. The LC system was controlled using ChemStation software (A09.01, Agilent). The separation was performed with a 250×3 mm ODS-3 column (Inertsil, Varian Inc, Palo Alto, CA, USA) at 40°C and with a gradient (0.5 mL/min) of ultra-pure water (mobile phase A) and acetonitrile (mobile phase B). The gradient was started at t=0 min with 100% (*v/v*) A, was stationary for 5 min and then changed linearly over 40 min to 100% (*v/v*) B (t=45 min). The injection volume was 5 µL.

The degradation products at different degradation times have been fully hydrolysed towards polyacrylic acid, glycolic acid and lactic acid. The hydrolysis was performed in a closed bottle with 2 mL sample and 200 µL 10 M NaOH solution for several days at 90°C.

The weight-average molecular weight (M_w) and concentration of polyacrylic acid was determined by SEC using a highly polar hydroxylated methacrylate 8×300 mm Suprema 1000 Å column (10 µm particle size), with a separation range of 1-1000 *kDa* (PSS, Mainz, Germany). The mobile phase (0.1 M NH_4Ac) was pumped at a flow rate of 1.0 mL/min. The SEC analysis was performed using an Agilent 1100 LC-DAD system, as described above. Refractive index (RI) detection was performed using a RI-71 detector (Showa Denko KK. Tokyo, Japan) with the following settings: fast response, positive polarity and 512 range. The RI signal was collected with ChemStation software (A09.01, Agilent). A series of polyacrylic acid sodium-salt standards (M_p 1.25, 2.93, 7.5, 16, 28, 62.9, 115 and 323 *kDa*, Polymer Laboratories, Shropshire, UK) was used to calibrate the SEC system. The molecular weight calibration curve is given by the relation; $\log(M) = -0.001420(t_R)^3-0.11796(t_R)^2+1.34386(t_R)+1.90512$, R^2=0.9995. The concentration calibration curve of polyacrylic acid was determined by injection of different polyacrylic acid standards (M_w 17.8 and 37.1 *kDa*) at different concentrations (0 to 10 mg/gr, corrected for Na concentration). The concentration calibration curve is given by the relation: Area(RI) = 0.2351(conc. PAA), R^2 = 0.9989.

The concentration of glycolic acid and lactic acid was determined on an Agilent 1100 LC-MS system, which consists of a binary pump, degasser, autosampler, column oven, diode-array detector and a time-of-flight-MS. The ESI-MS was run in negative mode, with the following conditions: *m/z* 50-3200, 215 V fragmentor, 0.94 cycl/sec, 350°C drying gas temperature, 12 L N_2/min drying gas, 45 psig nebuliser pressure and 4 kV capillary voltage. UV detection was

performed at 195 nm. The separation was performed with a 250×4.6 mm Prevail-C18 column (Alltech, USA) at room temperature and with a gradient of 50 mM sulfonic acid in ultra-pure water (mobile phase A) and acetonitrile (mobile phase B). The gradient was started at t=0 min with 99% (*v/v*) A, was stationary for 5 min and then changed linearly over 10 min to 90% (*v/v*) B (t=15 min). The flow rate was 0.5 mL/min and injection volume was 5 µL.

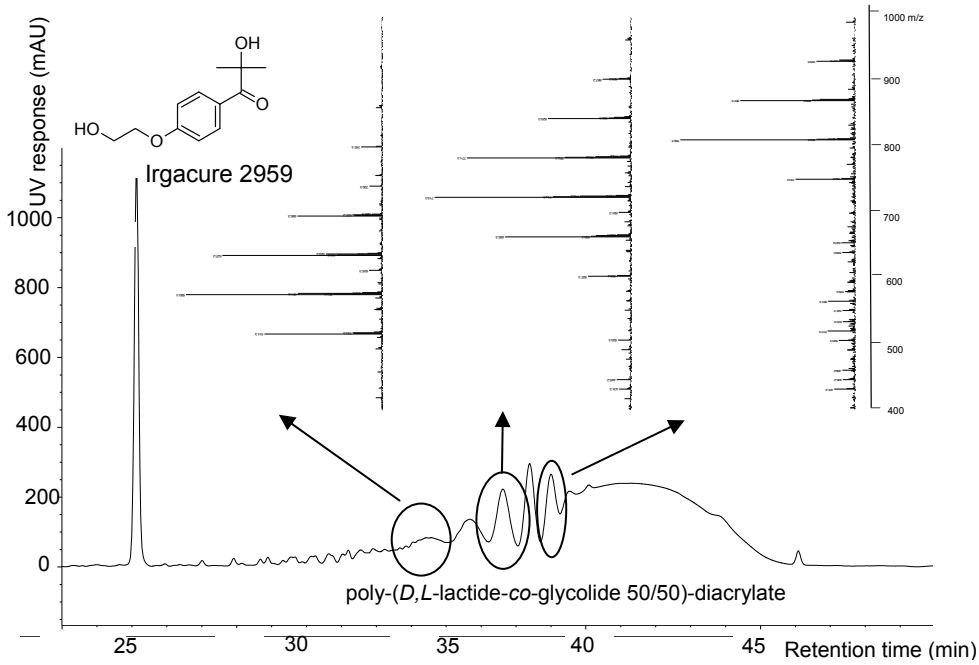

Fig. 4.2. UV-chromatogram (λ=200 nm) of uncured poly-(D,L-lactide-co-glycolide 50/50)-di-acrylate with 1% (w/w) Irgacure 2959. Inserted are extracted MS-spectra representing some oligomers series at different eluting times. See experimental section for LC-DAD-MS conditions.

4.3. Results

4.3.1. Chemical composition of poly-(D,L-lactide-co-glycolide 50/50)-di-acrylate

After synthesis, the uncured poly-(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate is analysed by ^{1}H-NMR and LC-DAD-MS to determine the actual chemical composition and the number-average molecular weight. The UV chromatogram

(λ=200 nm) of uncured poly-(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate with 1% (*w/w*) photo initiator Irgacure 2959 is depicted in *Fig.* 4.2. A detailed chromatogram with both Irgacure 2959 and poly-(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate oligomers is obtained. The relatively broad peaks are caused by the increasing numbers of isomeric compounds with increasing chain length. The low-molecular-weight oligomers could be easily identified by their MS-spectra, however, mass-resolving problems arise for the higher molecular weight oligomers that contain more than 10 glycolide and lactide repeating units. Nevertheless, the MS-spectra show different oligomeric series of poly-(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate with different numbers of glycolide and lactide repeating units as shown in *Fig.* 4.2. Both LC-UV-MS and ^{1}H-NMR analyses indicate >95% conversion of the hydroxyl endgroups into acrylate end-groups. The NMR analysis reveals a molar average composition of 10.8 glycolide, 10.8 lactide and 1.01 di-ethyleneglycol repeating units in the poly-(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate, which results in a number-average molecular weight (M_n) of 1619 *Da*.

4.3.2. Weight loss and pH change during the degradation

After cross-linking of the bi-functional poly(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate, the photo-cured films are degraded in vitro using a PBS buffer solution (pH 7.4) at 37°C. The weight loss of the films and the pH change of the medium *vs.* degradation time are given in *Fig.* 4.3. The cured poly(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate shows a continuous weight loss after 10 days of relatively constant weight, which resulted in a pH drop of the PBS buffer solution. At the highest rate of weight loss, the pH reached a minimum at pH 4, while the pH rose again at the latest stage of degradation. By day 35 the network was totally degraded; no solid material was visible.

4.3.3. Liquid chromatography – mass spectrometry analysis of the sol fraction

The water soluble degradation products are analysed by LC-DAD-MS as a function of degradation time using 2 different approaches. The direct approach is the LC-DAD-MS analysis of the degradation products as released over time, while the indirect approach is the analysis of glycolic acid, lactic acid and polyacrylic acid after complete hydrolysis of the sol fraction using LC-DAD-MS and SEC-RI. These two approaches provide detailed and complementary

information about the degradation process of cross-linked poly(*D,L*-lactide-co-glycolide 50/50)-di-acrylate, as is illustrated below.

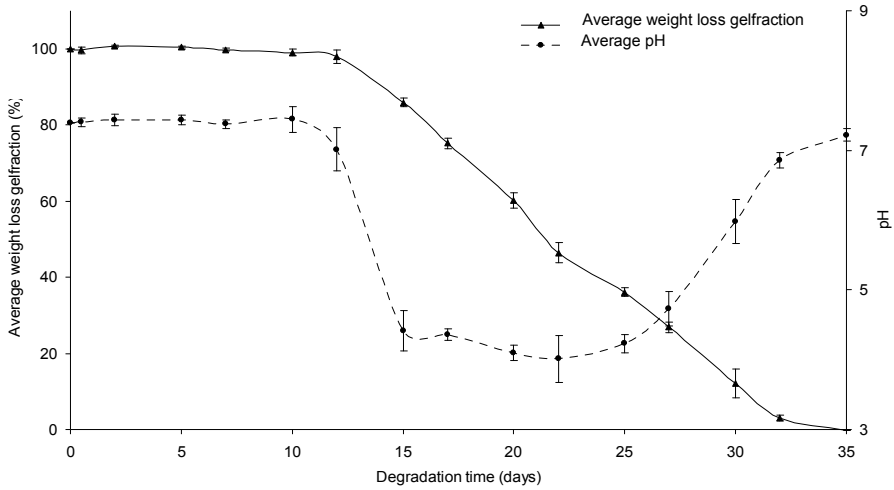

Fig. 4.3. Average weight loss of the network and the pH change of the PBS buffer at 37°C vs. degradation time.

4.3.3.1 Analysis of oligomers and residual photo-initiator released

Using the direct approach, the concentration of residual photo-initiator and different oligomeric series are determined in the sol samples at different stages of degradation.

The concentration of unreacted Irgacure 2959 was quantified by LC-DAD-MS analysis using pure Irgacure 2959 as reference. The residual concentration of unreacted Irgacure 2959, released during degradation, is 9.1% of the total concentration of photo-initiator used in the starting formulation. As the photo-initiator has the potential to react by the hydroxyl-functional group to form a grafted photo-initiator onto the polymeric backbone, the network shows low concentrations of extracted photo-initiator [43]. Besides the intact Irgacure 2959, two photo-initiator degradation products at low concentrations are observed; 1.8% of 4-(2-hydroxyethoxy)benzoic acid (M_w = 182 *Da*) and 1.9% of 4-(2-hydroxyethoxy)-benzaldehyde (M_w = 166 *Da*). The accumulative concentrations of photo-initiator Irgacure 2959 at different degradation times are given in *Fig.* 4.4. Very low concentrations of residual Irgacure 2959 are extracted during the first 10 days due to slow diffusion from the non-degraded polymer. Bulk release of Irgacure 2959 is observed over days 15-17 due to network

degradation. The burst release of Irgacure 2959 shows that the poly(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate network is fully accessible for the buffer solution over days 15-17.

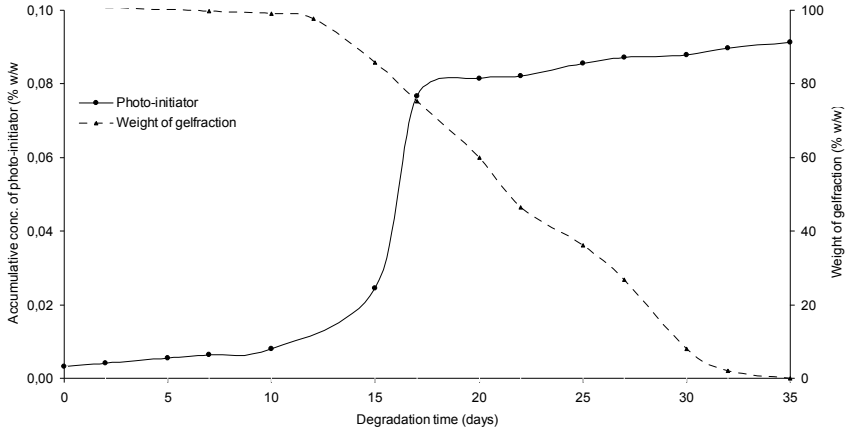

Fig. 4.4. The accumulative concentration of photo-initiator Irgacure 2959 as determined by LC-UV-MS analysis and average weight loss of the gel.

Besides low concentrations of residual photo-initiator, oligomeric series are observed in the sol samples at different degradation times. The MS-chromatograms of the degradation products at day 17 and day 25 (see *Fig.* 4.5) show the presence of different oligomers. To gain more insight into the compositions of these oligomers, the various oligomers are identified by careful elucidation of the observed *m/z* ions of the different chromatographic peaks.

In general, two oligomeric series with different compositions are observed during the degradation: series ***I*** consists of glycolide, lactide and di-ethyleneglycol units, while the other series ***II*** consists of glycolide and lactide units only. The soluble degraded chain fragments have a distribution in length and composition, as a result of the multiple degradation sites along the chains between cross-link junctions.

The MS-chromatograms show decreasing molecular weights of the oligomeric series with degradation time (see *Fig.* 4.5). The determined compositions of the oligomers of series ***I*** and series ***II*** at day 17 and 25 are presented in *Figs.* 4.6 and 4.7, respectively. The compositions of the series ***I*** oligomers show a broad distribution of glycolide units in the oligomers from 1 to 8 units, while a narrow distribution of lactide units in the oligomers from 1 to 4 units was observed. The broadness of the distribution does not change in time. However, the average number of glycolide units seems to decrease with increasing degradation time; at

day 17 the oligomeric series has an average of 2 lactide and 4 glycolide units, while at day 25 the average number of glycolide units has decreased to 2 glycolide units. As a result the average molecular weights of the oligomers decrease with increasing degradation time.

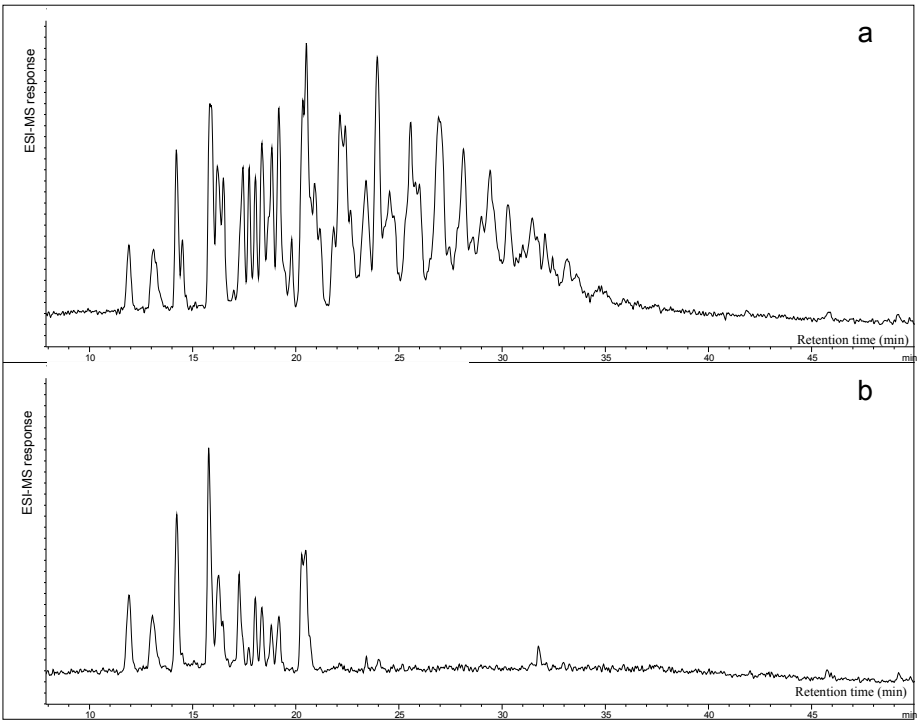

Fig. 4.5. MS-chromatograms of the sol-fraction at day 17 (a) and 25 (b), which indicate the change in molecular weight of the different oligomers. See experimental section for LC-MS conditions.

The same phenomenon is also observed for the oligomers of series ***II***, where the average number of glycolide units in the oligomers decrease from 4 glycolide units at day 17 to 2 glycolide units at day 25. At later stage of degradation the average number of lactide units in the oligomer series is systematically lower than the average glycolide units. This indicates that the hydrolytic scissions of the ester-bonds tend to primarily target the linkages between glycolic-glycolic and lactic-glycolic bonds [44], which results in faster release of glycolide containing oligomers.

The concentration of the oligomers at different degradation times is determined by LC-MS analysis. Oligomers of glycolide/diethyleneglycol/lactide are required for calibration of these oligomers, but are not available in various

molecular weights and compositions. Therefore, the concentrations of these oligomers are determined using the uncured poly-(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate as a reference. Due to differences in ionisation efficiency during the ESI-MS ionisation, arising from variation in compositions and molecular weights of the oligomers, a systematic deviation in the quantitative results could occur. A possible alternative to improve the experimental quantification of oligomers is the (on-line) coupling of LC to hydrolysis or pyrolysis, followed by chromatographic or spectrometric analysis of the monomers.

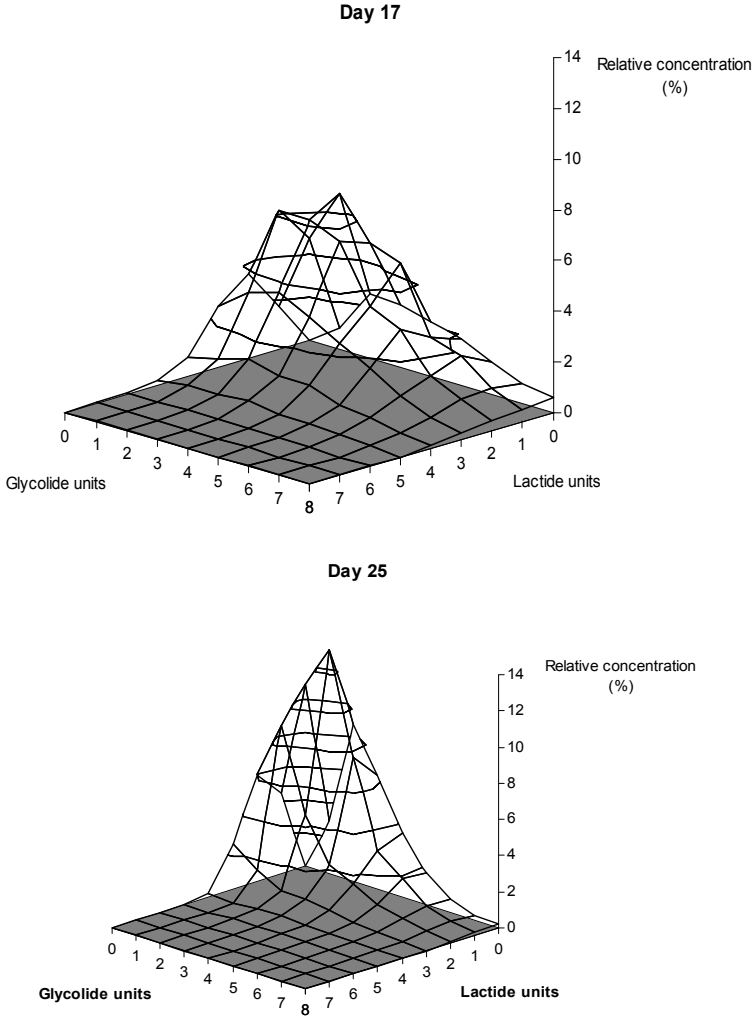

Fig. 4.6. Relative concentration of lactide and glycolide oligomers of series ***I*** *upon degradation at day 17 and day 25.*

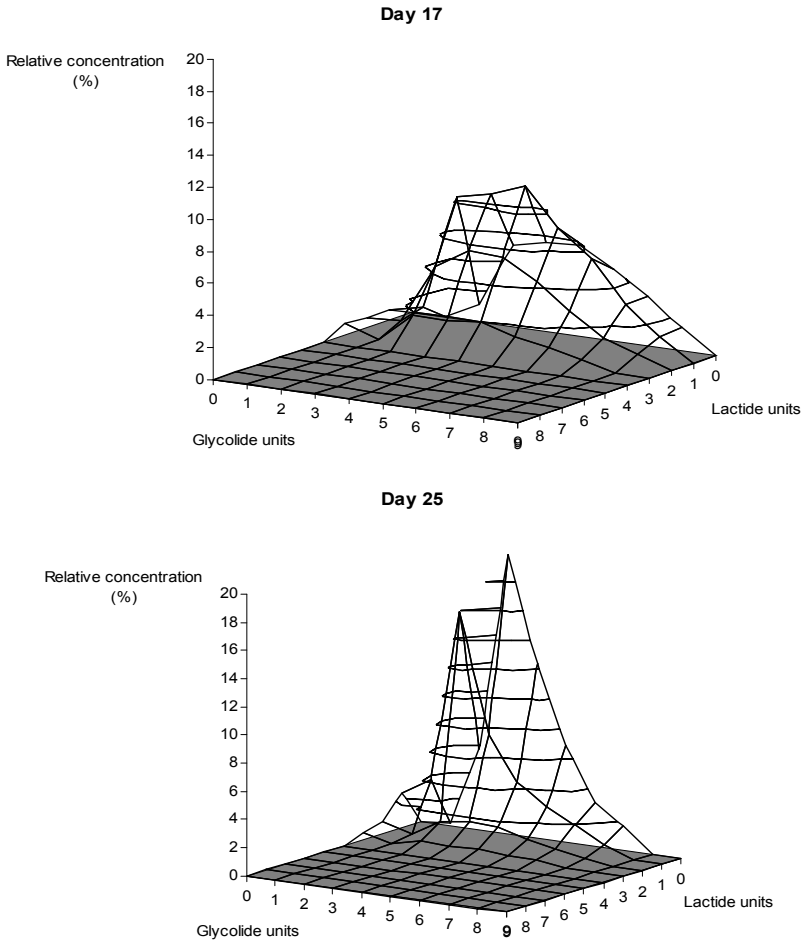

Fig. 4.7. Relative concentration of lactide and glycolide oligomers of series ***II*** *upon degradation at day 17 and day 25.*

The theoretical concentration of glycolide/diethyleneglycol/lactide oligomers released is between 94 and 117% (*w/w*). A recovery of more than 100% is due to the addition of water during the hydrolysis of the ester-group, while the absolute recovery depends on the number of water additions. The experimental concentration of oligomers released during degradation is 130% (*w/w*) of the original weight of the network, which agrees fairly with the theoretical concentration of oligomers released during degradation. However, the determined concentration of oligomers released could deviate as the glycolide/lactide oligomers can be partially attached to the polyacrylate backbone chains. The latter cannot be determined in these sol fractions as such,

as direct analysis of higher molecular weight polyacrylate chains with oligomeric side chains is not possible by the LC-MS method used [42].

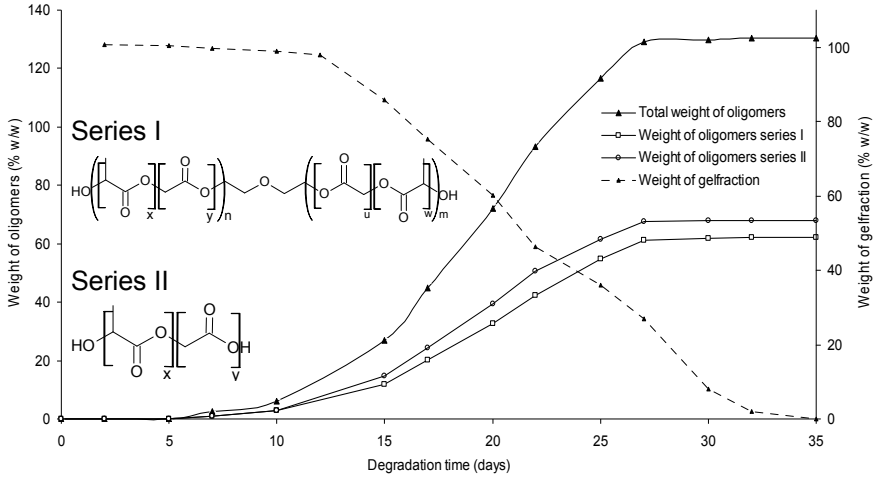

*Fig. 4.8. The percentages of the concentrations accumulated of oligomeric series **I** and **II** as determined by LC-MS analysis.*

The relative percentages of the concentrations accumulated of the different oligomeric chains released between the network junctions are given in *Fig.* 4.8. In general, the concentrations of soluble lactide/glycolide oligomeric compounds follow the weight loss during the degradation. The concentrations of series ***I*** and ***II*** are similar during the degradation of the network. However, comparison of the concentrations of oligomers and the weight loss during degradation indicates that at day 27-30 a systematic deviation occurs; the concentration of oligomers released at day 27-35 is lower than 5% (*w/w*) while a weight loss of about 27% (*w/w*) still remains at day 27. Since only low fractions of free glycolic acid (± 2.3% of the weight loss) and lactic acid (± 1.2% of the weight loss) are found in the degradation samples at day 27-35; the differences in weight loss and the concentration of oligomers released will be explained below by the release of polyacrylate backbone chains with glycolic/lactic side-chains.

4.3.3.2 Analysis of polyacrylate chains released

An indirect approach is used to gain more insight into the release of the polyacrylate chains with or without attached glycolide and lactide units. The sol

fractions at different degradation times are fully hydrolysed towards polyacrylic acid (PAA), lactic acid and glycolic acid. The concentrations of glycolic and lactic acids are determined using LC-MS with pure glycolic acid and lactic acid as references, while the concentration and average molecular weight of polyacrylic acid is determined by aqueous-phase SEC-RI analysis. The absolute concentration of polyacrylic acid chains released during degradation is 8.4% (*w/w*), while a theoretical concentration of 8.9% (*w/w*) is expected based on a perfect network structure (see *Fig.* 4.1). The absolute concentrations of glycolic and lactic acids, released during degradation, are 51.3 and 69.7% (*w/w)* respectively. The theoretical concentration of glycolic acid and lactic acid released is 50.7% (*w/w*) and 60.0% (*w/w*) respectively. The experimental and theoretical concentrations of polyacrylic acid, glycolic and lactic acids agree well. No indication of free acrylic acid is found using the described LC-DAD-MS method. This indicates that the acrylate endgroups are fully reacted via cross-linking or even via cyclisation into the network during photo-polymerisation. The cumulative concentrations of glycolic acid, lactic acid and polyacrylic acid chains determined at different degradation times are shown in *Fig.* 4.9. The concentration of polyacrylic acid *vs.* degradation time shows the release of the polyacrylic acid chains at day 30-35.

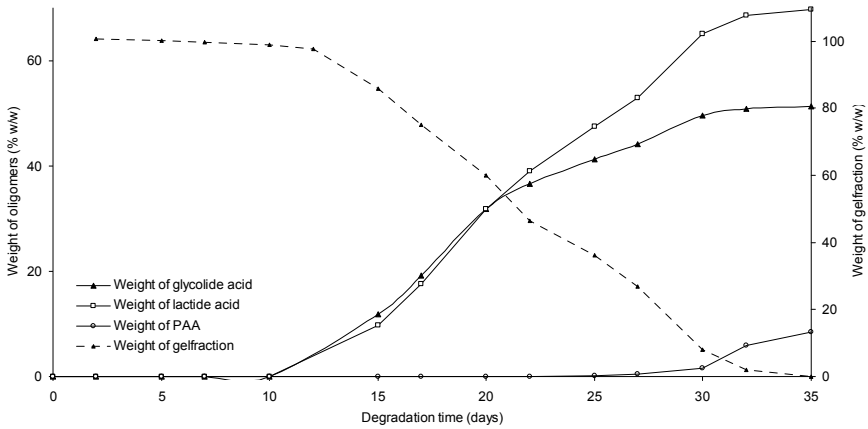

Fig. 4.9. The percentages of the concentrations accumulated of glycolic acid, lactic acid and polyacrylic acid (PAA).

In *Fig.* 4.10, the SEC-RI chromatograms of the fully hydrolysed degradation samples and the weight-average molecular weight determined of the polyacrylic acid backbone chains at day 22-35 are given. The higher average molecular weights of polyacrylic acid chains are released with longer degradation times.

As given in *Fig.* 4.9, the concentration of glycolic acid and lactic acid at day 30-35 is relatively high. However, the concentration of free glycolic and lactic acids in the sol fraction is respectively ± 2.3% and ± 1.2% of the weight loss. This indicates that relatively high fractions of glycolide and lactide are still attached to the released polyacrylate backbone chains at day 30-35. More specifically, the average number of glycolide and lactide units attached to each polyacrylate unit at day 32 is about 1 lactide/glycolide unit.

The molar ratio glycolide/lactide *vs.* the degradation time is given in *Fig.* 4.11, which shows the preferential degradation of glycolide units over lactide units. This is in agreement with the composition of the released oligomers during degradation. Secondly, *Fig.* 4.11 indicates that a systematically higher fraction of lactide than glycolide was attached to the polyacrylic acid backbone chains during the release. In conclusion, the release of the polyacrylic acid chains with glycolide and/or lactide side-chains is simply determined by the number and type of ester-groups that have been degraded hydrolytically to solubilise these acrylate chains with glycolide and/or lactide side-chains.

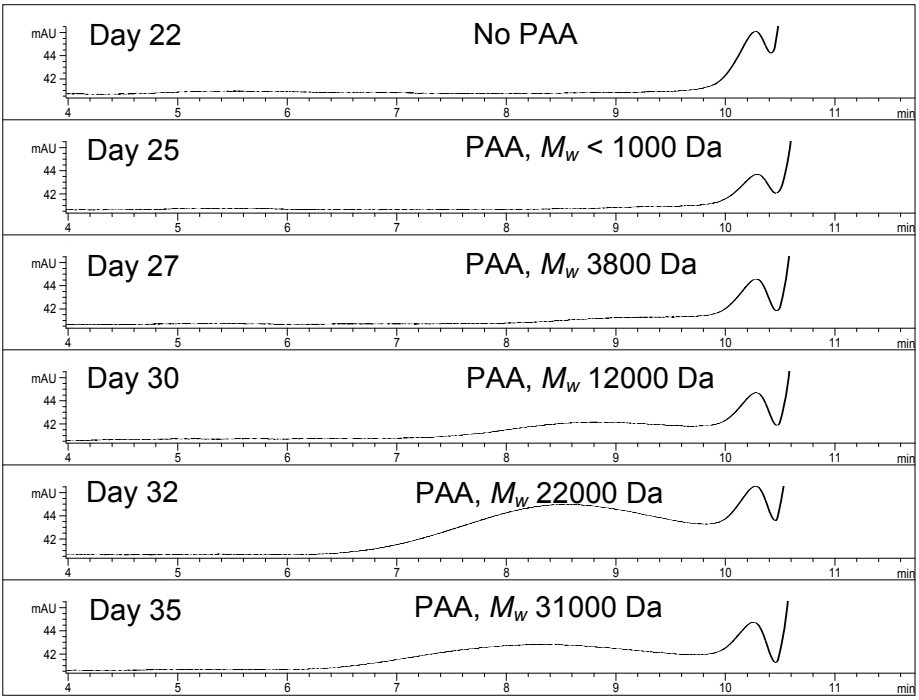

Fig. 4.10. SEC-RI chromatograms of fully hydrolysed samples at different stages of degradation, which show the presence and the weight-average molecular weight (M_w) of the polyacrylic acid chains (PAA). See experimental section for SEC-RI conditions.

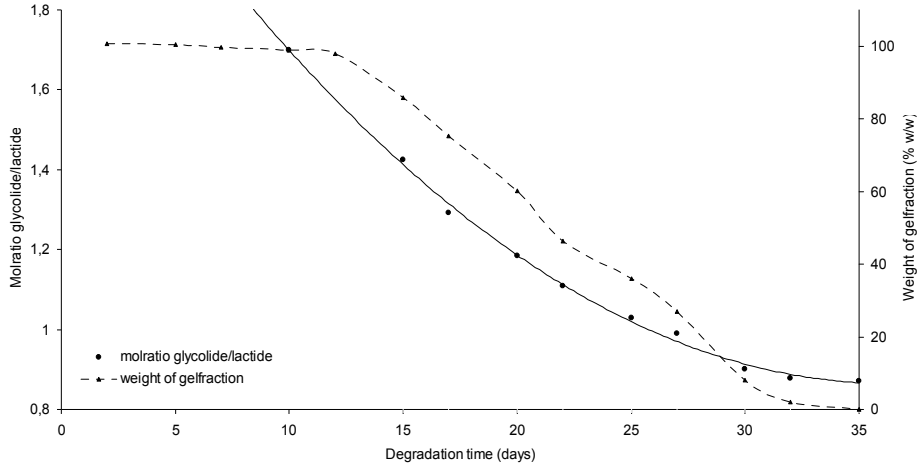

Fig. 4.11. The decreasing molar ratio of glycolide/lactide released during the degradation.

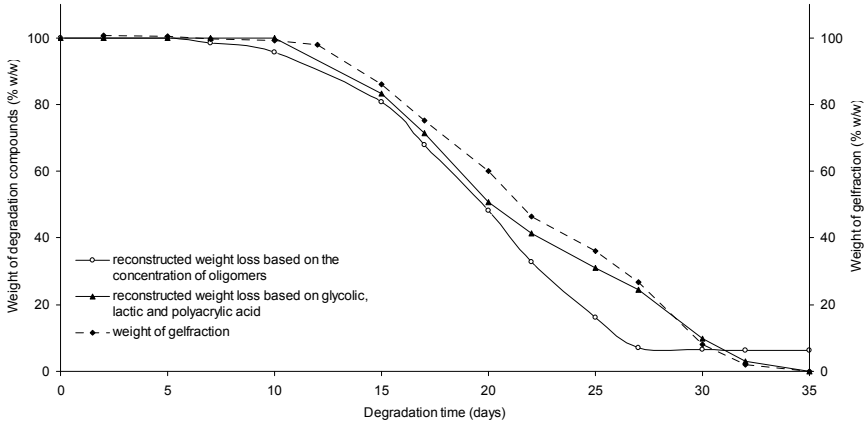

Fig. 4.12. The degradation curves, reconstructed from the data obtained by different approaches.

Both the direct and indirect approach gives information about the compositional and molecular weight drift of products released with degradation time. The degradation curves are reconstructed with the use of the quantitative results of the oligomers released and the quantitative results of polyacrylic acid and lactic and glycolic acids after hydrolysis. These curves (see *Fig.* 4.12) are in good agreement with the experimental weight loss during degradation. However, the degradation curve reconstructed from the oligomers quantified seems

systematically overestimated. This is probably related to the use of uncured poly-(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate as a reference for the quantification of the oligomers released combined with the ionization differences arising from variation in compositions and molecular weights of the oligomers, as outlined before. Nevertheless, chromatography and mass-spectrometry provided valuable qualitative and quantitative information about oligomers and polymers released upon degradation.

4.4. Discussion

Analysis of the sol fractions of an in vitro degraded poly(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate network in a PBS buffer solution at 37°C shows the release of different compounds during each stage of degradation.
From day 1-10, the weight and pH is relatively constant and only extraction of low concentrations of residual Irgacure 2959, and two photo-initiator degradation products, are observed.
After day 10, the pH drops to a constant level, while a constant loss of weight was observed. During this stage, oligomers with lactide/glycolide and/or di-ethyleneglycol building blocks are released at a constant rate. The oligomers of glycolide, lactide and diethyleneglycol units have hydroxylic endgroups, while the oligomers of glycolide and lactide units only, have one hydroxylic and one carboxylic endgroup. It is expected that both series are formed by two hydrolytic scissions of the ester-groups at the chains between network junctions.
After day 25, the release of polyacrylate chains with lactide/glycolide side chains results in an increasing pH at this late stage of degradation. It is assumed that these series are formed by the release of the lactide/glycolide/di-ethyleneglycol oligomeric series from the network.
The quantification of the compounds released makes it possible to calculate the concentration of carboxylic endgroups (mmol/L) at different stages of degradation. As shown in *Fig.* 4.13, three distinct regimes are observed. The hydrolysis seems to starts between 0 to 15 days. From day 15 to 25 the rate of hydrolysis gets constant and is in the order of 6.5±0.6 mmol/L•day. From day 25-27 onwards, the rate of hydrolysis decreases significantly to 2.1±0.2 mmol/L•day. After the degradation of the network, a total of about 105 mmol acids/L are formed as result of hydrolysis. As a result, it can be estimated that approx. half of the total available esters in the poly(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate network (190 mmol ester/L are totally available) are hydrolysed during the 35 days of in vitro degradation.

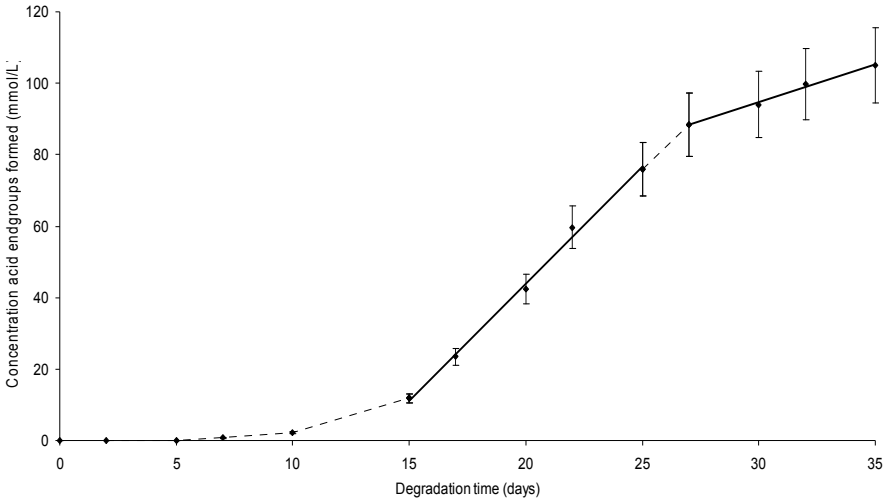

Fig. 4.13. The concentration of carboxylic groups accumulated (mmol/L) as obtained from analysis of the sol fractions.

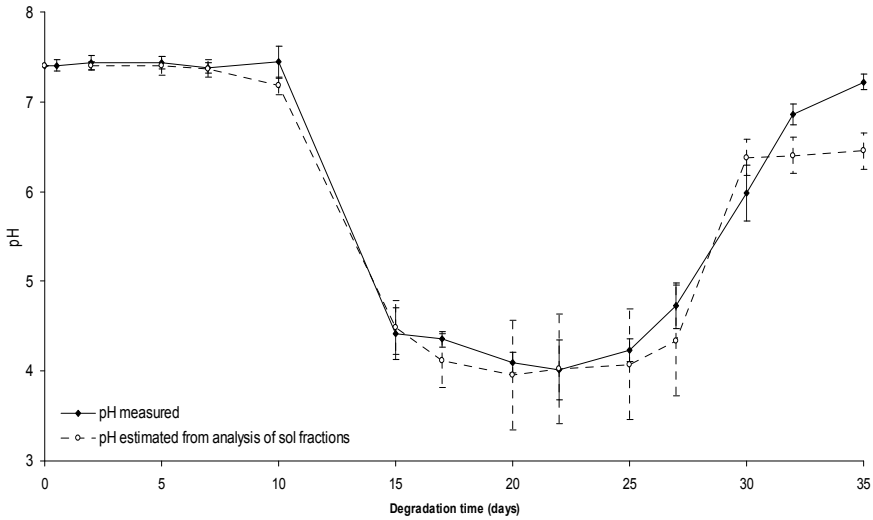

Fig. 4.14. The pH change measured and estimated from the analysis results during degradation time.

The pH change of the PBS buffer as result of the different concentrations of carboxylic endgroups released, as estimated using the Henderson-Hasselbalch equation [45,46] (pK_a lactic acid 3.85, pK_a glycolic acid 3.83, pK_a polyacrylic acid 4.5 [47]), is given in *Fig.* 4.14. Although the relatively high standard

deviation of the pH estimated, the pH trend during the degradation is similar for both the pH measured and estimated. In the final stage, a significant differences between the pH measured and estimated was observed. However, the pH change at day 25-32 indicates that the number of free acid endgroups decrease in time as a result of release of the polyacrylate backbone chain. From this it can be concluded that most of the hydrolytic scissions have already taken place in the bulk degradation stage, resulting in a loose network of mainly polyacrylate backbone chains with pendant glycolide and lactide unit side chains at day 22-25. These observations are in line with the observations made by Litvinov *et al.* [38], who studied a similar chemically cross-linked di-acrylate network by solid-state flow NMR relaxometry. They found large network break down prior to weight loss, resulting in loosely attached degraded fragments.

The hydrolytic degradation of poly(*D,L*-lactide-*co*-glycolide 50/50)-di-acrylate network as studied by liquid chromatography and mass spectrometry, showed at least five different stages in the degradation process (see also *Fig.* 4.15):

(1) *Extraction stage*. From day 0-5 the network shows a minimum of weight loss. Only release of low concentrations of residual Irgacure 2959 and two degradation products of the used photo-initiator at lower concentration level are observed.

(2) *Network attack stage*. From day 7-12 the network starts to degrade. Hydrolytic scissions of network chains cause formation of network defects. As result, extraction of low concentration of oligomers with lactide/glycolide and/or diethyleneglycol building blocks and a low fraction of Irgacure 2959 is observed.

(3) *Network penetration stage*. At day 15-17 the network is fully accessible to the buffer solution as indicated by the burst release of Irgacure 2959. The open network structure and the resulting release of oligomers of lactide/glycolide and/or diethyleneglycol building blocks cause a pH drop of the PBS buffer solution.

(4) *Bulk degradation stage*. From day 17-25 the systems degrade homogeneously and a constant release of lactide/glycolide and/or di-ethyleneglycol containing oligomers is observed. The pH is low, but relatively constant during this stage of constant release of oligomers. The hydrolysis rate is constant. Both oligomeric series (series ***I*** and ***II***) show a broad distribution of glycolide units in the oligomers up to n=8, while a narrow distribution of lactide units in the oligomers up to n=4 is observed. Both oligomeric series show a strong decrease in the average number of oligomeric repeating units with degradation time, particularly the average number of glycolide units.

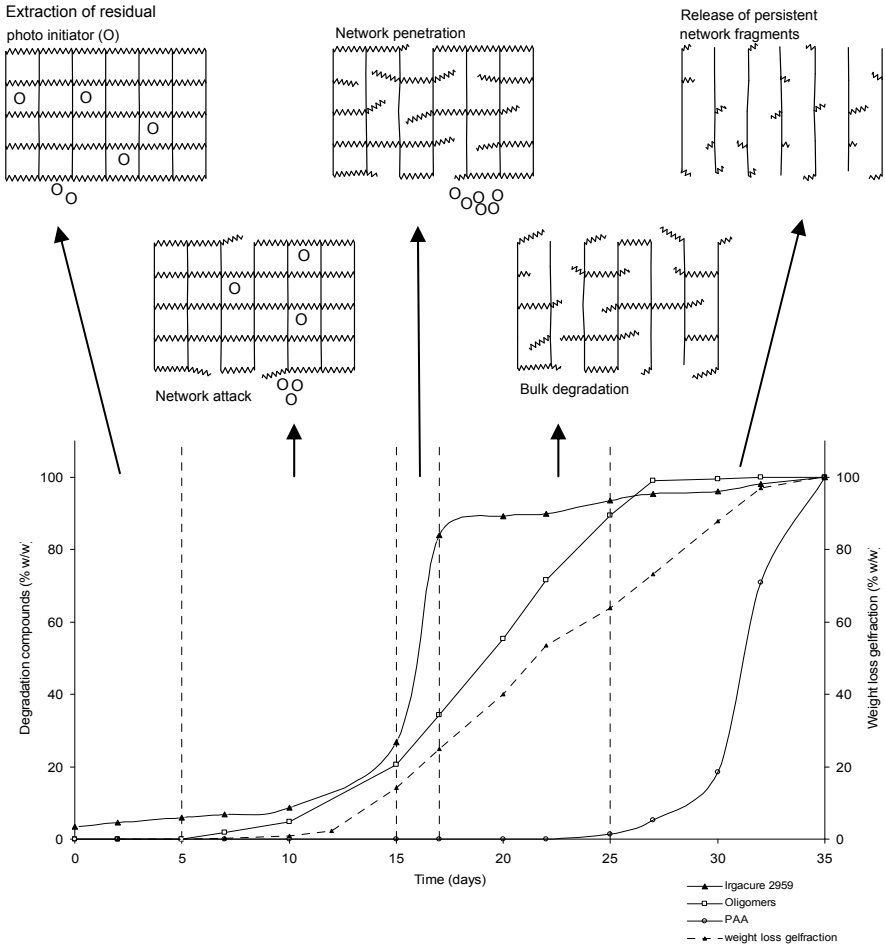

Fig. 4.15. Schematic representation of the five different stages at the hydrolytic degradation of photo-cured poly-(D,L-lactide-co-glycolide 50/50)-di-acrylate coating.

(5) *Release of persistent network fragments stage*. In the final stage at day 25 up to day 35, the release of oligomers is minimized, while the polyacrylate backbone chains start to dissolve in the medium. At this late stage, the weight-average molecular weight of polyacrylate chains increases with degradation time; ranging from 3800 *Da* at day 27 up to 31000 *Da* at day 35. This effect is caused by the broad distribution in molecular weight of the polymeric backbone chains in the network. The polyacrylate chains released contain side chains of glycolide and lactide units. The concentration of these side chains decreases with increasing

degradation time, but the molar ratio glycolide/lactide indicates an increasing percentage of lactide units attached to the polyacrylate chains. This is the result of the preferential degradation of glycolide units over lactide units, which is observed during all stages of degradation.

4.5. Conclusion

The chemical composition of the sol fraction of photo-cured poly(*D,L*-lactide-*co*-glycolide)-di-acrylate is determined at different degradation times using different approaches and chromatographic methods. The direct approach, which consists of the analysis of the degradation products by LC-DAD-MS, reveals detailed information about the residual Irgacure 2959 and the composition of the lactide/glycolide and/or diethyleneglycol oligomers originating from the chains between cross-link junctions. Complete hydrolysis of the degradation products, followed by LC-UV-MS and SEC-RI analysis gives detailed information about the polyacrylate chains with lactide/glycolide side chains released. Even with the assumptions made in the quantification of oligomers, a good fit is found between the quantitative results of both approaches presented and the experimental weight loss and pH change during degradation. The presented approaches provide detailed insight into the chemical degradation process that influences the degradation rate and the release of degradation products. As a result, the hydrolytic degradation of a biodegradable material, was categorised into five distinguish phases.

References

[1] R.L. Dunn, Biomedical Applications of Synthetic Biodegradable Polymers, Ed. J.O. Hollinger, CRC Press, Boca Raton (1995) 17.
[2] S.M. Li, M. Vert, Degradable Polymers: Principles and Applications, Ed. G. Scott and D. Gilead, Chapman and Hall, London (1995) 43.
[3] J.A. Brydson, Rubbery Materials and their Compounds, Elsevier, London (1988).
[4] H. Juni, M. Nakano, Crit. Rev. Ther. Drugs Carr. Syst. 3 (1987) 81.
[5] J.M. Anderson, M.S. Shive, Adv. Drug Deliv. Rev 28 (1997) 5.
[6] S.P. Schwendeman, Crit. Rev. Ther. Drugs Carr. Syst. 19 (2002) 73.
[7] R. Jain, N.H. Shah, A.W. Malick, C.T. Rhodes. Drug Dev. Ind. Pharm. 24, (1998) 703.

[8] K.D. Vlugt-Wensink, X. Jiang, G. Schotman, G. Kruijtzer, A. Vredenberg, J.T. Chung, Z. Zhang, C. Versluis, D. Ramos, R. Verrijk, W. Jiskoot, D.J. Crommelin, W.E. Hennink, Biomacromolecules, 11 (2006) 2983.
[9] V. R. Babu, M. Sairam, K. M. Hosamani, T.M. Aminabhavi, Int. J. Parm. 325 (2006) 55.
[10] Y.Y. Hang, T.W. Chung, T.W. Tzeng, Int. J. Pharm. 182 (1999) 93.
[11] S.J. Park. S.H. Kim, J. Colloid Interface Sci. 271 (2004) 336.
[12] T. Panoyan. R. Quesnel, P. Hildgen, J. Microencapsul. 20 (2003) 745.
[13] R. Quesnel, P. Hildgen, Molecules 10 (2005) 98.
[14] A.S. Sawhney, C.P. Pathak, J.A. Hubell, Macromolecules 26 (1993) 581.
[15] J. Elisseeff, K. Anseth, D. Sims, W. McIntosh, M. Randolph, R. Langer, Proc. Natl. Acad. Sci. USA, 96 (1999) 3104.
[16] A.K. Burkoth, K.S. Anseth, Biomaterials 21 (2000) 2395.
[17] Y.D. Park, N. Tirelli, J.A. Hubell, Biomaterials 24(2003) 893.
[18] J.D. Clapper, J.M. Skeie, R.F. Mullins, C.A. Guymon, Polymer 48 (2007) 6554.
[19] A. T. Metters, K.S. Anseth, C.N. Bowman, J. Phys. Chem. B. 105 (2001) 8069.
[20] D.D. Lewis, Biodegradable Polymers as Drugs Delivery Systems, Eds. M. Chasin and R. Langer, Marcel Dekker, New York (1990) 1.
[21] S.J. de Jong, E.R. Arias, D.T.S. Rijkers, C.F. van Nostrum, J.J. Kettenes-van den Bosch, W.E, Hennink, Polymer 42 (2001) 2795.
[22] M.F. Gonzalez, R.A. Ruseckaite, T.R. Cuadrado, J. Appl. Polym. Sci. 71 (1999) 1223.
[23] L. Lu, S.J. Peter, M.D. Lyman, H-L. Lai, S.M. Leite, J.A. Tamada, J.P. Vacanti, R. Langer, A.G. Mikos, Biomaterials 21 (2000) 1595.
[24] M.L.T. Zweers, G.H.M. Engbers. D. W. Grijpma, J. Feijen, J. Control. Rel. 100 (2004) 347.
[25] T.G. Park, W. Lu, G. Crotts, J. Control. Rel 33 (1995) 211.
[26] J.Y. Yoo, J.M. Kim, K.S. Seo, Y.K. Jeong, H.B. Lee, G. Khang, Biomed. Mater. Eng. 15(4) (2005) 279.
[27] E. Vey, A.F. Miller, M. Claybourn, A. Saiani, Macromol. Symp. 251 (2007) 81.
[28] E. Piskin, X. Kaitian, E.B. Denkbas, Z. Kucukyavuz, J. Biomater Sci. 74 (1995) 359.
[29] K. Tomic, W.S. Veeman, M. Boerakker, V.M. Litvinov, A.A. Dias, Journal of Pharmaceutical Sciences, 97 (2008) 3245.
[30] I. Osaka, M. Watanabe, M. Takama, M. Murakami, R, Arakawa, J. of Mass Spectrom. 41 (2006) 1369.
[31] S. Li, H. Garreau, M. Vert, T. Petrova, N. Manolova, I. Rashkov, J. Appl. Polym. Sci. 68 (1998) 989.
[32] S. He, M.D. Timmer, M.J. Yaszemski, A.W. Yasko, P.S. Engel, A.G. Mikos, Polymer 42 (2001) 1251.

[33] K. Urakami, A. Higashi, K. Umemoto, M. Godo, C. Watanabe, K. Hasmimoto, Chem. Pharm. Bull. 49 (2001) 200.
[34] J. Chen, J.A. Cardella, Macromolecules 32 (1999) 7380.
[35] A.T. Metters, C.N. Bowman, K.S. Anseth, American Institute of Chemical Enigeers 47(6) (2004) 1432.
[36] Y. Zang, C-Y. Won, C-C. Chu, Journal of Polymer Science Part A; Polymer Science, 37 (2000) 4554.
[37] A.T. Metters, K.S. Anseth, C.N. Bowman, Polymer 41(11) (2000) 3993.
[38] V. Litvinov, B. Plum, M. Boerakker, A. Dias, Macromol. Symp., 266 (2008) 6.
[39] H. Hayen, A.A. Dechamps, D. W. Grijpma, J. Feijen, U. Karst, J. Chromatogr. A 1029 (2004) 29.
[40] H. Philipsen, J. Chromatogr. A 1037 (2004) 329.
[41] I. Hilker, A. Schaafsma, B. Coussens, R. Peters, A. Nijnenhuis and A. Heise, European Polymer Journal 44 (2008) 1441.
[42] R. Peters, V.M. Litvinov, P. Steeman, A.A. Dias, Y. Mengerink, R. van Benthem, C.G. de Koster, Sj. van der Wal, P.J. Schoenmakers, J. Chromatogr. A 1156 (2007) 111.
[43] J.A. Burdick, T. M Lovestead, K.S. Anseth, Biomacromolecules 4 (2003) 149.
[44] T.G. Park, J. Control. Rel. 30 (1994) 161.
[45] L. J. Henderson, Am. J. Physiol. 21 (1908) 173.
[46] K.A. Hasselbalch, Biochemische Zeitschrift 78 (1917) 112.
[47] J.E. Gebhardt, D.W. Furstenau, Colloids Surf. 7 (1983) 221.