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High-performance liquid chromatographic separation and tandem mass spectrometric identification of breakdown products associated with the biological hydrolysis of a biomedical polyurethane

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Abstract

As part of ongoing investigations into the biological degradation of biomaterials, methods have been developed to isolate and chemically analyze polymer biodegradation products. The use of these methods can provide information on the biodegradation product profiles and yield concentration levels for the isolated products. The latter information is required to assess the toxicological nature of biomaterials and their related degradation products. In this study a model biomedical polyurethane was synthesized with toluene diisocyanate, polyester diol and ethylene diamine, and then incubated at 37°C in a biological solution containing enzyme. The biodegradation products were isolated from the in vitro system and prepared for HPLC analysis, by using a combination of ultrafiltration, freeze drying and liquid–solid extraction. The ultrafiltration and the liquid–solid extraction effectively removed protein contamination. The separation of more than 20 degradation products, with gradient HPLC, was optimized using a photodiode array detector. The separated degradation products were identified using a tandem mass spectrometer. The model polyurethane was labeled with ^{14}C in different segments, in order to assist in confirming the efficiency of the sample preparation and isolation methods. A detection limit of 2 ng was found. No toluene diamine – a suspected human carcinogen associated with some medical implants – could be found in the test samples. This represents a significant finding since the amount of this injected sample actually contained a total of 28 μg of degradation products isolated from the incubation medium. © 1997 Elsevier Science B.V.

Keywords: Biodegradation products; Polyurethane

1. Introduction

Polyurethane elastomers have been one of the most important groups of polymers used as biomaterials for the fabrication of medical implant devices such as blood filters, pacemaker leads, catheters, heart valves and cardiac-assist devices [1]. This acceptance in the medical device field has resulted primarily from their excellent physical properties and moderate bio-compatibility [1]. However, their use in long-term implant devices has been controversial due to their potential degradation in the biological environment. The biodegradation of polyurethanes has been documented in some cases to lead to the release of potentially toxic degradation products [2,3] and has been one of the limiting factors for their use in long-term implantation.

Typical polyurethanes are synthesized with three

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types of monomers: (1) a diisocyanate, (2) an oligomeric diol and (3) a chain extender. Aromatic diisocyanates such as toluene diisocyanate (TDI) have been extensively used for their synthesis. It has been reported that TDI-synthesized polyurethanes can be hydrolyzed and release toluene diamine (TDA) under acidic, alkaline or high temperature conditions [4,5]. TDA is a suspected human carcinogen [2,6] and has been extensively studied in animal implant studies [7–10]. In vivo experiments carried out by Chan and coworkers reported that TDA was released from implanted TDI-based polyurethane [11,12]. In these studies TDA was detected in urine samples, collected from women with polyurethane breast implants. The fact that the samples were hydrolyzed in the presence of acid at 105°C prior to the analysis has complicated the determination of the true source of TDA.

The detection of TDA and any derivative of TDA from degradation products is essential to the toxicological evaluation of implanted polyurethanes. To date there have been very few studies which have reported on the biodegradation product profiles of polyurethanes, following their exposure to a biological environment. In the work reported by Labow and Santerre [13,14], it has been found that cholesterol esterase (CE) is an effective enzyme in catalyzing the hydrolytic degradation of TDI-based polyurethanes. CE has been found in various tissues and particularly monocytes as they rapidly differentiate into macrophages around implantation sites [15]. These authors have also previously reported on the presence of several compounds released from polyurethanes, following the latter material's incubation with cholesterol esterase [16]. However, the analysis of these products carried out by Santerre et al. [16] was limited due to the difficulty in removing protein contaminants which resulted in the incomplete separation of the degradation samples.

Deproteinizing samples is an essential process to be carried out prior to the chemical analysis of biodegradation products generated from physiologically relevant protein-containing samples. This is particularly valid when using methods which apply reversed-phase high-performance liquid chromatography (HPLC) [17,18], since the proteins have a high tendency to adsorb onto the HPLC columns and to cause blockage [17]. Furthermore, in a gradient analysis mode, proteins have a tendency to aggregate and then later precipitate during the gradient run [19]. Previous work on the separation of polyurethane biodegradation products showed that the classical deproteinization methods, such as liquid–solid extraction following vacuum drying, or precipitation by adding organic solvents, did not result in sufficient removal of proteins contained in the samples [16]. Furthermore, deproteinization by adding acid or base to precipitate proteins may lead to further hydrolysis of the degradation products [20]. An alternate deproteinization method has been reported using Ultrafree (UF) filter units (Millipore, Bedford, MA, USA) with different molecular mass cut-offs. This approach removes high-molecular-mass proteins and protein-related residues [21,22]. A combination of this latter method with some of the classical deproteinization techniques is expected to improve the isolation of biomaterial degradation products from biological systems.

This study presents an analytical method for the isolation of biodegradation products derived from the interaction of polymers and specifically polyurethane with inflammatory enzymes. The method includes the development of steps for sample preparation, separation by reversed-phase HPLC and identification with tandem mass spectrometry (MS–MS). ^{14}C-Labeled polyurethanes were synthesized and used in degradation studies to confirm the product discovery process.

2. Experimental

2.1. Materials and synthesis

Cholesterol esterase (CE, lot No. 31010) was obtained from Genzyme (Cambridge, MA, USA). 2,4-Toluene diisocyanate (TDI), labeled with ^{14}C in the phenyl ring (NEN, Du Pont, lot No. 3104-168), was supplied in amber glass ampoules each containing 0.24 mCi in 0.55 ml of anhydrous toluene. 1,2-[^{14}C]-Ethylene diamine (0.25 mCi/0.262 ml per vial, lot No. 048F9239-1) was supplied from Sigma (St. Louis, MO, USA) in salt form in 2% aqueous ethanol. Prior to synthesis, the ethanol and water were removed by vacuum at 1.0 mm Hg and room temperature for 12 h. Aldrich supplied non-labeled

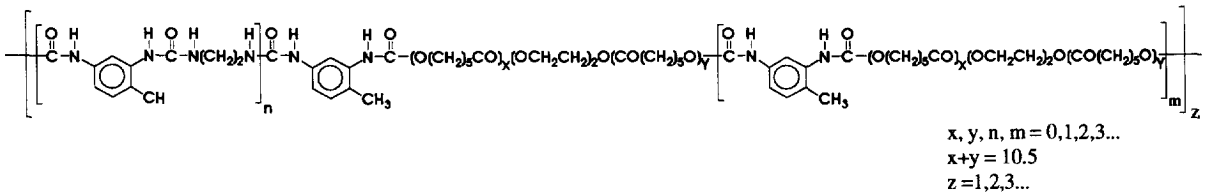

Fig. 1. Chemical structure of the polyester-urethane (TDI–PCL–ED).

TDI (98% purity), ethylenediamine (ED, 99+% purity), 2,4-toluenediamine (TDA, 98% purity), N,N-dimethylacetamide (DMAC, 99% purity) and polycaprolactone diol (PCL) with an average molecular mass of 1250. 1-Butanol (BuOH) with 99.5% purity and HPLC grade tetrahydrofuran (THF, 99.9% purity) were obtained from BDH (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) was received from Sigma with 99.9% purity. HPLC methanol (MeOH, 99.9%) was supplied by Mallinckrodt (Paris, KY, USA).

The synthesis and purification of radiolabeled polyurethanes ([^{14}C]-TDI–PCL–ED and TDI–PCL–[^{14}C]-ED) with a 2.2:1:1.2 stoichiometry of TDI, PCL and ED was described in detail elsewhere [16]. The physical characterization of the two radiolabeled polyurethanes [^{14}C]-TDI– PCL–ED and TDI–PCL–[^{14}C]-ED by gel permeation chromatography (GPC) and the determination of the specific activity in a scintillation counter (Rackbeta, LKB), was previously reported [23]. The chemical structure of this polyester–urethane is shown in Fig. 1.

BuOH was dried by distillation in the presence of sodium within 4 h of required use. Sodium wire was added into BuOH, and the mixture was boiled under reflux until the metal was dissolved. Then the BuOH was distilled off [24].

A model compound (BuOH–TDI–BuOH), with similar chemistry to one of the suspected degradation products, was synthesized to assist in the interpretation of mass spectroscopic characterization data for the degradation products. The model compound was synthesized with a 1:2 stoichiometry of anhydrous TDI and BuOH in DMAC (see Fig. 2). The reaction temperature was maintained between 60–70°C. The product solution was concentrated by evaporating DMAC under vacuum. The concentrated solution was precipitated in a mixed solvent of 25% (v/v) methanol in water. The precipitate was redissolved in methanol and precipitated in the mixed solvent again. Finally, the product was dried in an air-flow oven at 50°C for 2 days. BuOH–TDI–BuOH was characterized by a tandem mass spectrometer (see Section 2.5). Its purity was determined by HPLC (see Section 2.4).

2.2. Biodegradation

Three sample groups were prepared. Group A consisted of 500 polyurethane-coated glass tubes (25 mm×2.0 mm I.D.×3.0 mm O.D.) with 0.2 U/ml (0.06 mg/ml) of cholesterol esterase in 0.05 *M* sodium phosphate buffer (pH 7.0). A control group (B), contained the same number of coated glass tubes but was incubated with only the buffer solution. Polymer coating procedures were previously described [16]. A third group (C) was made up of 500 non-coated glass tubes and the CE solution which was used for group A. The latter group served as a test solution for validation of the deproteinization process. All samples were incubated for seven days at pH 7.0 and 37°C. Further details on the description of the experimental procedure were provided in previous publications [16,23].

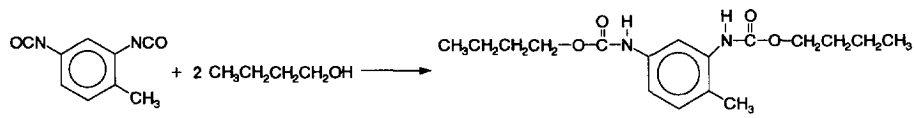

Fig. 2. Chemical structure of the model compound.

Table 1
HPLC mobile phases for the separation of degradation products

Time (min)	Flow-rate (ml/min)	Port A[a] (%)	Port B[b] (%)
0	1.0	5	95
130	1.0	70	30
150	1.0	100	0

[a] Methanol.
[b] Mixed solvent including 0.002 mol/ml ammonium acetate buffer (pH 7.1), with 4.76% methanol (v/v).

2.3. Isolation of degradation products

UF-CL and UF-20 filter units (from Millipore) were used to remove the solution components with molecular masses higher than 5000 and 10 000 respectively. Prior to using the filter unit to isolate the biodegradation products it was necessary to evaluate the effectiveness of its membrane in isolating the protein contaminants. Since the pore size of the UF-20 filter (MWCF 10 000) is larger than that of the UF-CL filter (MWCF 5000), the latter was used to test the effectiveness of removing CE. For this study, a 3.0 mg/ml solution of CE in the initial HPLC mobile phase [0.005 *M* potassium phosphate buffer (pH 7.0), containing 5% methanol (v/v)] was prepared for the evaluation. A TDA standard solution made up in the same incubation buffer was also filtered with the UF-CL filter units in order to estimate the loss of TDA following the filtration.

The incubation solutions from the three sample groups in the biodegradation study were collected and filtered with UF-20 filter units in order to deproteinize the samples, and then dried in a freeze dryer. HPLC grade methanol was added to the solid deposits and stirred for 2 h in order to extract the degradation products from the deposits. The liquid phase was separated by centrifugation. The extraction was repeated five times. The solid deposits were then extracted with HPLC grade THF in order to obtain products which may have a higher solubility in THF than in methanol. In the case of the degradation products released from the ^{14}C-labeled polyurethanes, the extraction solutions were counted in order to monitor the loss of radioactive products in the filtration and extraction process. Finally the organic phases were combined together and dried by a flow of nitrogen. HPLC samples were prepared by dissolving the dried extracts in the initial HPLC mobile phase (see below). The sample solutions were filtered with UF-CL filter units in order to ensure complete deproteinization.

2.4. High performance liquid chromatography (HPLC)

A Waters HPLC system was used for chromatographic separation of degradation products. It consisted of a 600E multisolvent delivery system, a UK6 injector and a 996 photodiode array (PDA) detector. A Millennium 2010 chromatography manager system (Waters) was used to acquire and process data from the HPLC experiment. A C_{18} μBondapak column (10.0 cm×8.0 mm) from Waters was selected to run all the chromatographic separation experiments. The gradient HPLC mobile phase programs are described in Tables 1 and 2. All the mobile phases were

Table 2
HPLC mobile phases for the separation of product 2

Time (min)	Flow-rate (ml/min)	Port A[a] (%)	Port B[b] (%)	Port C[c] (%)
0	1.0	0	100	0
15	1.0	2	98	
24	1.0	8	0	92
75	1.0	25	0	75

[a] Methanol.
[b] Mixed solvent including 0.002 mol/ml ammonium acetate buffer (pH 7.0) with 4.76% methanol (v/v).
[c] Water.

filtered and degassed by ultrasonication. HPLC chromatograms are displayed at 220 nm, based on the optimal absorbance of TDA (see Fig. 3) and its polymer-associated derivatives. The UV spectra of each peak at the apex and two offsets at ±30% from the apex were also obtained. This assisted in determining the purity of the fractions for selected peaks.

The HPLC fractions that contained individual degradation products were collected, based on the start and end of the product peaks in the chromatograms. These HPLC fractions were mixed with 10 ml LSC cocktail (Formula 989, Packard Instrument) and then counted in a liquid scintillation counter, in order to determine whether the degradation products contained released radiolabeled TDI or ED segments. Products containing elevated levels of radiolabeled TDI segments were collected for subsequent mass spectrometric analysis.

The purity of the model compound in Fig. 2 was determined, using a gradient mobile phase starting with 50% methanol and 50% water and finishing with 100% methanol within 40 min.

2.5. Mass spectrometry

Ion spray mass spectrometric experiments were carried out on an API-III triple quadrupole mass spectrometer (MS–MS) (Perkin–Elmer/Sciex, Concord, Canada). The third quadrupole was used as a mass analyzer to obtain the molecular mass analytes in MS and MS–MS analysis. In MS–MS analysis, the second quadrupole was used in a reaction region for collision-induced dissociation of a selected parent ion by the first quadrupole and the fragmentation of fragment ions are assigned by the third quadrupole. This permitted obtaining the MS–MS spectrum of a selected parent ion. The degree of the fragmentation was adjusted by the pressure of collision gas (argon) in the second quadrupole and was set such that the collision gas target (CGT) had a value in the range of 120–250.

The HPLC fractions collected from the HPLC separation were injected thought a 7125 injector (Rheodyne) with a varying volume in the range 1–20 μl, depending on the concentration of degradation products. Samples were delivered into the ionization source with HPLC grade methanol which was

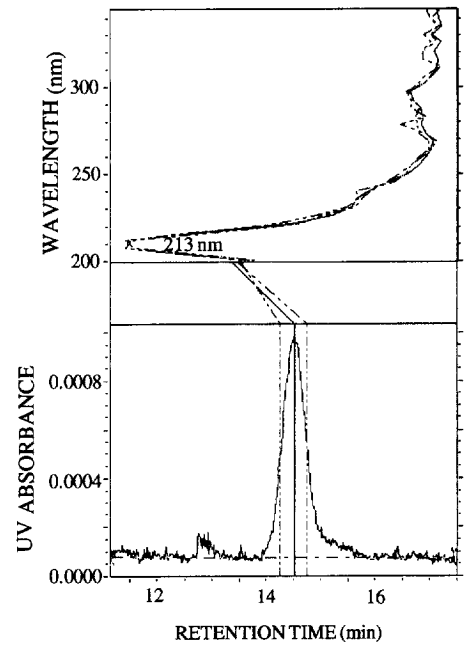

Fig. 3. HPLC chromatogram and UV spectrum of TDA standard.

pumped at a 0.02 ml/min flow-rate, using an LKB (Bromma, Sweden) HPLC pump. The voltage applied to the tip of the ion spray needle was 5.0 kV and the voltage applied to the orifice (where the ion passed into the mass spectrometer) was 80 V. The accuracy of the m/z measurement, within the range of 100–2000 amu, is better than ±0.5 amu. The data was acquired at a 0.001 second dwell time and a 0.2-amu step.

3. Results and discussion

3.1. Characterization of model compound

The chemical structure of the synthesized model compound (shown in Fig. 2) was confirmed by MS–MS analysis (see Fig. 4). The relative purity of the model compound was determined by HPLC, which showed a single dominant peak at 30.5 min in Fig. 5.

3.2. Validation of deproteinization process

Fig. 6 shows the UV spectra of the CE solution before (A) and after (B) the filtration and the TDA

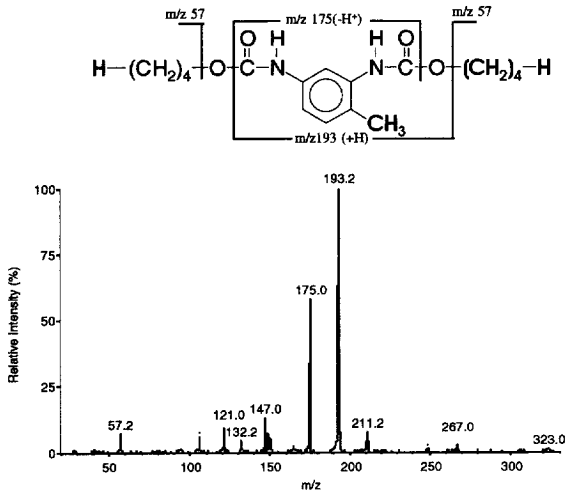

Fig. 4. Mass spectrum of the model compound.

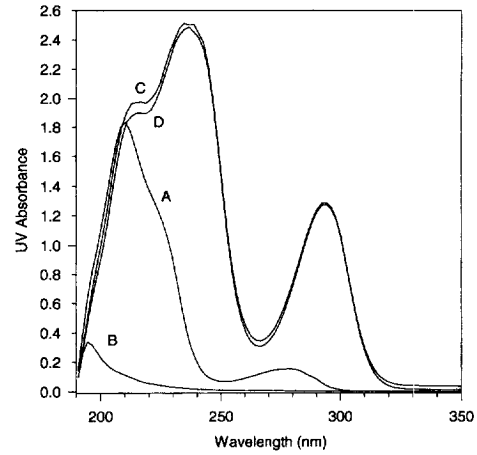

Fig. 6. UV spectra of CE before (A) and after (B) filtration and UV spectra of TDA standard before (C) and after (D) filtration.

solution before (C) and after (D) the filtration with the UF-CL filter units. These spectra indicate that the filtration with UF-20 and UF-CL filter units deproteinized the biological samples and validated their use for the subsequent separation of enzyme from the biodegradation products. The loss of TDA after filtration was less than 5%, based on the UV absorbance of the TDA before and after the filtration.

The radioactivity of the incubation solution in group A (5.7×10^4 counts/min) before and (4.3×10^4 counts/min) after the filtration showed a 20% loss in radioactivity due to filtration. This suggests that there was larger-size degradation products from the polymer which were stuck on the membrane of the filter while the small-size products such as suspected TDA (see Fig. 6) could go through the membrane.

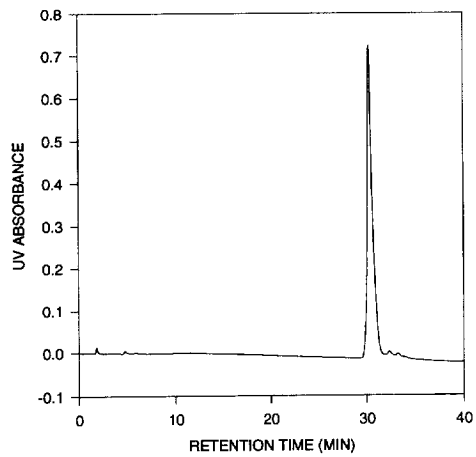

Fig. 5. HPLC chromatogram of the model compound

3.3. Chromatographic separation

The amount of released degradation product from the polymer was estimated from the specific radioactivity of the polymer [23], which permitted the conversion of the released radioactivity into the weight of the released products (see Table 3). As shown in Table 3, CE cleaved the polymer chains and released degradation products at approximately ten times the level of the degradation observed for group B, which was incubated in the buffer solution. The mixture of the degradation products were separated by HPLC in order to isolate individual degradation products which were subsequently identified by MS–MS.

Fig. 7(1) shows the HPLC chromatograms for groups A (polymer incubated with CE solution), B (polymer incubated buffer) and C (CE solution). The fractions corresponding to significant peaks in chromatogram A were collected, based on the width of their peaks, and counted for radioactivity. Fig. 7(2) and (3) show the radioactivity levels of all HPLC fractions associated with the degradation products of

Table 3
Constants presenting the release of biodegradation products in three groups

Groups	Products/polymer (w/w)	Products (μg)/polymer surface area (cm^2)
A	1.60%	2.80
B	0.16%	0.26
C	0.00%	0.00

[^{14}C]-TDI–PCL–ED and TDI–PCL–[^{14}C]-ED, respectively. Specific peaks with elevated radioactivity levels in Fig. 7(2) and (3) are related to the products which contain TDI and ED segments, respectively. Chromatogram A in Fig. 7(1) contains more than 20 peaks that are suspected to be related to the enzyme-catalyzed degradation of the polyurethane. The peaks for these products are not found at similar levels in either of the control chromatograms B or C in Fig. 7(1).

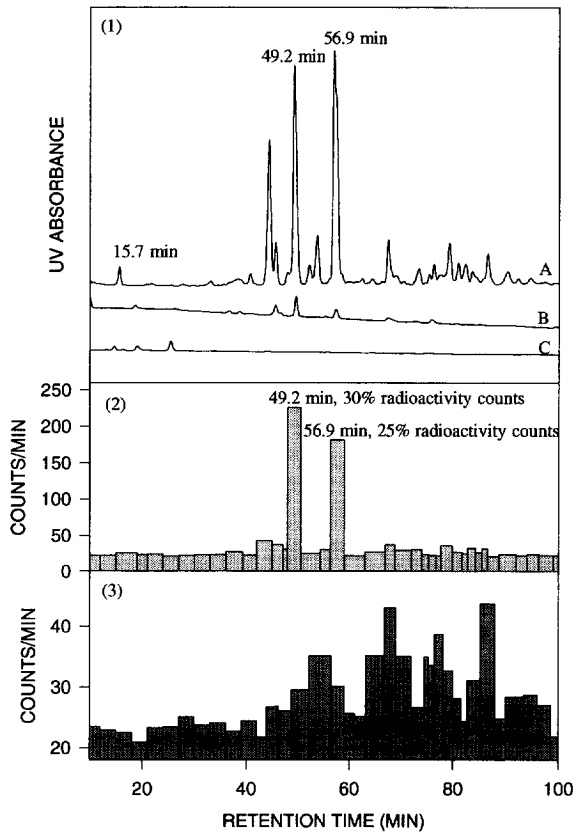

Fig. 7. (1) HPLC chromatograms of groups A (polymer incubated with CE solution), B (polymer incubated in buffer) and C (CE solution); (2) radioactivity counts for radioactive release from [^{14}C]-TDI–PCL–ED and (3) radioactivity counts for radioactive release from TDI–PCL–[^{14}C]-ED.

2,4-TDA was prepared in the initial mobile phase and was injected as an HPLC standard in order to obtain the retention time of 2,4-TDA and define the limit of detection. The retention time corresponded to the value reported in Fig. 3 which is 14.7 min, while the detection limit was defined at 2 ng. It can be seen in Fig. 7(1) that there is no peak observed for TDA at 14.7 min on either of the chromatograms for groups A and B. UV spectra of TDA before and after filtration, shown in Fig. 6, indicated that 95% of the TDA was recovered following the sample preparation process. Since the loss of TDA in the sample preparation process is negligible, the absence of the TDA peak in chromatogram A of Fig. 7(1) suggests that there was no free TDA found (or less than 2 ng of TDA) within the injected samples. Based on the specific radioactivity of the original polymer [23], it was estimated that the injected sample contained approximately 28 μg of a mixture of the degradation products. The background level of radioactivity observed for the fraction at 14.7 min in Fig. 7(2) further confirms the absence of TDA as a significant product in the mixture.

The peaks at 49.2 min (product 1) and 56.9 min (product 2) in chromatogram A of Fig. 7(1) are the two most abundant degradation products as detected by UV absorbance. Fig. 7(2) also shows that the majority of degraded radiolabeled TDI segments are contained within these two principal products.

Fig. 7(3) represents the distribution of radiolabeled -ED- degradation products from TDI–PCL–[^{14}C]-ED, which is specifically representative of the polyurethane's hard segment component (TDI–ED). Note that no significant radioactive peaks were detected at the retention time of 49.2 and 56.9 min. This indicates that the two principal products, identified in Fig. 7(1) are primarily associated with soft

segment components (TDI–PCL) of the polyurethane.

3.4. Mass spectroscopy

The fractions containing products 1 and 2 at 49.2 and 56.9 min respectively were collected and analyzed by MS–MS. Their mass spectra are shown in Fig. 8. For product 1, four ions show higher intensities at the mass-to-charge ratios (m/z) of 439, 456, 461 and 477 (see Fig. 8A), while the dominant ions of product 2 were found at 413, 430, 435 and 451 (see Fig. 8B). It was suspected that the multiple ions for each degradation product were present as a result of product association with different cations found in the sample itself and in the mobile phase. The ions would include H^+ (mass 1), NH_4^+ (mass 18), Na^+ (mass 23) and K^+ (mass 39). The suspected molecular ions at m/z of 413, 430, 435 and 451 for product 2 in Fig. 8B were selected as parent ions in order to study the fragmentation of the product and confirm the common relationship between the ions. Fig. 9A, B, C and D depict the daughter ion scans of the four parent ions of product 2. The ions at m/z of 413 (Fig. 9A) and 430 (Fig. 9B) show a similar fragmentation pattern, since several fragment ions were

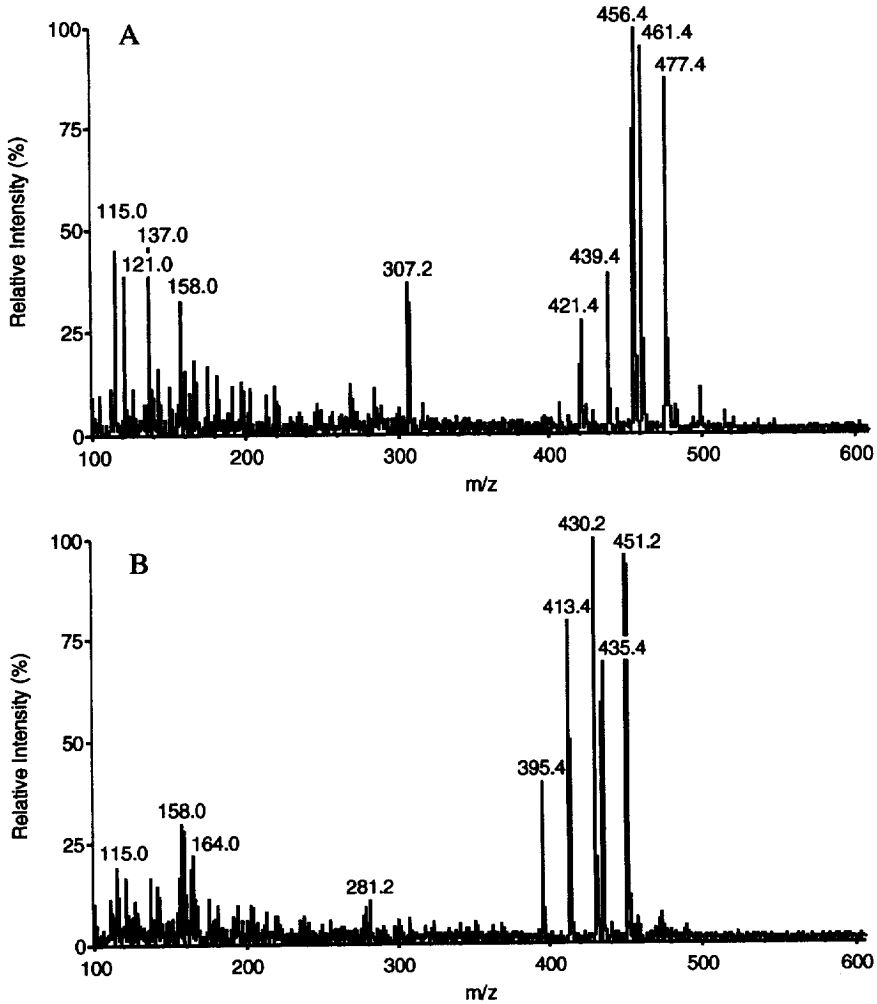

Fig. 8. Mass spectra of (A) degradation product 1 and (B) degradation product 2.

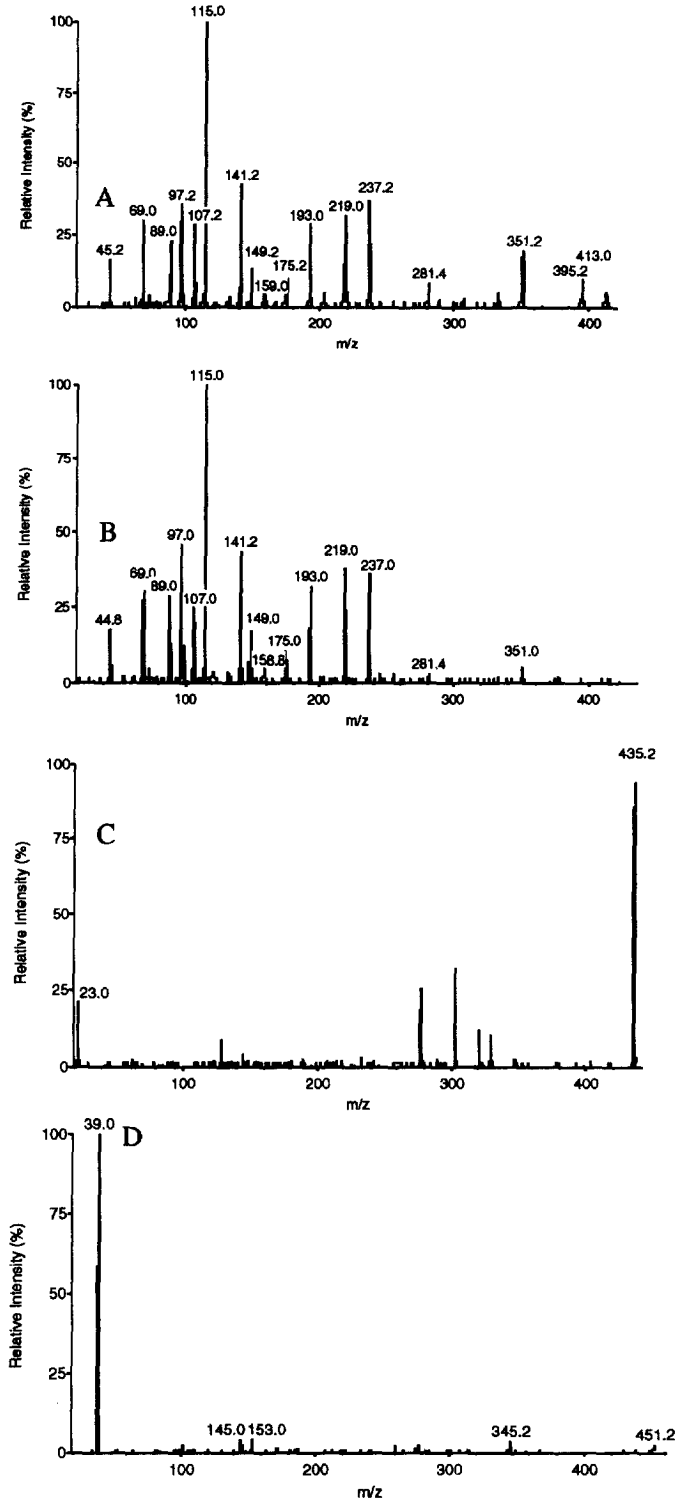

Fig. 9. Daughter-ion scan of ions m/z 413 (A), 430 (B), 435 (c) and 451 (D) of product 2.

observed in both MS–MS spectra. The association of H^+ (mass 1) and NH_4^+ (mass 18) with product 2 (mass 412) results in the generation of molecular ions at m/z 413 and 430. Ions Na^+ (m/z 23) and K^+ (m/z 39), respectively, were associated with the parent ions m/z 435 (Fig. 9C) and 451 (Fig. 9D). Since it was suspected that these counter ions have significantly different binding energies with product 2 from H^+ and NH_4^+, the fragmentation pattern for m/z 435 and 451 are different from those for m/z 413 and 430. A similar analysis has been done for product 1 in Fig. 8A and the four parent ions at m/z 439, 456, 461 and 477 have been attributed to product 1 associated with ions H^+, NH_4^+, Na^+ and K^+.

It was noted earlier from radioactivity counts (Fig. 7) that products 1 and 2 were specifically associated with the soft segment components (TDI–PCL) of the polyesterurethane. This implies the existence of urethane linkages in their structure. Since the two principal products 1 and 2 were attributed to derivatives of TDI with urethane substitutes, the fragmentation pattern of the model compound BuOH–TDI–BuOH (Fig. 2) was used to assist in the identification of fragment ions of the degradation products. The daughter ion MS–MS spectrum of BuOH–TDI–BuOH was shown in Fig. 4. The protonated molecule ion is located at m/z 323. Two product-ions, at m/z 175 and 193, are characteristic of the model's fragmentation to a compound with the toluene moiety connected to two urethane linkages. The two ion peaks can be used to recognize the chemical structure of the urethane–toluene–urethane component associated with the mass spectra of degradation product 1 (Fig. 10) and 2 (Fig. 9A).

The protonated molecule ion (MH^+, m/z 439) of product 1 was selected for the daughter ion scan. The mass spectrum is shown in Fig. 10. Two ions are noted at m/z 175 and 193, which correspond to the two fragment ions of the model compound (see Fig. 4). This finding confirmed the existence of a urethane–toluene–urethane component. The ion at m/z 115 is attributed to an oxycaproic acid group, which produced an ion at m/z 97 by losing H_2O and m/z 69 by losing H_2O and CO. The proposed chemical structure of product 1, which is given in Fig. 10, is consistent with these analytical data. The structure indicates that the enzyme cleaved the two adjacent ester bonds of the polyurethane's PCL segments, at both sides of the urethane–toluene–urethane component, in order to release product 1 from the polymer backbone.

The MS–MS spectrum for protonated degradation product 2 (Fig. 9A) also shows the presence of the two ions (m/z 175 and 193) found from the mass spectrum of the model compound (Fig. 4). Hence,

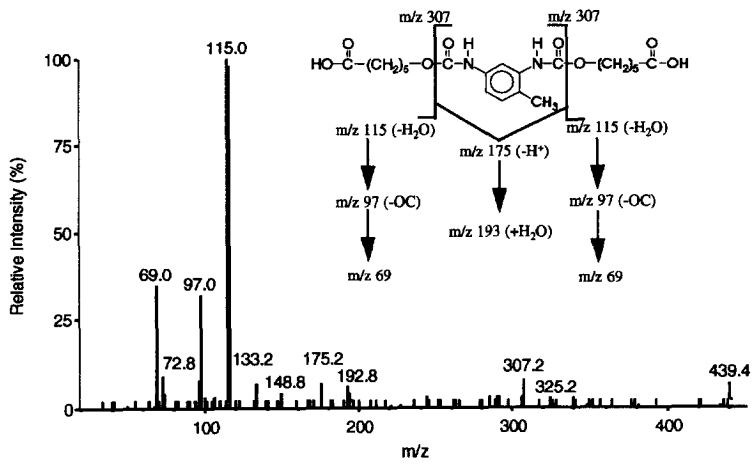

Fig. 10. Daughter-ion scan of protonated product 1 and chemical structure of product 1.

this product also contains the urethane–toluene–urethane component in the fragmentation profile. The three ions (m/z 69, 97 and 115) related to oxycaproic acid are also observed in Fig. 9A, but additional ions at m/z 45 and 89 were found. These latter ions have been attributed to the substitution of a diglycol component for one of the oxycaproic acid groups. This combination exists as a result of the TDI monomer having reacted with a diglycol terminated PCL monomer during the polymer synthesis. The proposed chemical structure of product 2 is shown in Fig. 11. It is known that diglycol was used in the synthesis of the PCL by Aldrich (personal communication with the technical service department of Aldrich Chemical) and that each PCL molecule contains one diglycol unit that is distributed among the oxycaproate units or at the end of the PCL (see Fig. 1). Therefore, product 2 was generated from the biological hydrolysis of the ester bonds of PCL segments containing hydroxyl caproate- and diglycol-terminated groups.

It should be noted that by exchanging the positions of the oxycaproic acid group and the diglycol group in product 2, two isomers are generated (see Fig. 11). To confirm the existence of these isomers, the HPLC separation was optimized with an alternate method (see Table 2). The HPLC chromatogram for this subsequent separation is shown in Fig. 12. Product 2 located at 56.9 min in Fig. 7(1) was further separated into two peaks with the new method and yielded two products with retention times at 64.2 and 65 min (Fig. 12). It is observed that the two products

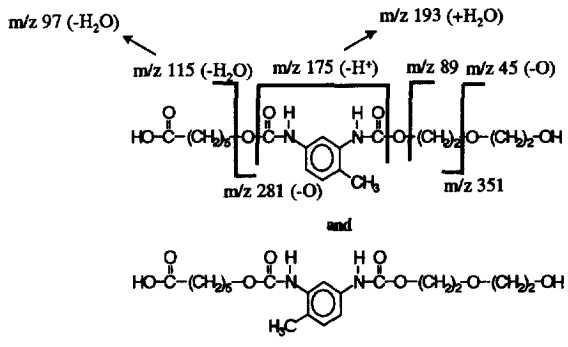

Fig. 11. Chemical structure of product 2.

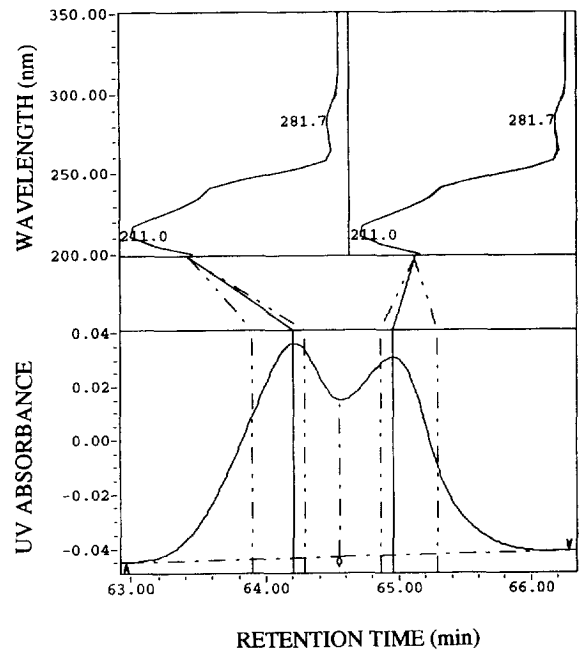

Fig. 12. HPLC chromatogram for the separation of the two isomers of product 2, including their associated UV spectra.

(isomers) have similar UV spectra. As well, these two fractions were analyzed by MS and exhibited the same fragmentation pattern as shown for degradation product 2 in Fig. 9.

4. Conclusion

The above methods were shown to be successful in analyzing the biodegradation products of a biomedical polyurethane, synthesized with TDI, the precursor to the suspected carcinogen TDA [7–10]. The method, including the isolation and chemical analysis of samples from the biological system, can be considered as an effective tool to characterize organic chemical compounds from the biodegradation of biomaterials and bio-erodible polymeric drug release systems. The sample isolation protocol, which combined freeze drying, solid–liquid extraction and ultrafiltration, removed most proteins and prepared the sample in a relatively pure form. The

biodegradation of polymeric materials can release many degradation products into solution, however, to date most studies which have investigated the biodegradation of polyurethanes [2,3,11,12] have only focused on the detection of diamine-containing products and more specifically TDA from a polyester–urethane [4,5]. Complete separation is a requirement for further product identification. To achieve this, the removal of protein is required. The classical methods of removing protein have often resulted in the conversion of primary degradation products into other species. The information derived from such data could provide a misleading interpretation of chemical structure for biomaterial-derived biodegradation products. The ultrafiltration step presented here has eliminated this concern and allowed for the identification of several products.

In this work, the reversed-phase HPLC provided a significant separation of more than 20 products associated with polyurethane biodegradation. The detection of peak purity by the use of the photodiode array detector led to the successful optimization of HPLC separation methods. In MS–MS analysis, the first scan for molecular ions further filtered the ions fragmented from the samples and impurity ions from the mobile phase and allowed for the analysis of a uniform compound in the second mass scan. This approach readily permits the samples to be further separated after the chromatographic separation. This is a great advantage for studying polymer biodegradation, since the complications, due to multiple enzymatic degradation products, often lead to difficulty in completing chromatographic separation.

Future work will use the developed methods for the identification of the all degradation products and the subsequent analysis of commercial biomedical implant materials.

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References

[1] M.D. Lelah, S.L. Cooper, Polyurethanes in Medicine, CRC Press, Boca Raton, FL, 1986.
[2] Expert Panel on the Safety of Polyurethane-covered Breast Implants, Canada, Can. Med. Assoc. J., 145 (9) (1991) 1125.
[3] C.P. Sharma, M. Szycher, Blood Compatible Materials and Devices, Technomic Publishing, Lancaster, USA, 1991.
[4] P. Amin, J. Wille, K. Shah, A. Kydonieus, J. Biomed. Mater. Res. 27 (1993) 655.
[5] C. Batich, J. Williams, K. Roy, J. Biomed. Mater. Res. 23 (1989) 311.
[6] U.S. Department of Health and Human Services, NIOSH Current Intelligence Bulletin, 53, 1989
[7] M. Umeda, GANN 46 (1955) 597.
[8] A.L. Giles, C.W. Chung, J. Toxical. Health 1 (1976) 433.
[9] R.H. Cardy, J. Natl. Cancer Inst. 62 (1979) 1107.
[10] J.M. Sontag, J. Natl. Cancer Inst. 66 (1981) 591.
[11] S.C. Chan, D.C. Birdsell, C.Y. Gradeen, Clin. Chem. 37(5) (1991) 756.
[12] S.C. Chan, D.C. Birdsell, C.Y. Gradeen, Clin. Chem. 37(12) (1991) 2143.
[13] R.S. Labow, D.G. Duguay, J.P. Santerre, J. Biomater. Sci. Polym. 6 (1994) 169.
[14] J.P. Santerre, R.S. Labow, J. Biomed. Mater. Res. 36 (1997) 225.
[15] R.S. Labow, E. Meek, J.P. Santerre, J. Biomed. Mater. Res. (1997) in press.
[16] J.P. Santerre, R.S. Labow, D.G. Duguay, D. Erfle, G.A. Adams, J. Biomed. Mater. Res. 28 (1994) 1187.
[17] T. Hanai, Liquid Chromatography in Biomedical Analysis, Elsevier, New York, 1991.
[18] G. Szpesi, HPLC Pharmaceutical Analysis II, Practical Applications, CRC Press, Boca Raton, FL, 1991.
[19] W.S. Hancock, J.T. Sparrow, HPLC Analysis of Biological Compounds, a Laboratory Guide, Marcel Dekker, New York, 1984.
[20] M.C. McMaster, HPLC A Practical User's Guide, VCH, New York, 1994.
[21] G.O.H. Peelen, J.G.N. de Jong, R.A. Wevers, Anal. Biochem. 198 (1991) 334.
[22] D.F. Hunt, R.A. Henderson, J. Shabanowitz, K. Sakaguchi, H. Michel, N. Sevilir, A. Cox, E. Applella, V.H. Engelhard, Science 255 (1992) 1261.
[23] G.B. Wang, R.S. Labow and J.P. Santerre, J. Biomed. Mater. Res. 36 (1997) in press.
[24] G. Hilgetag, A. Martini, Preparative Organic Chemistry, John Wiley, Toronto, 1972, p. 1098.