ABSTRACT

SUMANASINGHE, RUWAN DEEPAL. Stability of Poly(ethylene terephthalate) Endovascular Prostheses to Hydrolytic and Enzymatic Degradation. (Under the direction of Martin William King)

Poly(ethylene terephthalate) (PET) is a thermoplastic polymer which is widely used in the construction of woven and knitted endoprostheses for reconstructive arterial surgery. Analysis of many explanted PET vascular prostheses has given evidence of the existence of a long term in-vivo degradation of the PET material. Due to the increasing demand for less invasive methods of abdominal aortic aneurysm (AAA) repair using PET endoprostheses, there is a significant need for predicting the biostability of these materials by means of an accelerated in-vitro test. The purpose of this research has been to develop an in-vitro accelerated test protocol based on hydrolytic/enzymatic degradation of PET to predict the biostability of PET endovascular stent grafts. In addition, this study involves assessing whether the presence of nitinol stent material influences the rate of degradation of the PET graft. Non-sterile warp knitted prosthetic fabric specimens from Type 56 Dacron® PET fibers were subjected to degradation in neutral enzymatic and alkaline environments for periods of up to 9 weeks and 10 days, respectively. The alkali degradation resulted in a maximum weight loss of 80%, while degradation in enzyme showed a weight gain. Both, alkali at pH 13.4 and papain at pH 7.2 caused a change in crystallinity of the PET specimens. The behavior of the melt endotherms especially in alkali degraded specimens, indicated the presence of two types of crystalline areas in the fiber. These results correlated well with the observation made on explanted vascular prostheses. The degradation of PET in alkali was well confirmed by the exponential growth of the concentration of terephthalate anions in the degradation
solutions. Examination of the change in conformation of the ethylene glycol segments in PET fibers using FTIR gave evidence of a significant conformational change in alkali degraded specimens. Similar observations were made with specimens degraded in saline without nitinol stents after 3 and 6 weeks. A significant change in the conformation of \(-\text{CH}_2\text{-CH}_2-\) bonds occurred in enzyme degraded specimens after 3 and 6 weeks. With the alkali degraded specimens a linear correlation was shown between the loss in probe bursting strength and the duration of degradation which correlated well with the observations on explanted prostheses. SEM observations revealed the formation of pits elongated in the direction of the fiber axis after 2 days of alkali degradation. The enzyme particles were observed to be trapped and bound within the interstices and on the surface of fibers in specimens degraded with enzyme at \(50^\circ\text{C}\). Except for a minor discoloration due to alkali degradation and deposition of enzymes, the surface of the nitinol stent elements appeared to be unchanged. Based on the post-degradation bursting strength measurements, 2.6 days of \textit{in-vitro} degradation of PET endoprostheses in \(\text{pH} 13.4\) and \(65^\circ\text{C}\) produced results which correlated well with 162 ± 23 months of \textit{in-vivo} degradation. The nitinol stents did not appear to influence degradation of PET in any of the three environments.
STABILITY OF POLY(ETHYLENE TEREPHTHALATE) ENDOVASCULAR PROSTHESES TO HYDROLYTIC AND ENZYMATIC DEGRADATION

by
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1. Introduction and Objectives

1.1. Introduction
Since the observations made by A.B.Voorhees (1) in the 1960’s that synthetic fabrics could be used for reconstructive arterial surgery, synthetic fibrous materials such as Vinyon N®, nylon, Teflon®, Orlon® and Dacron® have been investigated for their physical characteristics and biological responses. More recently the use of a synthetic fabric in an endovascular prosthesis was proposed by C.I Dotter (2, 3, 4). Becker et al. (5) were the first to describe the deployment of a covered stent in a human. Animal studies on the treatment of abdominal aortic aneurysms (AAA) began with Parodi’s work in 1976. He used a knitted Dacron® (polyester) tube graft mounted on the outside of Palmaz stents. Parodi et al’s work continues in humans, and was first published in 1991 (6). Since then 75% of the grafts placed for the treatment of AAA have been technically successful. The first bifurcated endograft used in humans was designed by Chuter et al. Their experience in 14 dogs was published in 1993 (7).

The conventional treatment for aortic aneurysms involve a high risk, invasive surgical procedure during which the surgeon opens the abdomen to repair the damaged portion of the aorta and replaces it with a textile graft. Recovery from the procedure can take up to six weeks. Therefore aortic stent grafts implanted during a minimally invasive procedure offer several advantages, such as reduced operative trauma, shorter stay in hospital and the possibility of treating patients who would have been unfit for open surgery. Stent grafting for abdominal aortic aneurysms is made more difficult due to the fact that in over 95% of cases the aneurysm extends to the aortic bifurcation. Thus a tube stent graft is only rarely applicable, making a bifurcated system necessary, which adds to the complexity of the equipment and the procedure (3, 8).

Recent developments in endoprostheses have been based on the combination of stent and grafts technology. Basically the currently produced endoprostheses contain two distinct parts – the wire stent and a textile structure. The wire stent may be made from nitinol (nickel titanium alloy), which is a shape memory alloy that returns to its preset
dimensions above 37 °C, or from self-expandable, or from balloon expandable stainless steel \(^2, 9\). The shape of the stent may depend on the particular design. Many manufacturers use a zigzag shape nitinol wire frame elements. The wire frame elements can be connected to each other so that they lie around the textile structure. A number of these wire frames sets may be attached to each other by multiple ligatures or by entangling them with each other or welding them in order to distribute them uniformly along the length of the textile structure. When connected by ligatures, the ends of ligatures are fused by heat to prevent unraveling. The textile structure is usually of a woven construction and may be made out of polyester (Dacron\(^\text{®}\)), expanded polytetrafluoroethylene (PTFE) or less frequently polyurethane continuous filament yarn. The metallic stent is attached either inside or outside of the woven textile structure by multiple ligatures. The attachment points may be only at the proximal and distal ends or uniformly continue from one end to the other \(^{10}\).

1.2 Problem Statement

Despite the increasing number of uses and the advantages brought forth by the endovascular technique, clinical trials and research have been able to pinpoint some of the drawbacks connected with. One of the major problems, which has gained a great concern among surgeons, is the problem of periprosthetic leak \(^{11}\). Periprosthetic leaks can be caused by insufficient seal of the proximal or distal stents of the device and leaks from the stent graft itself, either at the connections of different components of the stent system or through tears in the prosthetic cover \(^{10, 12, 13}\). The pulsatile blood circulation and movement of aorta lead to micro movement of the stent wires. Most movement takes place in the large frames of the body middle ring in the stent grid. The twisting of the wires within the ligature lead to wear and finally fatigue with ruptures of the ligatures of frames in body middle rings \(^{14}\). The micro-movement of the wires can also cause abrasion between the textile structure and the wire surface leading to disintegration of the textile structure creating holes in it. A woven fabric construction plays a major role in providing adequate stability to an endoprostheses. Changes that occur in the textile structure such as yarn shifting, distortion, filament breakage and holes may consequently
lead to blood leakage in the long term\textsuperscript{(10, 11)}. These may result from defects in the fabric’s structure or from damage that occurs during deployment.

Among the types of materials used for the construction of endoprostheses, Dacron\textsuperscript{®} poly(ethylene terephthalate) (PET) has gained wide acceptance over the years for exhibiting excellent material properties and also proving to be biostable. Due to the increased number of arterial reconstruction using Dacron\textsuperscript{®} PET prostheses, in younger patients with longer life expectancies, increasing attention has been given to predicting the long-term \textit{in-vivo} stability of these materials. A number of research studies have been carried out to determine the cause of failure of grafts implanted in humans as well as to predict the time of complete degradation. In 1979 Rudakova et al \textsuperscript{(15)} predicted complete degradation of polyester fibers after 30 years of implantation in humans and dogs. From the studies carried out on retrieved Dacron\textsuperscript{®} PET grafts from humans, King et al \textsuperscript{(16)} predicted that a 25\% loss in molecular weight can be expected after 162 ± 23 months of implantation. It has been suggested that the most likely mechanism of failure of grafts implanted in humans is due to hydrolytic degradation of the PET polymer chain\textsuperscript{(17)}.

1.3. Purpose of Research
The degree of degradation of poly(ethylene terephthalate) fibers can be assessed by monitoring the bursting strength of the fabric, by determining the carboxylic end groups of the polymer, and by taking molecular weight measurements. Most of the studies carried out on the degradation of vascular prostheses have been based on long-term retrieval programs, which consume an overwhelming time period and also demand substantial amounts of funds. Due to this, a greater emphasis is now being given to formulating accelerated \textit{in-vitro} tests that will predict the long-term \textit{in-vivo} degradation of PET vascular prostheses.

\textit{The objective of this study is to devise a method of predicting the long-term biostability of the PET fabric used in the fabrication of endovascular prostheses by determining their rate of hydrolytic and/or enzymatic degradation in an \textit{in-vitro} accelerated aging test.}
In particular, this study will follow the changes in physical and chemical properties of PET prosthetic fabric during accelerated *in-vitro* tests. The findings of this study will be compared with the changes in chemical and physical properties of retrieved samples reported previously in order to conclude whether or not the accelerated test conditions reproduce the *in-vivo* degradation mechanism.

### 1.4. Outline

In the following chapters, a comprehensive review of PET vascular prostheses and the phenomenon of hydrolytic degradation will reveal the rationale behind the current investigation into the accelerated *in-vitro* degradation study. The scope of the experimental work involves the preparation of hydrolytic and enzymatic solutions, following the hydrolytic/ enzymatic degradation process for periods of up to 9 weeks, and assessing the rate of degradation of PET fabric specimens. Since endovascular devices invariably contain a metallic stent component, the rate of degradation of the PET graft fabric with and without the presence of nitinol stent wires will also be monitored so as to determine the effect of the stent on the PET degradation process.

### 1.5. Limitations

It was planned to carry out the degradation studies at elevated temperatures as well as at body temperature. One potential limitation is the difficulty in correlating an accelerated *in-vitro* test to long term *in-vivo* degradation. The most suitable method of correlating these two different conditions is to analyze the change in physical and chemical properties of the polymer when the polymer is exposed to these conditions. Similarities between the behavior of polymer physical and chemical properties would enable an assessment of the two different degrading conditions. The other limitation which existed in this study was the limited amount of material to carry-out random sampling during post-degradation evaluation. This especially influenced the results of the mechanical
tests, such as the bursting strength test, which require substantial number and amount of specimens to minimize the variation between repetitive measurements.
2. Review of Literature

2.1. Polymeric Biomaterials for Endoprostheses

2.1.1. Selection

Polymers are a promising class of biomaterials that can be engineered to meet specific end use requirements. They can be selected according to key characteristics such as mechanical resistance, degradability, permeability, solubility and transparency. Any polymeric biomaterial that is intended to be used for a vascular prosthesis should consist of the following properties (18, 19, 20).

(1) Non-thrombogenic.
(2) Allow little or no blood loss (hemostatic)
(3) Biocompatible.

Hemocompatible
- non-destructive to blood cells and enzymes
- should not cause depletion of the blood electrolytes \(^{(21)}\).
- no adverse immune responses
- should not alter plasma proteins

Non-cytotoxic
- should not damage or impair the function of cells.

Non-carcinogenic
(4) Sterilizable.
(4) Low rate of infection when exposed to bacteremia
(5) An optimum range of tensile strength
(6) An optimum level of resilience, fatigue endurance and biostability. This means it should retain its chemical and mechanical properties such as compliance and elasticity during functional use.
(7) Should possess a certain level of porosity.
From the point of view of biostability or bio-absorbability, polymeric biomaterials can be divided into two groups \(^{(22)}\).

- Bioabsorbable polymers
- Non-bioabsorbable polymers

A variety of terms associated with biodegradation of polymers are used including degradation, bioabsorption, bioerosion and resorption \(^{(22)}\). The term biodegradation can be used in a wide sense for any degradation occurring in a biological environment. A bioinert material does not elicit severe foreign body reactions, such as activation of the immune system or blood coagulation leading to thrombus formation. To date no material has been reported to be entirely bioinert \(^{(18, 22, 23)}\).

Microscopic degradation of polymeric materials can take place either by random chain scission (which remarkably reduces the MW and mechanical strength with an insignificant loss of mass) or by zipper like scission from end of the polymer chain, in which case mass and the molecular weight decrease linearly. If a detectable loss of mass takes place, even if it occurs at a much later stage than the molecular weight reduction, then the polymer can be regarded as bioabsorbable. A bioabsorbable polymer is degraded into water-soluble, low molecular weight compounds such as monomer units, which are then absorbed by surrounding body fluid. If these compounds interact with cell surfaces or penetrate cell membranes such an absorbable polymer might give adverse effects. Therefore the selection of a bioabsorbable polymer must be made with much more caution than for nonbioabsorbables \(^{(22)}\).

### 2.1.2. Desirable Properties

#### 2.1.2.1. Biocompatibility

Polymer biocompatibility refers to the reactions of polymer with blood, other body fluids and adjacent tissues, which will depend on the site and purpose of the implant. For vascular grafts, biocompatibility is determined largely by specific interactions with blood and its components \(^{(18, 19, 22)}\).
2.1.2.2. Surface Functional Characteristics
Polymers for the manufacture of vascular grafts or used for tissue engineering are selected with the aim of modifying their surface chemistry so as to optimize the cell polymer interactions. The polymer surface that does not absorb any plasma proteins must be considered to be blood compatible, with the additional advantage that a device fabricated from completely blood compatible materials does not require immune suppression of the patient after implantation. An essential surface characteristic of a prosthetic material is its hydrophilicity or hydrophobicity, lubricity, smoothness and surface energy. The surface must be non-thrombogenic. The surface charge and energy should then be considered, because they regulate the fluid/material interactions within the host. In general, a higher charge density is required to reduce protein adsorption and this in turn promotes thromboresistance of the surface. A higher charge is desirable when in continuous contact with blood.

Water absorption also plays a key role in strength, creep resistance, biostability and durability of materials, which may be affected by hydrolytic degradation. Vascular grafts should possess water repellent properties for long-term biostability and resistance to degradation, which is promoted by fluid adsorption.

2.1.2.3. Bulk Properties
The bulk morphology determines many of the physical properties of a biomaterial. Mechanical strength, density, elasticity, ageing and creep resistance play an important role in the selection of polymers for materials in tissue engineering and vascular grafts.

2.1.2.4. Tissue Engineering Matrices
A variety of natural and synthetic polymers have been used to fabricate tissue-engineering matrices. Bioabsorbable polymeric systems are particularly attractive in tissue engineering. One of the main advantages is that they eliminate the need for surgical removal of the polymer matrix.
2.1.2.5. Sterilization and Stability
Polymeric devices used as prostheses must be prepared by aseptic processing under good manufacturing practice conditions and sterilized or disinfected before medical use. The sterilization method (wet or dry heat, radiation or chemical treatment using eg. ethylene oxide) should not cause structural changes or lead to chain scission, cross-linking or a significant alteration in mechanical properties. Biostability is the basic criterion for use of a polymer in an implant unless of course it is a bioabsorbable implant, in which case wet and chemical sterilization methods are more of a challenge (18, 19, 22).

2.1.3. Performance and Evaluation
In determining the biostability of an implant, the material must be examined for chemical changes, such as chemical degradation or other changes in molecular structure, changes in mechanical properties, wear particles and cellular ingrowth (19).

A series of tests are usually carried out to determine the properties and performance of vascular prostheses. These have been outlined in Table 2.1.
Table 2.1. Performance evaluation of vascular prostheses

<table>
<thead>
<tr>
<th>In-vitro Evaluation</th>
<th>In-vivo Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Textile Properties</td>
<td></td>
</tr>
<tr>
<td>Fabric structure and fiber morphology</td>
<td>Surgery (Implantation)</td>
</tr>
<tr>
<td>Stitch count or woven fabric count</td>
<td>Follow up (Angiographic findings)</td>
</tr>
<tr>
<td>Filament diameter</td>
<td></td>
</tr>
<tr>
<td>Yarn linear density</td>
<td></td>
</tr>
<tr>
<td>Porosity</td>
<td>Graft Harvesting</td>
</tr>
<tr>
<td>Physical Properties:</td>
<td>Determination of changes in dimensional,</td>
</tr>
<tr>
<td>Water permeability</td>
<td>mechanical and chemical properties.</td>
</tr>
<tr>
<td>Bursting strength</td>
<td></td>
</tr>
<tr>
<td>Suture retention strength</td>
<td></td>
</tr>
<tr>
<td>Dilatation under internal pressure</td>
<td>Histological analysis</td>
</tr>
<tr>
<td>Elongation at break</td>
<td>(Patency and microscopic evaluation)</td>
</tr>
<tr>
<td>Chemical properties:</td>
<td>Enzyme analysis</td>
</tr>
<tr>
<td>Surface chemistry</td>
<td>Immunohistochemical markers</td>
</tr>
<tr>
<td>Fourier Transform Infrared (FTIR) Spectroscopy</td>
<td></td>
</tr>
<tr>
<td>Differential scanning calorimetry (DSC)</td>
<td></td>
</tr>
<tr>
<td>Levels of extractable</td>
<td></td>
</tr>
</tbody>
</table>

2.2. Poly(ethylene terephthalate) (PET)

2.2.1. Synthesis

PET is prepared in two stages from dimethyl terephthalate and ethylene glycol. In the first stage a precondensate is formed which consists of the di-glycol ester together with a certain amount of oligomers. This transesterification is catalyzed by small amounts of metal salts (Zn, Mn, Cd, Mg etc.) and is carried out at 200 °C. The methanol formed is continually removed by distillation to enhance the formation of sufficiently long chains. In the second stage, polycondensation of the precondensate is carried out at 280 °C under high vacuum (< 1 torr) with the addition of a heavy metal salt catalyst (usually Sb) and
the continual removal of the glycol byproduct \(^{(24)}\). The entire polymerization can be presented as below.

\[
\begin{align*}
2 \text{HOCH}_2\text{CH}_2\text{OH} & \quad + \quad \text{O} & \text{O} \\
& \quad + \quad \text{CH}_3\text{OC}-\overset{\cdot}{\text{C}}-\text{COCH}_3 & \quad \text{Dimethyl terephthalate} \\
& \quad + \quad \text{HOCH}_2\text{CH}_2\text{OC}-\overset{\cdot}{\text{C}}-\text{COCH}_2\text{CH}_2\text{OH} & \quad + \quad 2 \text{CH}_3\text{OH} \\
& \quad + \quad \left(\text{CH}_2\text{CH}_2\text{OC}-\overset{\cdot}{\text{C}}-\text{CO}\right)_n & \quad + \quad \text{HOCH}_2\text{CH}_2\text{OH} \\
\text{(Poly(ethylene terephthalate) – PET)} & \quad + \quad \text{(Ethylene glycol)}
\end{align*}
\]

Orientation of the non-crystalline phase i.e. presence and extent of molecular order has a significant effect on the physical and mechanical properties of the polymer. The volume of the amorphous areas of the polymer link the sections of crystalline polymer together in the form of tie molecules. The density of these tie segments and the extent to which they are extended out of their normal random coil conformation during fiber spinning and drawing, along with the secondary forces between the chains determine the physical properties of the product, such as tensile strength \(^{(25)}\).
2.2.2.  Properties

The physical and chemical properties of a typical fiber forming poly(ethylene terephthalate) polymer are presented in Table 2.2.

<table>
<thead>
<tr>
<th>Property</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Conformation</td>
<td></td>
<td>Nearly Planar (crystallized)</td>
</tr>
<tr>
<td>Molecular weight (of repeat unit)</td>
<td>g mol⁻¹</td>
<td>192</td>
</tr>
<tr>
<td>Weight Average Molecular weight</td>
<td>g mol⁻¹</td>
<td>30,000 – 80,000</td>
</tr>
<tr>
<td>Density</td>
<td>g cm⁻³</td>
<td>1.38</td>
</tr>
<tr>
<td>Melting Point</td>
<td>°C</td>
<td>260 - 265</td>
</tr>
<tr>
<td>Glass Transition temperature</td>
<td>°C</td>
<td>65 - 80</td>
</tr>
<tr>
<td>Heat of Fusion (ΔH)</td>
<td>KJ mol⁻¹</td>
<td>24.1</td>
</tr>
<tr>
<td>Dry Tenacity</td>
<td>N/ tex</td>
<td>0.44</td>
</tr>
<tr>
<td>Breaking Elongation</td>
<td>%</td>
<td>20</td>
</tr>
<tr>
<td>Tenacity (wet-dry)</td>
<td>%</td>
<td>100</td>
</tr>
<tr>
<td>Moisture at 65% RH</td>
<td>%</td>
<td>0.4</td>
</tr>
<tr>
<td>Modulus of Elasticity</td>
<td>N/tex</td>
<td>7.9</td>
</tr>
<tr>
<td>Shrinkage in boiling water</td>
<td>%</td>
<td>7</td>
</tr>
</tbody>
</table>

2.3.  Stability of Poly(ethylene terephthalate) (PET) to Degradation

The required properties which must be demonstrated by a polymer in contact with the body can be discussed from two view points, i.e. the effects of the material on the stability of the host and the effect of the host on the stability of the material (20). The large amount and infinite variety of additives in polymers are perhaps the most significant contributors to the biological response evoked after implantation. Aromatic polyesters such as poly (ethylene terephthalate) exhibit excellent biostability and have proven to be almost totally resistant to microbial attack. Polymers with heteroatoms in the
main chain are generally susceptible to hydrolytic cleavage, such as, at ester or amide bonds \(^{28}\).

In case of poly(ethylene terephthalate) (PET), primary biological attack is an enzymatically-catalyzed hydrolysis of ester bonds in the polymer chain. The first step of depolymerization is a surface erosion process (enzymes cannot penetrate into the polymer bulk) which leads to water soluble intermediates, which can then be assimilated by microbial cells and metabolized \(^{28}\). In many cases the term biodegradation is also used if the primary degradation step is caused by hydrolysis and not catalyzed by enzymes. The depolymerization intermediates are then metabolized by microorganism or resorbed by the body.

Non-physiological degradation of PET includes: thermal, oxidative, radiation induced and hydrolytic degradation \(^{20}\). The hydrolysis of PET at \(100^0 - 120^0\) C and 100% relative humidity proceeds 10,000 times faster than that of oxidation of PET in air within the same temperature range \(^{29}\).

The hydrolysable structure in PET is the ester group within the polymer backbone chain. The hydrolytic degradation of PET involves the chemical scission of an ester linkage in the main chain by a proton thus creating one carboxyl and one hydroxyl group \(^{30}\).

The hydrolysis of ester bonds must proceed via the adsorption of water at the surface of the polymer and diffusion into the interior of the polymer from the point of entry. Therefore knowledge of the rates of adsorption and diffusion are of practical as well as of theoretical importance \(^{31}\). The rate of diffusion of water into PET at temperatures above \(100^0\) C is 1000-fold higher than the rate of hydrolysis \(^{31}\). The amorphous regions of a polymer are affected first and more rapidly due to the difficulty aqueous solutions have in penetrating crystalline regions. Thus the crystallinity of a polymer is expected to increase during degradation. Eventually, the amount of
absorbed water absorbed by a semi crystalline polymer will decrease with increasing crystallinity and density \(^{(32)}\).

Allen et al. have shown that the initial crystallinity of PET has a vital influence on the rate of hydrolytic degradation of the polymer due to the role of the crystalline regions to act as barriers to moisture diffusion \(^{(32,33)}\). The degradation reaction is catalyzed in acidic solutions by hydrogen ions and in alkaline solutions by hydroxyl ions \(^{(34)}\). In neutral solutions (pH = 7.0) the reaction is catalyzed by hydrogen ions from the dissociation of the organic acid end group of the polymer itself. In this case, the reaction will become autocatalytic since each hydrolytic scission of a polyester molecule creates a new -COOH functional group \(^{(34,35)}\). Hydrolytic degradation in alkaline environments appears to be more severe than in acidic solutions due to the more reactive OH\(^-\) ions, compared to H\(_2\)O molecules, as reagent \(^{(35)}\).

Sodium or potassium hydroxide solutions normally attack only the surface of PET fiber; however changes in the fine structure of the inner part of the fiber after such treatment have also been observed \(^{(36)}\). The dielectric properties of the polymer and the formation of carboxylate ions on the fiber surface apparently present a barrier to the penetration of the polymer by hydroxyl ions \(^{(37)}\).

In acid environments the free H\(^+\) ions catalyze the hydrolysis, but these ions hardly penetrate into the filaments. As a result catalysis by the free H\(^+\) ions is mainly restricted to the fiber’s surface \(^{(29,35)}\).
The reactions are as follows \(^{(30,38)}\):

\[
\begin{align*}
\text{PET} & \quad \xrightarrow{H^+} \quad \text{Glycol end} \\
\text{H}_2\text{O} & \\
\text{Carboxylic end} & \quad \text{OH} + \text{CHO} \quad \text{OHH} \quad \text{CH}_2\text{CH}_2 \text{OH} + \text{HO} \quad \text{C} \\
\end{align*}
\]

2.4. *In-vivo* Hydrolytic Degradation of PET

Over the years, the literature on clinical studies has given evidence of early and late failures of knitted and woven Dacron PET arterial prostheses. It is claimed that these failures have been caused by design errors, defective material, incorrect storage, degradation due to sterilization conditions, handling techniques and surgical trauma. At the same time there is considerable evidence indicating inherent biodegradation of Dacron PET reported from implant retrieval studies, which have analyzed large numbers of explanted vascular prostheses \(^{(39)}\).
From the studies carried out by King et al. (16) on a series of five different models of knitted and velour prostheses made out of semidull Type 56 and 62 Dacron PET retrieved from humans, it was reported that a 25% loss in initial bursting strength can be predicted after 162 ± 23 months of implantation. They also found that the loss of bursting strength and molecular weight and the increase in the carboxyl group content followed a logarithmic decay. It was predicted that a 25% loss in molecular weight can be expected after 120 ± 15 months of implantation in humans. The evidence further confirmed that PET experiences a chain scission reaction in-vivo and thus the kinetics of this reaction do not appear to fit the simple depolymerization and diffusion models.

Riepe et al. (17) examined the influence of in vivo hydrolysis on the physical properties of polyester grafts and their correlation to the period of implantation in the human body. Examination of human implanted vascular grafts showed a decrease in the maximum bursting strength and an increase in carboxylic end group concentration in the polymer over implantation periods of 0-23 years. The in vitro experimental hydrolysis of amorphous PET foil and an unimplanted Meadox Cooley Double velour graft at 70°C in a neutral buffered solution (pH 7.3) also showed similar results.

King et al. (40) used thermal analysis and Fourier Transformed Infrared Spectroscopy (FTIR) to characterize changes in the microstructure of PET biotextiles retrieved from patients after 2-16 years of implantation. FTIR examinations revealed increased concentration of trans conformations on the surface of the fibers, while the gauche conformer concentration decreased, indicating an increase in crystallinity near the fiber surface. The Differential Scanning Calorimetry (DSC) studies identified the growth of larger crystalline domains at the expense of the smaller ones due to the slow annealing effect of the body at 37°C.

The kinetic specificity of PET degradation in living bodies including rabbits, dogs and humans, was investigated by Rudakova et al. (15). PET nets implanted subcutaneously for periods of 2.5, 3.6 and 8.0 years in rabbits, dogs and humans respectively were used for
the study. The following equation was derived for the calculation of time for complete degradation of filaments in the living body.

\[ \tau = \frac{d_o \rho}{K_{\text{obs}}} \]

where; \( \tau \) = time for complete degradation  
\( d_o \) = original mean diameter of filaments  
\( \rho \) = Density of polymer  
\( K_{\text{obs}} \) = Observed rate constant

The time dependent decrease of tensile strength was represented by,

\[ P = P_o \left(1 - \frac{K_{\text{obs}} t}{d_o \rho}\right)^2 \]

where; \( P \) = tensile strength at time \( t \)  
\( P_o \) = Original tensile strength

From the experimental and clinical results they were able to determine the rate constant for PET degradation in rabbits, dogs and humans. According to these rate constants the time required for complete degradation of PET fibers in both dogs and humans is 30 ± 7 years and the time for the loss of 50% of the initial strength under similar conditions requires 10 ± 2 years.

### 2.5. In-vitro Degradation of PET

The systematic development of in vitro models of the physiological environment is of the utmost important in understanding the basic mechanism in vivo. In-vitro modeling allows the physiological environment to be simplified such that effects of specific parameters such as pH, ionic strength, temperature and enzymes can be evaluated \(^{(41)}\). Accelerated in-vitro testing at elevated temperatures and/or pH conditions can be used to obtain basic correlation with long-term test results.
2.5.1. Hydrolytic Degradation of PET in Neutral Solutions

As discussed earlier, PET has ester groups; hence water can react with PET by breaking the ester links in the main chain. Since the early stages of degradation are of prime importance in determining the useful life of PET, the initial water/PET interaction needs to be understood.

One of the first papers on PET hydrolysis was written by McMahon who used the change of molecular weight to determine the extent of degradation \(^{(29)}\). Skeins of yarn Type 5100 – high tenacity, bright grade Dacron yarn of 1100 denier from Dupont were immersed in water at 99, 90, 82 and \(71^0\) C, and maintained at neutral pH. It was observed that after 77 days of exposure to 95% relative humidity at \(71^0\) C, there was a decrease of intrinsic viscosity from 0.60 to 0.57, and the loss of tensile strength was 5% with a 70% gain in elongation. It was also recorded that lower pH did not speed up hydrolysis.

During the aging of polyester, density increases have been observed which have more recently been associated with a chemicrystallization process \(^{(25)}\). Crystallinity changes during the degradation of polymeric materials are particularly important in relation to other chemical changes such as chain scission and hydrolysis. Mechanical property changes, such as tensile strength and brittleness, are in turn influenced by a combination of physio-chemical changes, such as temperature and relative humidity.

Allen et al. \(^{(42)}\) studied the effect of hydrolysis at 60, 70, 80 and \(90^0\) C on amorphous PET film at neutral pH. Hydrolysis at \(60^0\) C showed severe changes in chain scission during an exposure time of 3.5 years, but showed negligible change in crystallinity. They found that at \(70^0\) C under 100% RH, the polyester film lost 30% of its intrinsic viscosity with a 45% loss in weight average molecular weight. The crystallinity increased by 26% and remained constant after 75 days of degradation.

The hydrolysis of PET is often treated as a simple chain scission process, and its auto accelerating character is generally interpreted in terms of the build up of terminal acid groups \(^{(43)}\).
Hydrolysis studies carried out by Launay et al. (44) on amorphous granular PET at 100° C in distilled water used changes in molecular weight, crystallinity ratio and water content at equilibrium to reveal that a simple second order random chain scission process seemed to be sufficient to describe the behavior of hydrolysis. They also recorded a 70% increase in crystalline fraction over 30 days of exposure and a weight loss of 6% over the same period.

Weidner et al. (45) investigated the hydrolytic degradation of technical PET yarns by means of different methods such as size exclusion chromatography (SEC), viscometry, end group titration and matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS). Commercially available high tenacity PET multifilament yarn of about 1100 dtex was held at 50 cN tension and degraded in 90 ± 1°C distilled water for 18 weeks. They recorded a loss in weight average molecular weight of 70 - 75% over the total degradation period. The tensile strength of the yarn fell to less than 50% of the original value.

In their study of hydrolysis of the PET fabrics (Dacron Type 54), Sanders and Zeronian (37) recorded a 0.14% weight loss after hydrolyzing for 6 hours at 60° C. Their tensile test on the exposed fabric properties revealed a 4% increase in tenacity, 3% increase in initial modulus and a 3% decrease in elongation. The fiber diameter showed no change, but the fabric weight and air permeability recorded decreases.

2.5.2. Hydrolytic Degradation of PET in Alkaline Medium

During alkaline hydrolysis of PET, polymer cleavage can occur in two ways: unimolecular cleavage which involves only a single hydroxide ion and bimolecular cleavage where two hydroxide ions react with the chain essentially simultaneously (30, 35, 46). The former pathway does not lead directly to weight loss, whereas the later pathway does.
The pathways may be represented by the following equations (46).

\[
P + OH^- \rightarrow P_1 CO_2^- + P_2 OH
\]

\[
P + 2OH^- \rightarrow P \text{ (modified)} + \text{ oligomers}
\]

Where, \( P_1 \) and \( P_2 \) are polymer chain fragments and \( P \) is the unreacted polymer chain.

After cleavage by either pathway, polymer may be further hydrolyzed by unzipping to form monomer units alternately removing terephthalate anions and ethylene glycol. These reactions lead to weight loss at each step (35, 46).

\[
P_1 CO_2^- + OH^- \rightarrow P_2 OH + -O2C –Phenyl – CO_2^- \\
P_2 OH + OH^- \rightarrow P_1 CO_2^- + HOCH_2CH_2OH
\]

The studies carried out by Namboori (36) showed that at 60, 80, and 100 °C the percent weight loss of PET fiber depended on the initial concentration of alkali, and showed a linear relation with respect to time of treatment. He recorded a 5% weight loss after treating PET fibers at 60 °C in 10% NaOH for 60 minutes. The percent elongation at break decreased considerably, whereas the tenacity at break showed only a small change.

The effect of hydrolytic degradation on the structure of PET was investigated by Padhye and Nadaf (47). The hydrolysis carried out in alkali (NaOH) medium on PET film (Dupont de Nemours) showed an increase in density which was less than in the case of acid treatment. It also showed a higher increase in crystallinity than had been expected from density data. When the concentration of NaOH was increased from 5 to 15% at a constant temperature of 60°C, after 3 hours of degradation time the crystallinity increased slightly. With a 5% (wt/vol) NaOH concentration, the samples were completely destroyed after 6
hours at 90 °C. End group analysis showed that the alkali does not cause random chain scission, as in the case of acid hydrolysis.

The effect of NaOH treatment on polyester plain-woven fabrics was studied by Kish and Nouri (48). Rapid weight loss was shown to occur above 80 °C when the fabrics were treated for 60 mins in 1.288 g/L NaOH. The weight loss was proportional to the square of the fiber diameter. A considerable decrease in yarn tensile strength was observed with the increase in weight loss, although no change was observed in the density of the fibers. SEM studies revealed a progressive thinning of fiber diameter with the surface becoming pitted with valleys and holes.

Collins et al. (49) studied the changes in fine structure and physical properties of bright and semidull PET fibers upon alkali hydrolysis. Type 56T Dacron PET fabric (semidull) and multifilament yarn (bright) were hydrolyzed with 11.2% NaOH concentration at 60 ± 0.1 and 21 ± 2 °C to obtain up to 68% and 90% respective weight losses. The weight losses were recorded after 6 hours and 50 hours of degradation. Both samples showed a linear relationship between weight loss and treatment time. A relationship was derived for cylindrical fibers to predict the final radius based on the initial radius and percentage of weight loss. It was assumed that length and density remained constant.

\[
R_f = \left[ R_i^2 (1 – FWL) \right]^{0.5}
\]

where;  \( R_f \) = final radius  
\( R_i \) = initial radius  
FWL = fractional weight loss

Although it has been found that alkaline hydrolysis takes place at the fiber surface, which would suggest that the fine structure would not influence the rate of hydrolysis, Collins et al. found that as the density of bright PET yarns increased with heat setting the rate of weight loss decreased.
For the given test parameters, no major changes in differential molecular weight distribution (DMWD) were recorded. For all samples, the density of the hydrolyzed fibers increased with longer times of hydrolysis. The semidull fibers showed a significantly more rapid increase than the bright fibers.

The following equation represents the density of successive layers in the radii of the original and final (hydrolyzed) fibers. Again it is assumed that fiber length does not change during hydrolysis.

\[
\rho_t = \rho_1 \left(1 - \frac{r_c^2}{r_t^2}\right) + \rho_c \left(\frac{r_c^2}{r_t^2}\right)
\]

where; \( \rho_1, \rho_c \): densities of removed layer and remaining core  
\( \rho_t \): density of starting product  
\( r_c, r_t \): radii of remaining core and starting fiber

With the heat set semidull fibers, the melting endotherm appeared to become sharper and the enthalpy became larger on hydrolysis. SEM studies showed circular shaped pits on the fiber surfaces. The pit size increased as more weight loss was achieved. The exposed void containing areas were found to be places where there were high concentrations of TiO\(_2\) particles.

During the hydrolysis of PET, the % loss in weight, linear density, tenacity and elongation at break maintains a lower linear profile when the reaction temperature remains below the glass transition temperature (\(T_g\)) of PET. Above \(T_g\), these parameters show a rapid increase with temperature\(^{(48, 50)}\). This is confirmed from the work carried out by Datye et al\(^{(51)}\), who found that the transition temperatures for weight loss and linear density lie between 76 and 78 °C, whereas for tenacity and % elongation at break they lie between 50 and 67 °C. They recorded a 0.8% weight loss upon hydrolyzing PET yarn for 40 minutes in 1N NaOH at 65 °C. It is believed that the steep rise in weight loss above the glass transition temperature is partly due to the increased segmental mobility of the PET polymer chains\(^{(50)}\).
When considering the process of alkaline hydrolysis of polyester, the relative importance of different reaction parameters appears to lie in the following order \(^{(50)}\).

\[
\text{Treatment time} < \text{Concentration of alkali} < \text{Treatment temperature}
\]

Several studies \(^{(49, 50)}\) carried out to monitor the changes in the molecular weight of PET fibers upon alkaline hydrolysis reveal that an insignificant change was shown in molecular weight upon hydrolyzing with 10-12\% NaOH up to 69 minutes \(^{(49)}\). This may be due to the fact that in the process of alkaline hydrolysis the outer skin of the PET filament is removed first of all. These cleaved molecular chains are then further attacked by alkali, which causes them to dissolve into solution. The polymer chains present in the core of the fiber appear to remain practically unaffected, thus resulting in an insignificant decrease in molecular weight after short periods of hydrolysis \(^{(50)}\).

SEM studies carried out on alkali hydrolyzed PET fabrics by Dave et al. \(^{(50)}\) showed that the alkali attacks the entire surface of the fiber resulting in the formation of elongated pits or cavities on the surface. Both the depth and the frequency of occurrence of these pits increases with increasing weight loss \(^{(51)}\).

The investigations by Sanders and Zeronian \(^{(37)}\) revealed that in the case of PET films the pits were rounder in contrast to the pits in fibers which were elongated and oriented along the fiber axis. They suggested that the reason for this could be the drawing process used during fiber manufacture.

During the same study on Dacron Type 54 PET fabrics Sanders and Zeronian \(^{(37)}\) recorded a 30.9\% weight loss after hydrolyzing with 10\% NaOH at 60 °C for 6 hours. A 7\% loss of tenacity, 20\% increase in breaking elongation and a 5\% increase of initial modulus were also recorded. The 31\% fabric weight loss corresponded to a 45\% increase in air permeability after hydrolysis.
The work carried out by Haghighatkish and Yousefi (52) on the structural effects of alkaline hydrolysis of polyester fibers showed that there was a 13.5% weight loss upon hydrolysis of fully drawn PET yarn when exposed to 10% aqueous NaOH at room temperature (28 °C) for 100 hours. A linear relationship was indicated between the percentage weight loss and the degradation time period. The density and the crystallinity remained unchanged after the degradation. SEM studies revealed the formation of cavities on the surface of the fibers. The pits on the fully drawn yarn also appeared to be elliptical and elongated in the direction of the fiber axis.

2.5.3. Enzyme Catalyzed Hydrolysis

2.5.3.1. Introduction

Biostability refers to the ability of a material to resist biodegradation and maintain its properties in situ. Enzyme catalyzed enhancement of hydrolysis is one of the mechanisms by which a polymer can degrade in a living body (19).

Since enzymes have the characteristic ability to catalyze certain chemical reactions, there is logic in the hypothesis that enzymes can accelerate in vivo polymer degradation. It is obviously important to select a suitable enzyme, which is likely to have a degradative effect on PET since enzymes are substrate specific and operate within a specific pH and a temperature range.

Esterase and papain enzymes have been found to degrade poly(ethylene terephthalate) under in vitro conditions at 37 °C at pH 6.0 and 8.0, respectively (53). For this study the papain enzyme was chosen due to its stability over a wide range of temperatures and pH, in other words, for its wide specificity.
2.5.3.2. Characteristics of Papain

Specificity
Papain is one of the sulfhydryl proteases among chymopapain and lysozyme isolated from the green fruit of Carica papaya\(^{54, 57}\). It consists of a single folded peptide chain of 212 amino acid residues containing three disulphide bonds and one free functional –SH group at the active site\(^ {54, 58}\). Its molecular weight ranges from 23,000 to 23,400\(^ {57, 58}\). Papain exhibits both proteolytic and esterase activity. It preferentially cleaves peptide bonds involving basic amino acids and hydrolyses carboxylic esters\(^ {54-58}\). In addition to this, papain is capable of catalyzing transamidation, transpeptidation and transesterification reactions as well\(^ {54, 55}\).

Storage stability
Papain can be stored for many months as a lyophilized powder or as a crystalline suspension in NaCl solution at neutral pH at 4\(^ {0}\)C without any significant loss of activity\(^ {54}\). In solution, the mercury derivative may be kept for months without losing potential activity, whereas the active enzyme loses 1-2% of its activity per day, probably due to autolysis and/or oxidation\(^ {54-58}\). It has been reported that soluble papain loses its activity completely after 60 hours of storage at pH 7.5 and 5\(^ {0}\)C\(^ {59}\). As a dry powder, papain can resist 100\(^ {0}\)C for 3 days\(^ {55}\). Papain also shows considerable pH stability from 4-9 at 3\(^ {0}\)C\(^ {59}\). Under acidic conditions with pH values below 2.8, papain is found to suffer a drastic decrease of activity\(^ {54}\).

Activation and Inactivation
Papain, being a sulfhydryl enzyme, requires a free sulfhydryl group for its catalytic activity. The thiol group in the native crystalline protein is found to be blocked mainly in the form of mixed disulfide groups with half-cysteine\(^ {54, 61}\). Activation is achieved with mild reducing agents such as cysteine, sulfide, sulfite as well as cyanide\(^ {54, 61}\). The process of activation consists of the removal of a half-cysteine residue from the enzyme with simultaneous liberation of a free thiol group on the enzyme\(^ {54}\). Optimum activation of papain has been recorded to occur upon simultaneous application of a thiol compound like cysteine or thioglycolate and a heavy metal or heavy metal
binding agent like ethylenediaminetetraacetic acid (EDTA). Alternatively activation occurs by the addition of 2,3 – dimercaptopropanol (BAL), a compound which combines the function of both a thiol compound and a metal binder (54, 61). The activity of papain can be maintained at 100% for 6 hours by adding 0.001 M cysteine and EDTA (61). The standard activation conditions which are used in the activity assays of papain require a medium containing 0.005 M cysteine and 0.001 – 0.002 M EDTA (54).

Papain is reversibly inactivated in the presence of air and a low concentration of cysteine or heavy metal ions. However it can be reactivated in the presence of EDTA and a higher concentration of cysteine (54). It was shown that when papain is incompletely activated with cysteine, the bivalent ions such as Cd $^{2+}$, Zn $^{2+}$, Fe $^{2+}$, Cu $^{2+}$, Hg $^{2+}$ and Pb $^{2+}$ have no great preference for combining with papain as against cysteine (54, 61). On the other hand when papain is not activated with cysteine, Cd $^{2+}$ and Zn $^{2+}$ have stronger affinities up to 100 fold and 1000 fold respectively, towards papain (61). Since the removal of Zn $^{2+}$, can only be carried out by EDTA, and the inhibition of Hg $^{2+}$ can only be prevented by cysteine, both cysteine and EDTA are required to gain the optimum activation of papain (61).

When the synthetic substrate Nα - Benzoyl- L- Arginine Ethyl Ester (BAEE) has been used as the substrate, the optimum pH for soluble papain was observed at 37 °C (57) to be 7.2. With the same substrate, the maximum enzyme activity for soluble papain was recorded at 65 °C. The pH of the enzyme at this temperature is unknown (59). When casein was used as a substrate the maximum activity at 40 – 60 °C was recorded at pH 7.0 (60). The optimum pH at 3 °C was between 6 -7 (60).

**Units and specific activity of papain:**
One unit of enzyme is defined as the amount, which will hydrolyze one micromole of Nα - Benzoyl- L- Arginine Ethyl Ester (BAEE) per minute at 25 °C. The specific activity is expressed as the number of units of enzyme per milligram of protein (54).
2.5.3.3. *In-vitro* Enzymatic Degradation of PET

The *in vitro* enzymatic degradation of C\textsuperscript{14} labeled PET was studied by Smith et al. \(^{53}\) at 37 °C using four buffered enzymes namely, trypsin, α - chymotrypsin, papain and esterase. No differences in the dpm (disintegration per minute) time curves were observed for trypsin and chymotrypsin in their respective buffers. This indicated that these enzymes had no influence on PET degradation under the given conditions. With esterase, however, degradation was observed between 5 and 15 days compared to the buffer solution alone for a similar duration of time. Throughout the experimental period, the release of C\textsuperscript{14} from the PET polymer was significantly greater, with increasing activity in the presence of papain for the first 8 days, followed by a reduction to a level similar to the first few days.
3. Materials and Methods

3.1. Fabric Preparation and Characterization

This section provides the details of the fabrics used for the hydrolytic/enzymatic degradation experiments and includes a brief description of the preparatory treatment processes. The material used for this study was a nonsterile, Type 56 Dacron polyester warp knitted fabric used for manufacturing endovascular prostheses and supplied by BARD Vascular Systems Division – C.R. BARD Inc.

In order to remove dust, oil and other contaminants, the fabric samples were treated with a solution of 2% Triton X-100 and 0.8% sodium carbonate for 30 minutes at 60 °C using a ATLAS Launder-ometer (ATLAS Electric Device Company, USA). Square fabric samples, each 6 inch x 6 inch in size, were placed in each container, which carried 350ml of detergent solution. After the detergent wash, each fabric specimen was rinsed in 350ml of doubled distilled water for 30 minutes at 60 °C using the same instrument. The specimens were then rinsed twice with doubled distilled water at room temperature and oven dried for 15 minutes at 105 °C to remove moisture.

The fabric specimens were analyzed for fabric count, mass per unit area, filament diameter and yarn linear density.

3.1.1. Filament Diameter

Individual filaments were removed randomly from fabric specimens for filament diameter measurements. The diameter at 3 different places along 10 filaments selected at random from different yarns was measured using a polarizing compound light microscope with a HITACHI HV-D3 digital camera at a magnification of 250×. The microscope was calibrated and the measurements were carried out using NCRC image analysis software (Nonwovens Co-operative Research Center, College of Textiles, NC State University).
3.1.2. Yarn Linear Density

The number of filaments in the yarns on the face and back of the fabric were counted using a MONOZOOM 7 compound microscope (Bausch and Lamb) fitted with CCD-IRIS/RGB video camera at a magnification of 21.5×. The average filament diameter, D (in µm), the number of filaments in the yarn, n, and the standard relative density of the PET filaments $d$ (in g/cm³) were used to calculate the nominal linear density of the yarns using the equation (3.1)\textsuperscript{(63)}.

\[
\text{Yarn nominal linear density (dtex)} = \frac{\pi n d}{100} \times \left(\frac{D}{2}\right)^2
\] (3.1)

3.1.3. Mass Per Unit Area of the Fabric

The average mass per unit area in g/m² of the warp knitted fabric specimens were calculated according to the ASTM D 3776-96 standard test method for mass per unit area (weight) of fabric \textsuperscript{(64)}. Since limited amount of fabric was available, the option –C of the test method was followed. Six fabric specimens having an area between 228-231 cm² were individually weighed and the mass per unit area was calculated using the equation (3.2).

\[
\text{Mass per unit area (g/m²)} = \frac{10^6 G}{L_s W}
\] (3.2)

Where;

$G$ = Mass of specimen in grams
$L_s$ = Length of specimen in mm
$W$ = Width of specimen in mm
3.1.4. Porosity

The porosity can be defined as the proportion of void spaces within the boundaries of a solid material compared to its total volume \(^{(65)}\). The porosity value, \(P\), for the prosthetic fabric was calculated from the average mass per unit area of the fabric, \(M\) (g/cm\(^2\)), the average thickness of the fabric wall, \(h\) (cm), and relative density of the polyester fibers, \(d\) (g/cm\(^3\)) using the equation (3.3). The thickness of the fabric specimens were measured using a Digital SDL carpet thickness gauge (SDL International Ltd, Stockport, England) according to the method described in standard test method ISO 7198 1998(E) for Cardiovascular implants – tubular vascular prostheses \(^{(66)}\).

\[
P = 100\left(1 - \frac{M}{hd}\right)
\]  \hspace{1cm} (3.3)

3.1.5. Fabric Count

The warp knitted fabrics were viewed through a Monozoom-7 compound microscope (Bausch and Lamb) fitted with a CCD-IRIS/RGB video camera at a 21.5× magnification. The number of courses per inch and wales per inch were counted according to the ASTM standard method D3887-96 – Standard specification for tolerances for knitted fabrics \(^{(67)}\). In both cases the mean value for each count was calculated from ten independent measurements in randomly selected samples.

The fabric analysis data is shown in Table (3.1). The format used is mean ± standard deviation.
Table 3.1. Fabric Characterization

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabric structure</td>
<td>Warp knitted</td>
</tr>
<tr>
<td>Stitch type</td>
<td>2 bar Tricot</td>
</tr>
<tr>
<td><strong>Yarn</strong></td>
<td></td>
</tr>
<tr>
<td>Filament diameter</td>
<td>11.73 ± 0.01 µm</td>
</tr>
<tr>
<td>Yarn linear density</td>
<td>29.23 ± 3.23 dtex</td>
</tr>
<tr>
<td><strong>Fabric count</strong></td>
<td></td>
</tr>
<tr>
<td>Wales per cm</td>
<td>18.20 ± 1.62</td>
</tr>
<tr>
<td>Courses per cm</td>
<td>35.80 ± 2.15</td>
</tr>
<tr>
<td>Mass per unit area (g/m²)</td>
<td>70.37 ± 1.10</td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>73.82 ± 1.64</td>
</tr>
</tbody>
</table>

3.2. Pre-degradation Enzymatic Analysis

3.2.1. Determination of the Optimum Temperature for Degradation

A thermal stability test of the papain enzyme was carried out along with a sample of the prosthetic fabric. Woven Type 56 Dacron PET fabric specimens, each weighing 0.4 g were cut from a sterile straight arterial graft (De BAKEY® USCI Corporation NY, C.R. Bard Company) and kept in 1.6% phosphate buffered papain solution (pH 7.2) at one of the five temperatures; that is 37, 50, 55, 60 and 65 °C. The fabric to enzyme weight ratio was maintained constant at 1:1 for test samples.

For controls, the same concentrations of buffered solutions were maintained simultaneously at the above temperatures. The observation with regards to the stability of the papain solution without fabric specimens revealed that the enzyme aggregates into small particles at 60 and 65 °C after one day. There was no sign of aggregation at other temperatures for up to two days. In the case of enzyme solutions containing the fabric specimens, aggregation was observed after 21 hours at 55 °C, and after 12 hours at 60
and 65 °C. However the samples kept at 37 and 50 °C showed no sign of aggregation for up to two days.
The formation of aggregates and the observations that enzymes were binding to the fabric in clumps were presumed to be a sign of reduction in enzyme activity.

3.2.2. Measurement of Enzyme Activity

3.2.2.1. Experimental

A series of tests were carried out to determine the papain enzymatic activity prior to the start of the actual degradation study. A four factor experimental design was set up to simulate the proposed degradation conditions with the four variables; fabric, temperature, time and activation, each controlled at two levels (Table 3.2). Papain enzyme solutions were prepared by dissolving 0.0667g of papain (in crude powder form) in 100 ml of 8.3 mmol/L phosphate buffer at pH 7.2. From a total of sixteen different conditions, eight were maintained at 37 °C and the rest at 50 °C. In half of each of these groups, the papain enzyme was initially activated by adding 0.05 M cysteine dissolved in 0.725 ml of 0.01 M EDTA in which the pH had been adjusted to 6.2. The fabric used was non-sterile Type 56 Dacron polyester warp knitted fabric (BARD Vascular Division – C.R. Bard), and each specimen weighed 0.0667g. All the fabric specimens had been subjected to a detergent wash, as described earlier under fabric preparation and characterization.

The incorporation of nitinol stent elements was omitted from experimental design based on the fact that nitinol does not contain any bivalent ions, which could cause an inhibition of papain. The enzyme activity was measured using a titrimetric rate determination method after each period of degradation, either one or two days (54, 58, 59, 61, 68).
Table 3.2. Experimental design to measure papain enzymatic activity

<table>
<thead>
<tr>
<th>Vessel ID</th>
<th>Presence of fabric</th>
<th>Temperature (°C)</th>
<th>Duration (days)</th>
<th>Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>37</td>
<td>1</td>
<td>Non</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>2</td>
<td>Non</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>1</td>
<td>Activated</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>No</td>
<td>2</td>
<td>Activated</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>1</td>
<td>Non</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Yes</td>
<td>2</td>
<td>Non</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Yes</td>
<td>1</td>
<td>Activated</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Yes</td>
<td>2</td>
<td>Activated</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>No</td>
<td>50</td>
<td>1</td>
<td>Non</td>
</tr>
<tr>
<td>10</td>
<td>No</td>
<td>2</td>
<td>Non</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>No</td>
<td>1</td>
<td>Activated</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>No</td>
<td>2</td>
<td>Activated</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Yes</td>
<td>1</td>
<td>Non</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Yes</td>
<td>2</td>
<td>Non</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Yes</td>
<td>1</td>
<td>Activated</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Yes</td>
<td>2</td>
<td>Activated</td>
<td></td>
</tr>
</tbody>
</table>

3.2.2.2. Enzymatic Assay of Papain for Esterase Activity

The activity of papain is estimated by its ability to cleave $\text{N}_\alpha$ - Benzoyl- $\text{L}$- Arginine Ethyl Ester (BAEE). Free carboxylic groups are then measured by titration with NaOH. The following reagents were prepared for the assay:

(A) 50 ml of 80 mM $\text{N}_\alpha$ - Benzoyl- $\text{L}$- Arginine Ethyl Ester (BAEE)

(B) 100 ml of 20 mM Ethylenediaminetetraacetic acid (EDTA)

(C) 25 ml of 50 mM $\text{L}$- Cysteine in reagent B adjusted to pH 6.2 at 25 °C with 1M NaOH.

(D) 25 ml of 3M sodium chloride

(E) 50 ml of 20 mM sodium hydroxide titrant.

The following amounts of the reagents were added to the titration vessel:
Reagent A (BAEE)  7.0 ml  
Reagent C (L-Cysteine)  1.0 ml  
Reagent D (NaCl)  1.0 ml  

The pH was adjusted to 6.3 by adding reagent E (NaOH) at 25 °C. Then 1 ml of papain solution was added to the titration vessel. The pH of the reaction mix was monitored and the time recorded when the pH reached 6.2. The pH was maintained at 6.2 by the addition of small volumes of reagent E and the time recorded (in minutes) when a total of 50µl of reagent E is consumed. The process is repeated for approximately 5-10 minutes till a total volume of 250µl of reagent E is consumed. The enzyme activity can be calculated from the equation (3.4) and (3.5).

\[
\text{Units/ml enzyme} = \frac{(0.05)(\text{Normality of NaOH})(1000)(\text{df})}{(T)(1)} \quad (3.4)
\]

\[0.05\] = Volume (in ml) of reagent E used to maintain the pH at 6.2.  
\[1000\] = Conversion from millimoles to micromoles  
\[\text{df}\] = Dilution factor  
\[T\] = Time (in min.) required to maintain the pH 6.2 per 50µl aliquot  
\[1\] = Volume (in ml) of enzyme used.

\[
\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}} \quad (3.5)
\]
3.3. Conditions for Hydrolytic and Enzymatic Degradation of PET

The nonsterile Type 56 Dacron PET warp knitted fabric specimens together with Nitinol stent elements (Teramed Inc. MN, USA) (Figure 3.1 and 3.2) were subjected to the following degradation conditions for different time periods following the detergent wash and the determination of initial oven dry weight.

3.3.1. Negative Controls
Samples of the fabric and Nitinol stent wires were stored at room temperature in a dark environment and were subjected to concurrent tests.

3.3.2. Hydrolytic Environment
Test specimens were continuously immersed in 100ml of 0.01M phosphate buffered saline solution at pH 7.4 at 37°C and 65°C for 3, 6 and 9 weeks (Table 3.3). After the specific degradation period, the samples were removed and rinsed three times with deionized water.

3.3.3. Alkaline Environment
As a positive control, fabric specimens and nitinol stent elements were immersed in 100 ml of 1% sodium hydroxide solution (pH = 13.43) at 65°C for 2, 4, 8 and 10 days (Table 3.3). After each degradation period the fabric specimens were removed from the solution and rinsed three times in deionized water. The remaining alkali solution was subjected to UV/VIS spectroscopic analysis to determine the concentration of dissolved terephthalate anions.

3.3.4. Enzymatic Environment
Both fabric specimens and Nitinol stent elements were treated with 100 ml of phosphate buffered papain enzyme (pH 7.2) at 37 and 50°C for 3, 6 and 9 weeks (Table 3.3). The material to enzyme ratio of 1:1 was maintained at all times. The enzyme solution was preactivated prior to the start of the degradation by adding 3-5 ml of 0.01M EDTA solution containing 0.026-0.029 g of cysteine \(^{59, 67}\). In other words, the
activated solutions were prepared based on the mole ratio of 1 : 2.5 : 12.5 between enzyme, EDTA and cysteine respectively (67). In order to maintain the activity of the enzyme during the entire degradation period, the enzyme solutions were replenished with activated enzymes after every 48 hours.

In all the above environments the solutions were maintained at 140 shakes/min using an orbital shaker water bath (BOEKAL Scientific, PA, USA). The amount of nitinol added was based on approximately 1:1 fabric: stent weight ratio. This was estimated to be the approximate mass ratio of these two materials incorporated into a typical transrenal bifurcated endovascular device.

Figure 3.1. Light microscopic image showing the Type 56 Dacron polyester fabric (magnification 95×).
### Table 3.3. Experimental design for hydrolytic/ enzymatic degradation

<table>
<thead>
<tr>
<th>Test/Control Materials</th>
<th>Environments</th>
<th>Degradation Conditions</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pH</td>
<td>Temp. (°C)</td>
</tr>
<tr>
<td>Dacron PET fabric</td>
<td>Negative Control</td>
<td>7.4</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Phosphate buffered saline</td>
<td>7.4</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>1% NaOH</td>
<td>13.4</td>
<td>65</td>
</tr>
<tr>
<td>Dacron PET with Nitinol stent elements</td>
<td>Papain enzyme</td>
<td>7.2</td>
<td>37</td>
</tr>
</tbody>
</table>

Figure 3.2. Light microscopic image of nitinol stent element (magnification 8.5x)
3.4. Post-degradation Evaluation

3.4.1. Percent Weight Loss

The initial and final weights of the fabric specimens were determined according to the method given in ASTM D-2495-01, Standard test method for moisture in cotton by oven drying \(^{(69)}\). The initial weight measurements were carried out after subjecting the fabric specimens to a detergent wash. In the case of the enzymatic degraded specimens, an additional detergent wash was followed after degradation to remove any enzymes adhering to the surface of the fabric. The percentage weight loss was calculated by using Equation 3.6.

\[
\text{Percent weight loss (\%) = } \frac{(\text{initial weight} - \text{final weight})}{\text{initial weight}} \times 100
\]  

\(3.6\)

3.4.2. Thermal Analysis

The thermal properties of the control and degraded Type 56 Dacron PET fabric specimens were measured by differential scanning calorimetry using a Perkin-Elmer DSC 7 equipped with a TAC7/DX thermal analysis controller (Connecticut, USA). About 3-5mg of fabric was placed and crimped in an aluminum pan and scanned to obtain the premelt and melt curves for each specimen. The premelt onset temperature, melt peak temperature and heat of fusion were measured using Perkin Elmer Pyris software V3.72. The degree of crystallinity was calculated using Equation 3.7 \(^{(70)}\).

\[
\text{Degree of Crystallinity} = \frac{\Delta H_m - \Delta H_c}{\Delta H_m(100\%)}
\]  

\(3.7\)

Where, \(\Delta H_m(100\%) = 140 \text{ J/g for 100\% crystalline PET}^{(71, 72)}\)

\(\Delta H_m = \text{Measured enthalpy of melting}\)

\(\Delta H_c = \text{Measured enthalpy of crystallization}\)
3.4.3. Fourier Transform Infrared Spectroscopy (FTIR)

In an attempt to detect any changes in PET microstructure during the degradation, the degraded and control fabrics were scanned using a Fourier Transform Infrared Spectrophotometer (Nicolet Model 510P) fitted with an attenuated total reflectance (ATR) attachment and a DTGS detector. A total of 128 scans were aggregated between 700 and 4000 cm\(^{-1}\) with each spectrum at 2 cm\(^{-1}\) resolution.

The peak heights of the bands at 1100 cm\(^{-1}\), 1340 cm\(^{-1}\), and 970 cm\(^{-1}\) which correspond to –O-CH\(_2\)-, -CH\(_2\)-O- conformers and -CH\(_2\) – CH\(_2\)- conformers in ethylene glycol in the non-crystalline portions in PET \((73-78)\) respectively were measured using OMNIC v5.2 software. These peak heights were expressed as a ratio relative to the peak height of the thickness band at 872 cm\(^{-1}\) (aromatic out of plane C-H absorption band) in order to analyze the changes in microstructure of PET due to hydrolytic/enzymatic degradation.

3.4.4. UV/VIS Spectroscopy

UV/VIS spectrometers are commonly used to measure the absorption of light in the visible and ultraviolet region, that is, in the 190 - 900 nm range. Absorption of radiation in this region of the spectrum causes the transition of electrons from molecular bonding orbitals to the higher energy molecular anti-bonding orbitals \((79)\). A typical ultraviolet and visible spectrum can be conveniently described in terms of the position of the peak \((\lambda_{\text{max}})\) and the intensity of that absorption \((\varepsilon_{\text{max}}, \text{the extinction coefficient})\) \((80)\). In contrast to the infrared spectrum, the UV/VIS spectrum is not primarily used to show the presence of individual functional groups but to show the relative concentration of functional groups as well as the presence of an aromatic ring \((80)\).

In this study UV/VIS spectroscopy was used to determine the concentration of the terephthalate anion in the alkali degraded solutions. Bimolecular cleavage of PET caused
by alkaline hydrolysis is known to produce terephthalate anions \(^{(35, 46)}\). This unzipping of the PET polymer to monomer units leads directly to a weight loss of the polymer. It was anticipated that determining the concentration of the terephthalate anions in solution following alkaline degradation of PET, would facilitate an understanding of the rate of degradation and enable a correlation with the weight loss of the fabric.

As a first step in determining the concentration of terephthalate anion, a calibration curve obtained using a series of known concentrations. Standard solutions having concentrations of \(1.0 \times 10^{-5}, 1.6 \times 10^{-5}, 2.5 \times 10^{-5}, 5.0 \times 10^{-5}\), and \(1.0 \times 10^{-4}\) M were prepared by dissolving appropriate weights of terephthalic acid in 1% NaOH solution. These solutions were then scanned using a Cary3E Varian UV/VIS spectrophotometer (Varian Analytical Instruments, CA, USA) in the wavelength range of 190 – 400 nm. A maximum absorbance peak was observed at the wavelength of 239 – 240 nm for each standard solution (Table 3.4). The absorbance values at the peak were used to construct a calibration curve of absorbance against concentration (g/l) of disodium terephthalate (Figure 3.3).

<table>
<thead>
<tr>
<th>Concentration (g/l)</th>
<th>(\lambda_{\text{max}}) (nm)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0017</td>
<td>239</td>
<td>0.1311</td>
</tr>
<tr>
<td>0.0027</td>
<td>240</td>
<td>0.2136</td>
</tr>
<tr>
<td>0.0042</td>
<td>239</td>
<td>0.3204</td>
</tr>
<tr>
<td>0.0083</td>
<td>240</td>
<td>0.6467</td>
</tr>
<tr>
<td>0.0166</td>
<td>239</td>
<td>1.2531</td>
</tr>
</tbody>
</table>
Equation 3.8 was obtained from the regression analysis of the above calibration curve.

\[ Y = 76.059 \times X \]

Figure 3.3. Calibration curve for disodium terephthalate

Each solution taken after a specific period of alkali degradation was scanned using the above-mentioned spectrophotometer. Prior to scanning, the solutions were filtered by suction using nylon membrane filter papers (pore size 0.2 \( \mu \)m) to remove any residual matter. It was necessary to dilute the solutions from 2, 4 and 8 days degradation by 100 times and 10 days degradation by 250 times so as to keep the maximum absorbance below 1.5 \(^{(68)}\). All solutions gave a maximum absorbance at a peak wavelength of 239 – 240 nm. The absorbencies recorded were used in conjunction with Equation 3.8 and Beer-Lambert’s law to calculate the actual concentration of disodium terephthalate in the degraded solutions.
According to Beer’s law, the absorption of radiant energy is proportional to the total number of molecules in the light path \(^{79, 81}\).

Beer Lambert’s law can be expressed as \(^{81}\);

\[
A = -\log T = \log \left( \frac{P_0}{P} \right) = \varepsilon bc
\]  

(3.9)

Where

- \(A\) = Absorbance
- \(P_0\) = Radiant power of solvent
- \(P\) = Radiant power of analyte
- \(T\) = Transmittance
- \(b\) = Path length (cm) of the transparent cell
- \(c\) = Concentration of absorbing analyte
- \(\varepsilon\) = Molar absorptivity (molar extinction coefficient – mol/l)

Equation 3.9 can be simplified to;

\[
A = Kc
\]  

(3.10)

Where \(K\) = Calibration coefficient

By correlating the slope of Equation 3.8 with \(K\), Equation 3.10 can be written as;

\[
A = 76.059 \, c
\]  

(3.11)

The maximum absorbance values were recorded for each solution at 239 –240 nm, and the actual concentrations of terephthalate anion in degraded alkali solutions were calculated using Equation 3.11 in g/L. The concentration of each degradation was used to calculate the weight of dissolved terephthalate anion in 100 ml of the degradation
solution in order determine the loss of weight in PET specimens. This loss of weight was due to the cleavage of terephthalate segments from the PET long chain molecule.

3.4.5. Probe Bursting Strength

Bursting strength measurements were carried out on the control and degraded specimens using a probe puncture test described in standard test method ISO 7198 1998(E) for Cardiovascular implants – tubular vascular prostheses (82). The specimens were flattened to remove any wrinkles or folds and clamped over the 1.14 cm diameter circular hole in the clamping plates. A probe with a circular cross section of 0.79 cm diameter and a hemispherical head traveled at a rate of 20.0 mm/min through the specimen using a compression cage mounted on a Sintech mechanical testing machine (MTS, Eden Prairie, MN) (Figure 3.4). The maximum forces measured from three repeated tests were averaged and divided by the area under the test (3.78 cm$^2$) to give the mean bursting strength of each sample.

3.4.6. Microscopic Analysis

In order to ascertain the extent of degradation, the surface of the fabric, filaments and nitinol stent elements were viewed under a scanning electron microscope (SEM). Images were acquired from a Philips 505T Scanning Electron Microscope (FEI – Hillsboro, OR, USA) using an accelerating voltage of 15 kV. The fabric specimens and nitinol stent elements from selected degraded samples were mounted on aluminum stubs using self-adhesive tabs. The edges of the aluminum stubs were coated with conducting silver paint to provide a continuous conducting medium. Subsequent to mounting, the samples were coated with gold/palladium using HUMMER V sputter coating instrument (ANATECH Ltd, Springfield VA) to obtain an average uniform coating thickness of 15nm. Multiple random micrographs were obtained at several magnifications.
Figure 3.4. Compression cage mounted on the Sintech mechanical testing machine showing (A) probe (B) clamping plates.
4. Results and Discussion

4.1. Change of Initial Weight of PET Fabrics upon Degradation

The percentage weight loss calculated relative to the initial oven dry weight of the Dacron fabric specimens gave clear evidence of the degree of severity of the hydrolytic and enzymatic degradation of the PET prosthetic fabric.

The Dacron fabric specimens degraded with 1% NaOH at 65 °C showed a significant weight loss over the 10 day degradation period. The percent weight loss observed from both treatment conditions, that is fabric specimens treated with and without nitinol wire elements, had a similar trend in the change of percent weight loss over the degradation period (Figure 4.1). A regression analysis carried out on the results revealed that a 50 % loss of initial weight can be expected after 5.89 and 6.10 days of alkali degradation of Dacron PET fabrics alone and in the presence of nitinol wire elements respectively. Negligible amount of change in weight was observed with the buffered saline specimens at 37 and 65 °C. Details of the results are in Appendix II. Exposure to enzymes at 37 and 50 °C produced a weight gain due to the adhesion of the enzyme to the fabric. Details of the results are in Appendix II.
Figure 4.1  Effect of alkali degradation on the initial weight of Dacron PET prosthetic fabric specimens ▲ Dacron fabric ■ Dacron fabric plus stent.
4.2. Effect of Degradation on Thermal Properties

The results from the differential scanning calorimetric (DSC) measurements are presented in Tables 4.1 and the premelt and melt curves are shown in Figures 4.2 and 4.3.

With respect to the thermal data on alkali degraded PET fabric specimens, examination of the heat of fusion from the melt curves and the calculated crystallinities revealed that there was a decrease in crystallinity from the original for the first 4 days of degradation. The crystallinity of the fiber increased again after 4 days (Table 4.1). No pre-crystallization occurred during heating of the Dacron PET specimens. At the same time a shoulder in the melt endotherm of the control fiber indicated the presence of two types or sizes of crystals. These regions have been defined as type I or stable fraction and type II or less stable fraction (40, 49, 81). The comparison of endotherms (Figure 4.2 and 4.3) revealed that the shoulder shifted closer to the melting peak and eventually disappeared after 10 days of degradation. In addition a minor decrease of melting temperature was seen after 10 days.

These observations of the melting endotherms and the degree of crystallinity of alkali degraded Dacron fabric specimens correlate well with the observations made on human polyester arterial prostheses implanted for 192 months (40). The shifting and the disappearance of the shoulder after 10 days of degradation indicates that the stable fraction of crystallites are located in the exterior portion of the fiber with the unstable crystallites present in the inner region.

Thermal analysis of both the saline and enzyme treated samples also showed a decrease in crystallinity with the exposure. Details of their results are in Appendix III.
Table 4.1. Differential scanning calorimetric data on control and alkali degraded PET fabrics.

<table>
<thead>
<tr>
<th>Treatment conditions</th>
<th>Period of degradation (Days)</th>
<th>Temp. at shoulder (°C)</th>
<th>Melting temp. (°C)</th>
<th>Heat of fusion, $\Delta H$ (J/g)</th>
<th>Crystallinity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>250.999</td>
<td>258.033</td>
<td>56.824</td>
<td>40.588</td>
</tr>
<tr>
<td>Alkali degraded</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fabric</td>
<td>2</td>
<td>250.841</td>
<td>256.700</td>
<td>51.585</td>
<td>36.850</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>251.474</td>
<td>258.366</td>
<td>51.266</td>
<td>36.618</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>251.474</td>
<td>259.033</td>
<td>60.566</td>
<td>43.261</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Non</td>
<td>254.700</td>
<td>56.272</td>
<td>40.194</td>
</tr>
<tr>
<td>Fabric + stent</td>
<td>2</td>
<td>251.632</td>
<td>258.859</td>
<td>55.500</td>
<td>39.640</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>252.106</td>
<td>257.033</td>
<td>54.688</td>
<td>39.063</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>252.581</td>
<td>258.700</td>
<td>57.553</td>
<td>41.109</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>254.162</td>
<td>258.837</td>
<td>57.813</td>
<td>41.295</td>
</tr>
</tbody>
</table>
Figure 4.2 Melting endotherms of control and alkali degraded Dacron PET prosthetic fabrics degraded without nitinol.
Figure 4.3 Melting endotherms of control and alkali degraded PET fabric specimens degraded with nitinol stent elements.
4.3. Analysis of Microstructural Changes in PET

The FTIR spectra obtained on the control and degraded Dacron PET fabric specimens correlated with the spectra obtained by Cole et al.\(^{(76, 77)}\) on oriented PET specimens with the beam polarized perpendicular to the draw direction. Several major peaks were identified in the control Dacron PET fabric specimen which are characteristic of PET\(^{(73 - 76)}\).

With respect to the control specimen a high intensity peak at 1715 cm\(^{-1}\) was observed which has been identified as that corresponding to carbonyl stretching\(^{(73, 75, 76)}\). This band has been observed to be present in the spectra generated from PET in which highly ordered trans glycol conformations are found in the crystalline phase\(^{(76, 77)}\). The band at 1409 cm\(^{-1}\) has been assigned to an in-plane vibration of the benzene ring which is usually used as a reference band to normalize spectra\(^{(76)}\). This band has been found to be sensitive to conformational changes. It has been found that this band exists in spectra generated on PET in which gauche conformations of ethylene glycol moieties are predominant in the amorphous phase\(^{(76)}\). The absorbance band at 1340 cm\(^{-1}\) has been observed to correspond to the trans conformation of glycol groups\(^{(73, 75 - 77)}\). This band has been identified as a characteristic band for drawn fibers\(^{(75, 77)}\). Also it has been found to occur in the spectra obtained from PET having a less ordered trans structure in which the peaks associated with terephthalate moieties of the molecule resemble those observed in amorphous regions\(^{(76)}\). The absorbance peak at 1247 cm\(^{-1}\) has been assigned to the \(\text{–C-O–}\) stretching vibration in complex in-plane ring ester modes\(^{(75, 76)}\). It has been suggested\(^{(62)}\) that changes in this band may be connected to the internal rotation of the bond between the ring and carbonyl carbon and also at the ester linkage of PET. A double peak was observed in the region of 1099 cm\(^{-1}\) and 1120 cm\(^{-1}\). The band at 1099 cm\(^{-1}\) has been associated with symmetric \(-\text{C-C–}\) stretching of the glycol group\(^{(75)}\) and also found to arise from the gauche glycol conformation in the amorphous phase\(^{(76, 77)}\). The band at 1018 cm\(^{-1}\) has been assigned to an in-plane bending mode of the ring C-H bonds\(^{(73)}\). This band has also been found to exist in the spectra of PET in which gauche conformations of ethylene glycol moieties are predominant in the amorphous phase. The
absorbance band at 971 cm\(^{-1}\) has been assigned to glycol C-O stretching and associated with trans conformers\(^{(73 - 75)}\). This band has been observed to exist in the spectra of PET having a less ordered trans structure\(^{(76, 77)}\). The bands at 872 cm\(^{-1}\) and 724 cm\(^{-1}\) have been associated with ring C-H out of plane deformation and a combination of ring C-H and C = O out of plane deformation respectively\(^{(73, 74, 76)}\).

When compared with the spectra which have already been analyzed and categorized for PET\(^{(76, 77)}\), the bands in the control and degraded PET specimens of this study have revealed that the glycol moieties exist as trans and gauche conformations in the amorphous phase and as trans (extended) conformation in the intermediate phase of the fiber.

Studies carried out on bulk PET obtained via coalescence from polymer – cyclodextrin inclusion compounds\(^{(78)}\) have revealed that the vibrations at 971 cm\(^{-1}\) correspond to –CH\(_2\) – CH\(_2\) - conformers of ethylene glycol in the non-crystalline portions of PET. Further, it has been pointed out that the vibration at 1370 cm\(^{-1}\) relates to –O-CH\(_2\) - and -CH\(_2\)-O- conformers in ethylene glycol in the non-crystalline portion in PET. In crystalline phase, all –CH\(_2\) – CH\(_2\) -, –O-CH\(_2\) - and -CH\(_2\)-O- bonds are found to exist in trans conformation\(^{(78)}\). In the constrained non-crystalline phase –CH\(_2\) – CH\(_2\) - bond exists in trans conformation while –O-CH\(_2\) - and -CH\(_2\)-O- bonds are predominately in gauche conformation. It has been suggested that the amorphous phase contains –O-CH\(_2\) - and -CH\(_2\)-O- mainly in trans and –CH\(_2\) – CH\(_2\) - bond predominately in gauche conformation.

Major bands at 1715, 1100, 1340, 971 and 872 cm\(^{-1}\) were considered in order to determine the changes in microstructure due to hydrolytic/enzymatic degradation of the PET specimens. The characteristic band at 1370 cm\(^{-1}\) was not prominent and was not considered for comparison.

A qualitative comparison of the spectra of alkali degraded PET fabrics without nitinol showed that the positions of 1100 cm\(^{-1}\) and 1340 cm\(^{-1}\) bands changed to 1099 cm\(^{-1}\) and
1339 cm\(^{-1}\) respectively after 4 days of degradation (Figure 4.4 and 4.5). In the fabric specimens degraded with nitinol, this was observed after 2 days. The band at 1715 cm\(^{-1}\) shifted to 1717 cm\(^{-1}\) in both fabrics treated with and without nitinol stents after 8 and 4 days, respectively. The upward shift of this band has been found to involve the conversion of gauche glycol conformers to trans with little change in conformation of the carbonyl group\(^{76,77}\). The thickness band at 872 cm\(^{-1}\) remained unchanged during the entire degradation period. The intensities of 971, 1100 and 1340 cm\(^{-1}\) were measured and expressed as a ratio to the thickness band at 872 cm\(^{-1}\) (Table 4.2). The calculations showed a marked increase of all three peak intensities after 8 and 4 days in fabrics degraded with and without nitinol stents respectively. As discussed earlier, since 971 cm\(^{-1}\)\(^{78}\) and 1100 cm\(^{-1}\)\(^{75}\) correspond to the \(-\text{CH}_2 – \text{CH}_2-\) conformers of ethylene glycol and also since 1340 cm\(^{-1}\) was found to associate with \(-\text{O-CH}_2-\) and \(-\text{CH}_2-\text{O-}\) conformers, it can be inferred that a change in the conformational populations of ethylene glycol segments took place due to alkali degradation. Both 1340 and 971 cm\(^{-1}\) been have been found in spectra arising from highly ordered and less ordered trans structures found in crystalline phases of PET\(^{76,77}\). The increase of intensities in both these bands indicated an increase in trans conformers of \(\text{O-CH}_2-, \text{-CH}_2-\text{O-}\) and \(-\text{CH}_2 – \text{CH}_2-\) bonds. This suggested that an increase in crystallinity had taken place in the exterior regions of the fiber.

The results and interpretation of the FTIR analysis of the saline and enzyme degraded specimens are to be found in Appendix IV.
Table 4.2. Intensities of FTIR bands corresponding to ethylene glycol conformers in control and alkali degraded Dacron PET fabric specimens.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Period of degradation (days)</th>
<th>Peak Intensities (at cm⁻¹)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A₈₇₂</td>
<td>A₁₃₄₀/₈₇₂</td>
<td>A₉₇₁/₈₇₂</td>
<td>A₁₁₀₀/₈₇₂</td>
</tr>
<tr>
<td>Control</td>
<td>None</td>
<td>0.0122</td>
<td>1.721</td>
<td>0.369</td>
<td>4.934</td>
</tr>
<tr>
<td>Alkali degraded</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fabric</td>
<td>2</td>
<td>0.0121</td>
<td>1.554</td>
<td>0.355</td>
<td>4.496</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.0102</td>
<td>1.784</td>
<td>0.382</td>
<td>5.049</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.0098</td>
<td>2.551</td>
<td>0.602</td>
<td>7.510</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.0074</td>
<td>2.392</td>
<td>0.550</td>
<td>6.946</td>
</tr>
<tr>
<td>Fabric + stent</td>
<td>2</td>
<td>0.0124</td>
<td>1.831</td>
<td>0.387</td>
<td>5.346</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.0083</td>
<td>2.506</td>
<td>0.590</td>
<td>6.843</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.0089</td>
<td>2.360</td>
<td>0.562</td>
<td>7.022</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.0072</td>
<td>2.431</td>
<td>0.570</td>
<td>6.986</td>
</tr>
</tbody>
</table>
Figure 4.4 FTIR spectra of control and alkali degraded Dacron fabric specimens degraded without nitinol stents.

Figure 4.5 FTIR spectra of control and alkali degraded Dacron fabric specimens degraded with nitinol stents.
4.4. UV/VIS Spectroscopy as a Tool to Determine the Rate of Degradation

The concentration of terephthalate anion in the solutions of alkali-degraded samples were calculated using the Equation 3.11. The maximum absorbances for the solutions were observed at 239 – 240 nm wavelength. The concentration of the terephthalate anion increased exponentially over the degradation period of 10 days (Figure 4.6). The concentrations resulting from both nitinol unassociated and associated fabrics were comparable, indicating that nitinol wire elements have no or minimum effect on the alkali degradation of PET prosthetic fabrics.

The regression analysis carried out on the relationship between percent weight loss and the concentration of terephthalate anions showed that at 50% weight loss, a terephthalate anion concentration of 0.8555 g/L and 0.9584 g/L can be expected from the degradation of PET fabric specimens with and without nitinol respectively (Figure 4.7). Similar to the observations on the weight loss measurements these results confirmed that the nitinol stents did not significantly influence the rate of the degradation of PET fabrics.

The weight loss of the fabric calculated from the absorbance measurements were always found to be less than that from the actual weight loss measured gravimetrically (Table 4.3). This could be due to the fact that the absorbance measurements do not account for the concentration of ethylene glycol segments in the solution.
Figure 4.6 Concentration of terephthalate anion in solutions resulted from alkali degradation of ▲ fabric □ fabric with stent.
Figure 4.7 Relationship between weight loss of PET fabric specimens and concentration of dissolved terephthalate anion in solutions resulted from the treatment of ▲ fabric ■ fabric with stent.
Table 4.3. Comparison of absolute weight losses of PET fabric specimens resulted from actual weight measurements and UV/VIS spectroscopic analysis.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Duration of degradation (days)</th>
<th>Weight loss (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>UV/VIS Spec.</td>
</tr>
<tr>
<td>Fabric</td>
<td>2</td>
<td>0.0229</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.0849</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.1194</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.2450</td>
</tr>
<tr>
<td>Fabric + stent</td>
<td>2</td>
<td>0.0325</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.0970</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.1194</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.2384</td>
</tr>
</tbody>
</table>

4.5. Loss of Bursting Strength

The change in bursting strength of the PET fabric specimens degraded with alkali was calculated as the percent loss of bursting strength relative to that of the control. The analysis showed a linear increase of percent loss of initial bursting strength over the degradation time of 10 days (Figure 4.8). Statistical analysis based on $\alpha = 0.05$ level revealed that there is a significant difference between the loss of bursting strength of fabric specimens degraded with and without nitinol for 2 and 8 days. However no significant difference was shown between the specimens degraded with and without nitinol stents for 4 and 10 days. The trend lines obtained on the loss of bursting strength over duration of degradation almost coincided with each other giving similar relationships (Figure 4.8). The standard deviation between the sets of three replicates was observed to be between 0-1.5 % for both types of specimens. Because these are relatively small values the error bars cannot be observed in Figure 4.8. Based on the linear relationship between loss of bursting strength and degradation period, a 25% loss of initial bursting strength can be expected after 2.59 days and 2.63 days of alkali
degradation of Dacron PET fabrics alone and in the presence of nitinol wire elements, respectively. A similar loss of initial bursting strength was predicted for implanted PET arterial prostheses after 162 ± 23 months (40).

For the saline degraded specimens, the bursting strength increased over the 9 weeks degradation period. Detailed results are in Appendix V. In addition, with regards to the enzyme degraded fabric specimens, a gain in bursting strength was shown. Detailed results are found in Appendix V.

\[
y = -9.5132x \\
R^2 = 0.9437
\]

\[
y = -9.6431x \\
R^2 = 0.9594
\]

Figure 4.8 Loss of initial bursting strength of PET fabrics degraded in alkali at pH 13.4 ▲ PET fabric ■ PET fabric with stent
4.6. Analysis of Surface Changes of PET Fibers by SEM

The scanning electron micrographs obtained on the PET prosthetic fabrics degraded with alkali showed a clear progression of the degradation process (Figures 4.9 - 4.16). At the end of 2 days of alkali degradation, the fibers showed small pits on their surface (Figure 4.10 and 4.11). These pits vary in depth and tend to elongate and orient along the length of the fiber axis. With the progress of degradation and at increasing weight losses, these pits grew in size particularly in the direction of the fiber axis (Figure 4.10 - 4.13). The subsequent increase in weight loss was accompanied by the erosion and the formation of pits on the rest of the surface of the fiber (Figures 4.14 - 4.16). Both the depth and the frequency of the pits increased with increasing weight loss. A close examination of the surface revealed that the erosion and cavitation was not uniform and randomly distributed along the fiber surface. In addition the fiber diameter also showed a remarkable decrease as the weight loss increased.

It has been recorded \(^{49}\) that if titanium dioxide (TiO\(_2\)) is present in the fiber matrix, as hydrolysis progresses, deep cavities will be formed parallel to the axis of the fiber. These large cavities have been found to be caused by the hydrolysis of the amorphous regions and TiO\(_2\) particles were reported to be found in the vicinity of severely pitted regions \(^{49}\). The elliptical or the specific elongation of the pits along the fiber axis can be due to the orientation of amorphous regions during the drawing process of the filaments. With regards to the nitinol stents, no change of surface characteristics due to the presence of the nitinol was observed over the entire degradation period (Figure 4.17 - 4.20).

No surface damages were shown in saline degraded fabric specimens and nitinol stent elements. The detailed results are presented in Appendix VI.
Figure 4.9  SEM microphotograph of control Dacron PET fiber (Magnification 5000×).

Figure 4.10  SEM photomicrograph of Dacron PET fiber degraded without nitinol for 2 days in alkali (pH 13.4) at 65 °C (Magnification 5000×).
Figure 4.11 SEM photomicrograph of Dacron PET fiber degraded with nitinol for 2 days in alkali (pH 13.4) at 65 °C (Magnification 5000×).

Figure 4.12 SEM photomicrograph of Dacron PET fiber degraded without nitinol for 4 days in alkali (pH 13.4) at 65 °C (Magnification 5000×).
Figure 4.13  SEM photomicrograph of Dacron PET fiber degraded with nitinol for 4 days in alkali (pH 13.4) at 65 °C (Magnification 5000×).

Figure 4.14  SEM photomicrograph of Dacron PET fiber degraded without nitinol for 8 days in alkali (pH 13.4) at 65 °C (Magnification 5000×).
Figure 4.15  SEM photomicrograph of Dacron PET fiber degraded without nitinol for 10 days in alkali (pH 13.4) at 65 °C (Magnification 5000×).

Figure 4.16  SEM photomicrograph of Dacron PET fiber degraded with nitinol for 10 days in alkali (pH 13.4) at 65 °C (Magnification 5000×).
Figure 4.17  SEM photomicrograph of control nitinol stent element (Magnification 78×)

Figure 4.18 SEM photomicrograph of nitinol stent element after 2 days of alkali degradation (pH 13.4) at 65 °C (Magnification 78×)
Figure 4.19  SEM photomicrograph of nitinol stent element after 4 days of alkali degradation (pH 13.4) at 65 °C (Magnification 78×)

Figure 4.20  SEM photomicrograph of nitinol stent element after 10 days of alkali degradation (pH 13.4) at 65 °C (Magnification 78×)
5. Summary and Conclusions

This research on the stability of PET endoprostheses to *in-vitro* hydrolytic/enzymatic degradation was necessitated by three main factors. One major reason was the demand for less invasive surgery to repair abdominal aortic aneurysms (AAA) using endoprostheses. The advantages such as less loss of blood, less strain on the patient especially with older patients which is achieved by the elimination of open surgery, shorter stay in hospital and easier device deployment have caused endoprostheses to be more popular among surgeons. The second reason was due to the increasing interest in using other types of PET material as a substitution for Dacron PET, since DuPont has removed its polymers from the biomaterial supply chain. As Dacron PET is getting more and more scarce, more manufacturers are investigating possible substitutes to be used to produce endoprostheses. The above two facts have given rise to the third reason which is the increased focus on developing an accelerated *in-vitro* test to compare the biostability of Dacron PET endoprostheses against other devices constructed out of other types of PET. An accelerated test not only facilitates comparing two types of PET, but will also enable researchers and manufacturers to predict the long term biostability of a certain PET material based on the already published data on clinically explanted Dacron PET prostheses.

Therefore the overall focus of this research was to formulate an *in-vitro* accelerated test protocol to predict the long term biostability of Dacron PET endoprostheses by determining its rate of hydrolytic and enzymatic degradation. In addition, it also focused on examining the influence of nitinol stents on the hydrolytic/enzymatic degradation of Dacron PET.

A literature search was performed which looked into different ways of carrying out hydrolytic and enzymatic degradation. Based on these published data, the treatment conditions and the degradation durations were inferred for each environment. The alkali degradation was carried out on fabric specimens with and without nitinol at pH 13.4 at 65
0°C for 2, 4, 8 and 10 days. The enzyme conditions included treating the fabric in a similar way with phosphate buffered papain at a pH of 7.2 at 37 and 50 °C for 3, 6 and 9 weeks. The saline degradation conditions were similar to the above but carried out at 37 and 65 °C at a pH of 7.4.

Fabric specimens degraded in alkali with and without nitinol showed a linear relationship between percent weight loss and degradation time. The close similarity of correlation coefficients indicated the non existence of any influence from nitinol stents on PET degradation. Specimens degraded in saline at 37 °C showed weight losses less than 0.16% while the ones degraded at 65 °C were below 0.27%. The enzyme degraded specimens behaved entirely differently from the rest. A net weight gain was reported in all specimens after 9 weeks at both temperatures. The gain of weight increased with the degradation time except with specimens degraded at 37 °C, which exhibited a decrease followed by a gain of weight. The major reason for the weight gain was the binding and deposition of papain enzyme to the fiber surface and in the interstices of the yarns. The accumulation of enzymes which increased the weight gain with degradation time, was well confirmed by the SEM analysis done on specimens degraded at 50 °C. This binding of the enzyme could have been promoted by the saturating conditions of the PET on the reaction kinetics.

The thermal analysis of alkali degraded specimens gave evidence of similarities between the in-vitro and in-vivo degradation. The crystallinity of the fiber decreased during the first 4 days followed by an increase exceeding the initial values. The behavior of the melt endotherm shape suggested a similar mechanism of degradation may occur in vitro as observed previously in vivo. In both saline and enzyme degraded specimens, the crystallinity decreased after 9 weeks. This decrease was more prominent in the enzyme degraded specimens. No change in melt endotherm resulted from either of these two conditions.
Alkali degradation of Dacron PET with nitinol seemed to induce significant conformational changes in ethylene glycol moieties after 8 days, while those without nitinol stents showed similar conformational changes after 4 days. These changes indicated an increase in surface crystallinity. A substantial change in trans conformations of ethylene glycol moieties was observed in specimens which were in saline at 37 °C for 3 and 6 weeks without nitinol stents. Degradation in papain at both temperatures caused predominant changes in gauche –CH₂ – CH₂- bond conformations and minor changes in trans –O-CH₂- , –CH₂-O- and –CH₂ – CH₂- bond conformations in PET fibers after 3 and 6 weeks.

The concentration measurements of terephthalate anion in alkali degradation solutions using UV/VIS spectroscopy gave an exponential growth over the whole degradation period confirming the degree of degradation. No difference was observed between specimens with and without nitinol.

A linear correlation existed between the loss of probe bursting strength and the degradation time. A 25% of loss in initial bursting strength was observed approximately after 2.6 days of degradation for specimens with and without nitinol. A similar loss was observed in clinically explanted prostheses after 162 ± 23 months of implantation. This suggests that 2.6 days of in-vitro degradation at pH 13.4 corresponds to 162 ± 23 months of in-vivo degradation. The saline degraded specimens showed a net gain in initial bursting strength after 9 weeks. The maximum was recorded for each specimen after 6 weeks of degradation. An increase in initial bursting strength was shown in both specimens degraded in papain at 37 °C throughout the entire period of exposure. This was also evident with specimens treated without nitinol at 50 °C. However, the ones treated with nitinol appeared to have a loss in bursting strength throughout the nine weeks of degradation.

SEM analysis on alkali degraded specimens clearly depicted the severity of degradation. Pits elongated in the direction of the fiber axis were seen on the surface of the fiber after
2 days. These pits grew in shape, size and frequency with increasing weight loss. Except for a minor discoloration, no sign of surface deterioration was evident on the nitinol stents. In contrast, the saline degraded specimens and the nitinol stents associated with them appeared to have undergone no change. The specimens treated with enzyme at 50°C were observed to be mostly contaminated by residual enzyme particles. Close examination of the fabric structure revealed that the enzyme particles had accumulated in the interstices of the yarns as well as on the surface of the fibers. However no surface damage was observed on either the fiber or the nitinol stents.

Based on the results of this study it can be recommended that accelerated in-vitro degradation can be successfully carried out to determine the long term in-vivo stability of Type 56 Dacron polyester endoprosthesis. According to observations made in this study, an accelerated test using NaOH at pH 13.4 and 65°C can be performed to predict the long term biostability of the material. The correlation to in-vivo data were based solely on the loss of bursting strength of Dacron PET endoprostheses after 162 ± 23 months of implantation. In the case when the PET material is degraded alone, a 22 % loss in weight, 25 % loss in bursting strength and 0.37 g/L concentration of terephthalate anion in degradation solution after 2.60 days will indicate 162 ± 23 months of in-vivo stability. When the Dacron PET fabric is degraded with nitinol stent elements for the same duration, a 21 % loss in weight, 25 % loss in bursting strength and 0.46 g/L concentration of terephthalate anion in the degradation solution will indicate 162 ± 23 months of in-vivo stability.

Furthermore, this study revealed that there is a minimal influence from nitinol stents on hydrolytic and/or enzymatic degradation of Type 56 Dacron PET endoprostheses.
6. Recommendations for Future Research

Based on the work carried out during this research study, it was evident that Dacron PET can be degraded rapidly in alkaline solutions having a high pH. Therefore it would be feasible to derive a kinetic model for the PET degradation process which would lead to a more correct way of predicting the rate of degradation. This will also assist in correlating in-vitro experimental data with the data on explanted vascular prostheses. The study suggested that the use of enzymes is an alternative way of attaining degradation. However the rate of degradation could be comparatively slow due to the inherent hydrophobicity of PET and the low diffusibility of molecules into the fiber. This may be overcome by selecting a more active enzyme or by using a compatible mixture of enzymes which is stable at temperatures above 60^\circ C. Rather than using material in large amounts, it would be prudent to use small amounts of material for degradation since the enzymes can easily get saturated which leads to a slower rate of degradation. Further, if the rate of degradation can be increased, then it can be effectively and efficiently analyzed within a shorter period of time. A study that would focus on the degradation mechanisms and rate of degradation would pave the way for understanding the process of bulk degradation of PET prosthetic fabrics when exposed to enzymes. When an endovascular prosthesis is implanted in the body it interacts with various peroxide and oxidizing agents released from macrophages and lymphocytes. Further studies will have to be carried out to find means of simulating these conditions in an accelerated in vitro test.
7. References


29. McMahon W, Birdsall HA, Johnson GR, Camilli CT. Physical properties


APPENDICES
Appendix I. Analysis of Enzyme Activity

The enzyme activity of both natural and initially activated papain was calculated using Equation 3.5 (Figure 4.21- 4.24) based on the experimental set-up shown in Table 3.2. The loss of initial activity calculated after 1 and 2 days (Figure 4.23 and 4.24) at 37 and 50 °C were compared based on the difference in loss of activity when fabric is incorporated with the enzyme and when the enzyme is initially activated with cysteine.

At both temperatures, after two days, the loss of initial activity of inactivated enzyme was greater when the enzymes were incorporated with fabric compared to the enzyme which was not exposed to the fabric (Figure 4.25 and 4.26). This increase in loss of activity was observed to be much greater at 50 than at 37 °C. However when the enzyme was initially activated, the papain maintained at 50 °C with the PET fabrics showed considerably less loss of initial activity compared to that of at 37 °C. From the former observation, it is apparent that papain can positively participate in the degradation of the PET fabric at both temperatures.

The later phenomenon suggests that papain not only exhibits a higher esterase activity on fabric at 50 °C, but it can also maintain its activity when it is initially activated. With regards to the effect of initial activation of enzyme on the fabric compared to that of inactivated enzyme, papain showed an increase in loss of initial activity at both temperatures when it was initially activated. This increase in loss of activity however was greater at 50 °C. This proved that the initial activation of the enzyme could have a positive degradative effect on the PET fabric at both temperatures.
Figure 4.21  Change of Esterase activity of natural and initially activated Papain kept at 37°C for two days ▲ Natural ■ Activated.
Figure 4.22 Change of Esterase activity of natural and initially activated papain incorporated with PET fabric and kept at 37 °C for two days. ▲ Natural enzyme with fabric ■ Activated enzyme with fabric.
Figure 4.23  Change of Esterase activity of natural and initially activated Papain kept at 50 °C for two days ▲ Natural ■ Activated
Figure 4.24 Change of esterase activity of natural and initially activated papain incorporated with PET fabric and kept at 50°C for two days. ▲ Natural enzyme with fabric ■ Activated enzyme with fabric.
Figure 4.25  Effect of the presence of Dacron PET fabric and the initial enzyme activation on esterase activity of Papain at 37 °C.
Figure 4.26 Effect of the presence of Dacron fabric and the initial enzyme activation on esterase activity of Papain at 50 °C.
Appendix II. Change of Initial Weight of PET Fabrics upon Saline and Enzyme Degradation.

The percent loss of initial weight of fabric specimens upon degradation with phosphate buffered saline at 37 and 65 °C were observed to be significantly lower compared to that of alkali degradation. Even after treating PET fabrics for 9 weeks with phosphate buffered saline at 65 °C, the fibers seemed to lose little or no weight (Figure 4.28). This was observed to be true with the fabrics treated at 37 °C (Figure 4.27). However the fabrics treated at 65 °C showed slightly greater weight loss than that at 37 °C irrespective of whether they included nitinol stent elements.

With regards to the Dacron fabric specimens degraded with enzyme, most specimens showed a gain in initial weight. Except the fabric specimens treated at 37 °C for three weeks, others showed a percent gain in initial weight (Figure 4.29). In the case of the fabrics treated at 50 °C, the percent gain of weight increased with the degradation period (Figure 4.30). This phenomenon was experienced on both samples treated at 37 and 50 °C, irrespective of whether they were incorporated with nitinol stent elements.
Figure 4.27 Effect of phosphate buffered saline degradation at 37 °C on the initial weight of Dacron PET prosthetic fabric specimens ▲ Dacron PET fabric ■ Dacron PET fabric plus stent.
Figure 4.28 Effect of phosphate buffered saline degradation at 65 °C on the initial weight of Dacron PET prosthetic fabric specimens ▲ Dacron PET fabric ■ Dacron PET fabric plus stent.
Figure 4.29 Change of initial weight of Dacron PET fabric specimens upon degradation with papain at 37 °C ▲ Dacron PET fabric ■ Dacron PET fabric plus stent.
Figure 4.30 Change of initial weight of Dacron PET fabric specimens upon degradation with papain at 50 °C ▲ Dacron PET fabric ■ Dacron PET fabric plus stent.
In addition, the fabric specimens treated at both temperatures appeared to be discolored. This color change was more prominent in samples treated at 50 °C. Apart from losing their original color, they were also observed to be stiff and had lost their original flexibility. The gain of weight, loss of original color and flexibility experienced during enzymatic degradation of PET fabrics can be justified based on the inherent behavior exhibited by the particular enzyme. The above observations suggest that papain enzyme tightly binds to the Dacron PET fabric. The inability to remove most of the enzyme from the surface of the fabric even after a thorough detergent wash suggests that it is not just a coating of the enzyme which imparts the above properties to the fabric. The binding between the enzyme and the fibers appeared to be much stronger, such that it was impossible to remove by the detergent wash.

When considering the chemical modes of enzyme catalysis, papain in particular contains two ionizable residues – a Cystiene residue (Cys-25) and a Histidine (His-159) in its active site (66, 80). The examination of the pH profile for papain with inflection points at pH 4.2 and pH 8.2 has revealed that the ionizable residue – nucleophilic Cysteine (having a pH of 3.4 due to the effect of other ionizable groups) is deprotonated at the optimum pH for papain (80). This nucleophilic cystiene is found to actively participate in the catalytic activity of papain. The cysteine, binds covalently to the substrate to form a reactive intermediate (80). In order for an enzyme to exert catalytic activity, its binding to a substrate must be weak. Extremely tight binding of the substrate causing excessive enzyme-substrate complex stability could very much hinder the catalytic activity resulting from non-recycling of catalyst and increase of the activation barrier (80).

The observations experienced on the interaction of papain with PET fabric specimens could be due to the excessive binding of papain to the PET long chain molecules. The cause of excessive binding of the papain enzyme to the fabric could be related to the concentration of PET in the reaction bath. When the papain enzyme is saturated with PET the equilibrium of the binding reaction between the enzyme and the PET favors the enzyme-PET complex. This leads to a stable enzyme-PET complex disrupting the
catalytic activity of papain. The result will be a greater reduction in the rate of degradation. Since the rate of degradation cannot be determined by analyzing the weight loss, molecular weight measurements should give a clearer indication of the extent of the enzymatic degradation.
Appendix III. Effect of Saline and Enzyme Degradation on Thermal Properties

The thermal analysis data of the Dacron PET fabrics degraded with buffered saline are presented in Table 4.4. Similar to the alkali degraded specimens, no pre-crystallization was observed in the endotherms. In contrast to the alkali degraded PET fabric specimens, the melting endotherms obtained on PET fabrics degraded with saline showed no sign of shifting of the endotherm shoulder. (Figure 4.31 and 4.32). This behavior was observed with all the treatment conditions including the samples which are associated with nitinol stents. However the nitinol unassociated samples, which were treated for three weeks at both temperatures, showed a sharpening of the endotherm (Figure 4.31 and 4.32). This sharpness disappeared after 6 weeks of degradation. The overall crystallinity of the PET fibers did not alter significantly throughout the saline degradation. Except for the PET fabric specimen with nitinol treated for 9 weeks at 65 °C, all the other samples, which were treated for nine weeks showed minor decreases in their crystallinity, compared to that of the control (Table 4.4).
Table 4. Differential scanning calorimetric data on control and saline degraded PET fabrics.

<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Specimens</th>
<th>Period of degradation (weeks)</th>
<th>Temp. at shoulder (°C)</th>
<th>Melting temp. (°C)</th>
<th>Heat of fusion, ΔH (J/g)</th>
<th>Crystallinity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>None</td>
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<td>258.033</td>
<td>56.824</td>
<td>40.588</td>
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Figure 4.31 Melting endotherms of PET fabric specimens degraded in saline at 37°C showing (A) PET fabrics (B) PET fabrics plus nitinol stent elements.
Figure 4.32 Melting endotherms of PET fabric specimens degraded in saline at 65 °C showing (A) PET fabrics (B) PET fabrics plus nitinol stent elements.
The thermal analysis data of the Dacron PET fabrics degraded in buffered papain are presented in Table 4.5. As seen from the table, the crystallinity of both fabric specimens treated with and without nitinol stent elements at both temperatures changed after three weeks of degradation. Except for fabric specimens treated at 50 °C, the crystallinity of others remained approximately the same up to six weeks. After 9 weeks a drastic decrease in crystallinity was observed with all fabric specimens. A significant decrease was shown with the specimens treated with nitinol at 50 °C.

The melting endotherms obtained on these fabric specimens showed no shifting of the shoulder compared to that of the control. However, the specimens treated at 37 °C without nitinol stents showed a double peak endotherm after nine weeks of treatment (Figure 4.33). In the case of the specimens treated with nitinol, the double peak endotherms were observed after six weeks of degradation. In addition the endotherms were also observed to be sharpened after six to nine weeks of degradation with both types of specimens. Except for the specimen degraded at 50 °C with nitinol stents for 3 weeks, all the other specimens showed no sign of pre-crystallization exothermal peaks.

Similar to the samples treated at 37 °C the specimens treated at 50 °C showed no shifting of the endotherm shoulder (Table 4.5 and Figure 4.34). The PET specimens degraded with nitinol showed a clearly distinguishable shoulder after 9 weeks. The melting peaks of the samples treated without nitinol stent elements showed a sharpening after 3 and 6 weeks of degradation, where as a similar trend was observed with samples treated with nitinol after 6 weeks of degradation. The observations on the non-shifting of the melting endotherm shoulder suggested that the outer layers of the PET fibers which constitutes the stable crystallites have not been degraded. However the double peak endotherm observed after 6 and 9 weeks with the specimens degraded at 37 °C indicated the formation of two distinguishable crystalline fractions. Though this phenomenon was not experienced clearly with the samples degraded at 50 °C, the sharpening of endotherms revealed that there was a restructuring of the crystalline areas in the fibers after 3 weeks.
Table 4.5. Differential scanning calorimetric data on control and enzyme degraded PET fabrics

<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Specimens</th>
<th>Temp. degradation (weeks)</th>
<th>Temp. at shoulder (°C)</th>
<th>Melting temp. (°C)</th>
<th>Heat of fusion, $\Delta H$ (J/g)</th>
<th>Crystallinity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>250.000</td>
<td>258.700</td>
<td>47.205</td>
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Figure 4.33  Melting endotherms of PET fabric specimens degraded in papain at 37 °C showing (A) PET fabrics (B) PET fabrics plus nitinol stent elements.
Figure 4.34  Melting endotherms of PET fabric specimens degraded in papain at 50 °C showing (A) PET fabrics (B) PET fabrics plus nitinol stent elements
Appendix IV. Microstructural Changes in PET due to Saline and Enzyme Degradation

For the fabric specimens degraded with saline at 37 °C, no shifting was observed at 1715 cm⁻¹ and 872 cm⁻¹ bands. However 1100 cm⁻¹ and 1340 cm⁻¹ bands shifted to 1099 cm⁻¹ and 1339 cm⁻¹ after 3 weeks of degradation followed by a reshift to the original position after 9 weeks (Figure 4.35 and 4.36). The peak intensities calculated for these specimens (Table 4.6) show a change in conformation of ethylene glycol segments with respect to specimens degraded without nitinol. The increase in intensities of 1340 and 971 cm⁻¹ ethylene glycol trans bands, up to 6 weeks in specimens degraded without nitinol stents indicated that a change in surface crystallinity had taken place. In comparison to the control, the samples treated with nitinol stents showed no change of ethylene glycol conformers.

With the fabric specimens degraded at 65 °C without nitinol, the 1100 cm⁻¹ band shifted to 1099 cm⁻¹ after 9 weeks while other bands remained unchanged. With the specimens treated with nitinol, the band at 1715 cm⁻¹ shifted to 1716 cm⁻¹ while 1100 cm⁻¹ and 1340 cm⁻¹ shifted by one unit to a lower position (Figure 4.37 and 4.38). No shifting was observed in the thickness band. The peak intensities calculated for these specimens (Table 4.6) revealed a considerable change in the conformation of ethylene glycol moieties in the specimens degraded with nitinol. Increase in 971 and 1340 cm⁻¹ band intensities in specimens degraded with nitinol stents for 9 weeks indicated an increase in surface crystallinity.

With regards to the PET fabrics degraded without nitinol stents in papain at 37 °C, the absorbance bands at 1100 cm⁻¹ and 1340 cm⁻¹ were shifted by one unit to lower positions after 3 weeks. The 1340 cm⁻¹ band re-shifted to the initial position after 9 weeks. In the fabric degraded with nitinol stents, both bands shifted similarly after 3 weeks, followed by a re-shifting to their initial positions after 9 weeks (Figure 4.39 and 4.40) In contrast, the specimens degraded at 50 °C with and without nitinol stents showed no shifting of absorbance bands (Figure 4.41 and 4.42).
In addition, the intensities calculated for both specimens treated at both temperatures in papain showed minor changes at 971 cm\(^{-1}\) and 1340 cm\(^{-1}\) while substantial changes occurred in the 1100 cm\(^{-1}\) band (Table 4.7). This indicated that the changes of –CH\(_2\) – CH\(_2\)- bond conformations were more pronounced while only minor changes occurred in – O-CH\(_2\)- and -CH\(_2\)-O- bond conformations. Minor changes in 971 and 1340 cm\(^{-1}\) suggested that minimal change of crystallinity had taken place. The peak intensities at 971 cm\(^{-1}\) for the fabric specimens degraded with nitinol stents at 50 \(^{\circ}\)C for 9 weeks were observed to be lower. This may be due to the fact that the absorbance measurements were hindered by the interaction of enzyme particles covering the fabric surface.

The shifting of absorbance bands at 1340 cm\(^{-1}\) and 1100 cm\(^{-1}\) in all specimens degraded with alkali, saline and enzyme was associated with concurrent increases in absorbance at their respective peaks relative to that of control. However, in neither band was a decrease in absorbance associated with shifting of the peaks.
Table 4.6. Intensities of FTIR bands corresponding to ethylene glycol conformers in control and saline degraded Dacron PET fabric specimens.

<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Temp. (°C)</th>
<th>Period of degradation (weeks)</th>
<th>Peak Intensities (at cm⁻¹)</th>
<th></th>
</tr>
</thead>
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<tr>
<td>Specimens</td>
<td></td>
<td></td>
<td>A₈₇₂</td>
<td>A₁₃⁴₀/₈₇₂</td>
</tr>
<tr>
<td>Control</td>
<td>None</td>
<td>None</td>
<td>0.0122</td>
<td>1.721</td>
</tr>
<tr>
<td>Saline degrad.</td>
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<td></td>
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<tr>
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<td></td>
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<td>9</td>
<td>0.0100</td>
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<tr>
<td>Fabric + stent</td>
<td>3</td>
<td>3</td>
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<tr>
<td></td>
<td></td>
<td>9</td>
<td>0.0125</td>
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</table>
Figure 4.35  FTIR spectra of control and saline degraded Dacron fabric specimens degraded without nitinol at 37 °C.

Figure 4.36  FTIR spectra of control and saline degraded Dacron fabric specimens. These fabrics were degraded with nitinol at 37 °C.
Figure 4.37 FTIR spectra of control and saline degraded Dacron fabric specimens degraded without nitinol at 65 °C.

Figure 4.38 FTIR spectra of control and saline degraded Dacron fabric specimens degraded with nitinol at 65 °C.
Table 4.7. Intensities of FTIR bands corresponding to ethylene glycol conformers in control and enzyme degraded Dacron PET fabric specimens.

<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Specimens</th>
<th>Period of degradation (weeks)</th>
<th>Peak Intensities ( at cm(^{-1}))</th>
<th>A(_{872})</th>
<th>A(<em>{1340}/A</em>{872})</th>
<th>A(<em>{971}/A</em>{872})</th>
<th>A(<em>{1100}/A</em>{872})</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>None</td>
<td>None</td>
<td>0.0122</td>
<td>1.721</td>
<td>0.369</td>
<td>4.934</td>
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<tr>
<td>Enzyme degrad.</td>
<td>Fabric</td>
<td>3</td>
<td>0.0098</td>
<td>1.745</td>
<td>0.378</td>
<td>5.296</td>
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<td></td>
<td></td>
<td>6</td>
<td>0.0100</td>
<td>1.780</td>
<td>0.380</td>
<td>5.390</td>
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<td></td>
<td></td>
<td>9</td>
<td>0.0133</td>
<td>1.729</td>
<td>0.376</td>
<td>5.188</td>
<td></td>
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<tr>
<td></td>
<td>Fabric + stent</td>
<td>3</td>
<td>0.0097</td>
<td>1.773</td>
<td>0.381</td>
<td>5.381</td>
<td></td>
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<tr>
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<td></td>
<td>6</td>
<td>0.0136</td>
<td>1.765</td>
<td>0.368</td>
<td>5.147</td>
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<td></td>
<td></td>
<td>9</td>
<td>0.0102</td>
<td>1.706</td>
<td>0.363</td>
<td>4.863</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fabric</td>
<td>50</td>
<td>3</td>
<td>0.0114</td>
<td>1.728</td>
<td>0.360</td>
<td>5.272</td>
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<td></td>
<td></td>
<td>6</td>
<td>0.0128</td>
<td>1.719</td>
<td>0.391</td>
<td>5.469</td>
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<td>9</td>
<td>0.0096</td>
<td>1.625</td>
<td>0.354</td>
<td>4.542</td>
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<td></td>
<td>Fabric + stent</td>
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<tr>
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<td></td>
<td>9</td>
<td>0.0083</td>
<td>1.699</td>
<td>0.133</td>
<td>4.711</td>
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</table>
Figure 4.39 FTIR spectra of control and enzyme degraded Dacron fabric specimens degraded without nitinol at 37 °C.

Figure 4.40 FTIR spectra of control and enzyme degraded Dacron fabric specimens degraded with nitinol at 37 °C.
Figure 4.41  FTIR spectra of control and enzyme degraded Dacron fabric specimens degraded without nitinol at 50 °C.

Figure 4.42  FTIR spectra of control and enzyme degraded Dacron fabric specimens degraded with nitinol at 50 °C.
Appendix V. Loss of Bursting Strength in PET fabric specimens upon Saline and Enzyme Degradation

The percent changes in initial bursting strength of saline degraded samples are presented in Figure 4.43 and Figure 4.44. A percent gain of initial bursting strength was observed with both nitinol associated and unassociated PET fabric specimens. After three weeks all fabric specimens showed a gain above 10%. Except with the nitinol associated fabric specimen degraded at 65°C, all the other specimens showed a minor increase of gain after 6 weeks of degradation. However this increase in gain decreased after 9 weeks in all the specimens. Except for the samples degraded for 9 weeks at 37°C, all the other specimens showed a difference in loss of bursting strength between nitinol associated and unassociated specimens. As seen from the figures a considerably higher variation was observed in all the results compared to the alkali treated specimens. One reason for this was the limited availability of samples to carry out repeated tests to minimize variation between measurements.

A similar trend of gain in initial bursting strength was observed with the specimens degraded in buffered papain. All samples degraded at 37°C had a gain above 6% after 3 weeks of degradation and this increase in gain continued till the end of the entire degradation period (Figure 4.45). The final gain recorded was approximately 11%-12% of the initial bursting strength. In the case of the fabric specimens treated at 50°C, only the fabrics treated without nitinol stents showed an increase in gain over the degradation period (Figure 4.46). However this increase was much lower than that of the samples treated at 37°C. The fabrics treated with nitinol stent elements had a loss in initial bursting strength. This loss increased over six weeks of degradation. Similar to the saline degraded specimens, these results also showed a substantial amount of variation. The variations were comparably higher in the fabric specimen degraded at 50°C for 9 weeks with nitinol stents. The gain of bursting strength could be attributed to the increase of stiffness of the fabric caused by the enzyme adhering to the fabric surface. However the loss of bursting strength observed in the specimens degraded with nitinol at 50°C
indicated that some degree of degradation had taken place. The specimens degraded with and without nitinol stents appeared to have a difference in loss of bursting strength at each period of degradation.

Figure 4.43 Change in initial bursting strength of PET fabrics degraded in buffered saline at 37 °C ▲ PET fabric ■ PET fabric with stent
Figure 4.44 Change in initial bursting strength of PET fabrics degraded in buffered saline at 65 °C ▲ PET fabric ■ PET fabric with stent
Figure 4.45 Change in initial bursting strength of PET fabrics degraded in buffered papain at 37 °C ▲ PET fabric ■ PET fabric with stent
Figure 4.46 Change in initial bursting strength of PET fabrics degraded in buffered papain at 50 °C ▲ PET fabric ■ PET fabric with stent
Appendix VI. Analysis of Surface Changes in Saline and Enzyme Degraded PET Fibers

In contrast to the alkali degraded samples, the fabric degraded with buffered saline solutions at both 37 and 65 °C did not show any formation of cavities or pits over the entire degradation period (Figure 4.47 - 4.50). Similar observations were experienced with the nitinol stent elements which were associated with these fabric specimens (Figure 4.51 and 4.52).

Similarly, no change of surface characteristics were found in PET fabric specimens degraded with and without nitinol stents in enzyme at 37 °C (Figure 4.53 and 4.54). After nine weeks of degradation there were some signs of enzyme deposition on the fiber surface (Figure 4.55). The surface of the nitinol stent elements was clear without any deposition of enzyme particles after 9 weeks (Figure 4.56 and 4.57).

In contrast, the specimens degraded at 50 °C showed deposition of enzyme particles on their surfaces after 3 weeks (Figure 4.58). There was no difference in the degree of deposition of enzyme on the fiber between specimens degraded with and without nitinol. The amount of enzymes adhering to the fiber surface increased over the degradation period (Figure 4.58-4.61). Further, the enzyme particles were observed to be trapped in between the fibers in addition to surface deposition (Figure 4.61). The surface of the nitinol stents were covered with a layer of enzyme after 3 weeks and this continued to increase till the end of the degradation period (Figure 4.62-4.63). Closer examination of the enzyme layer revealed that the enzymes are tightly bound to the nitinol wire surface in some areas (Figure 4.64).
Figure 4.47  SEM photomicrograph of Dacron PET fiber degraded without nitinol for 9 weeks in buffered saline (pH 7.4) at 37 °C (Magnification 5000×).

Figure 4.48  SEM photomicrograph of Dacron PET fiber degraded without nitinol for 3 weeks in buffered saline (pH 7.4) at 65 °C (Magnification 5000×).
Figure 4.49  SEM photomicrograph of Dacron PET fiber degraded without nitinol for 9 weeks in buffered saline (pH 7.4) at 65 °C (Magnification 5000×).

Figure 4.50  SEM photomicrograph of Dacron PET fiber degraded with nitinol for 9 weeks in buffered saline (pH 7.4) at 65 °C (Magnification 5000×).
Figure 4.51  SEM photomicrograph of nitinol stent element after 9 weeks of degradation in buffered saline (pH 7.4) at 37 °C (Magnification 78×).

Figure 4.52  SEM photomicrograph of nitinol stent element after 9 weeks of degradation in buffered saline (pH 7.4) at 65 °C (Magnification 78×).
Figure 4.53  SEM photomicrograph of Dacron PET fiber degraded without nitinol for 3 weeks in buffered papain (pH 7.2) at 37 °C (Magnification 5000×).

Figure 4.54  SEM photomicrograph of Dacron PET fiber degraded without nitinol for 9 weeks in buffered papain (pH 7.2) at 37 °C (Magnification 5000×).
Figure 4.55  SEM photomicrograph of Dacron PET fiber degraded with nitinol for 9 weeks in buffered papain (pH 7.2) at 37 °C (Magnification 5000×).

Figure 4.56  SEM photomicrograph of nitinol stent element after 3 weeks of degradation in buffered papain (pH 7.2) at 37 °C (Magnification 78×).
Figure 4.57  SEM photomicrograph of nitinol stent element after 9 weeks of degradation in buffered papain (pH 7.2) at 37 °C (Magnification 78×).

Figure 4.58  SEM photomicrograph of Dacron PET fiber degraded without nitinol for 3 weeks in buffered papain (pH 7.2) at 50 °C (Magnification 5000×).
Figure 4.59  SEM photomicrograph of Dacron PET fiber degraded without nitinol for 9 weeks in buffered papain (pH 7.2) at 50 °C (Magnification 5000×).

Figure 4.60  SEM photomicrograph of Dacron PET fiber degraded with nitinol for 9 weeks in buffered papain (pH 7.2) at 50 °C (Magnification 5000×).
Figure 4.61 SEM photomicrograph of Dacron PET fabric degraded without nitinol for 9 weeks in buffered papain (pH 7.2) at 50 °C (Magnification 625×).

Figure 4.62 SEM photomicrograph of nitinol stent element after 3 weeks of degradation in buffered papain (pH 7.2) at 50 °C (Magnification 78×).
Figure 4.63  SEM photomicrograph of nitinol stent element after 9 weeks of degradation in buffered papain (pH 7.2) at 50 °C (Magnification 78×).

Figure 4.64  SEM photomicrograph showing the enzyme bound to the surface of stent element after 9 weeks of degradation in buffered papain (pH 7.2) at 50 °C (Magnification 1250×).