# CHAPTER 5

# *IN VITRO* BIODEGRADATION

## 5.1 Experimental Procedure

The three commercial sutures, all in size 2-0: MONOCRYL (Ethicon), MAXON (Davis&Geck) and PDS II (Ethicon), together with the random terpolymer of L-lactide, ε-caprolactone and glycolide were chosen for *in vitro* biodegradation studies.

All glassware items were sterilized before use by steam autoclaving at 120°C for 20 minutes. A phosphate buffer saline (PBS) pH 7.40 was used as the immersion medium at a temperature of 37°C.

### 5.1.1 Phosphate Buffer Saline (PBS) Immersion Medium (pH 7.40)

Following the procedure described in the "European Pharmacopeia" [18], a phosphate buffer saline immersion medium of physiological pH 7.40 was prepared from 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8.0 g of sodium chloride dissolved in 1 $dm^3$ of deionized water. The pH of the solution, which was almost 7.40, was adjusted to exactly 7.40 with 1 M aqueous NaOH solution.

### 5.1.2 Sample Preparation

Three different 2-0 commercial sutures were studied: MONOCRYL, MAXON and PDS II. Twenty sets of each MAXON and PDS II (size 2-0) sutures and fifteen sets of MONOCRYL (size 2-0) suture were accurately weighed. Each set consist of three pieces of 30 cm monofilament sutures (obtained from one and a half package of each commercial sutures).

For the PLCG random terpolymer, it was made in the physical form of monofilament fiber (diameter $\approx$ 0.39 mm). Ten fibers, each about 120 cm long were accurately weighed. The fifty five sets of commercial sutures and ten PLCG fibers samples were then separately immersed in 50 ml screw-top glass bottles, each containing 30 ml of the pH 7.40 phosphate buffer saline solution. The bottles were immediately placed in the incubator (see Fig. 5.1) thermostatically controlled at 37.0 $\pm$ 1.0$^{\circ}$C, for the *in vitro* biodegradation experiments.


Fig. 5.1 Incubator used for the *in vitro* biodegradation experiment.

### 5.1.3 Sampling Procedure

After designated time interval for *in vitro* biodegradation studies, one bottle containing each type of the commercial suture and the PLCG terpolymer was taken out from the incubator. The fiber samples were then filtered off, washed carefully with deionized water and dried to constant weight in a vacuum oven at room temperature. Their weight were accurately recorded and their % weight retention calculated.

In addition to their % weight retentions, the fiber's mechanical properties (tensile testing), surface topography (scanning electron microscopy), melting point and heat of fusion (differential scanning calorimetry) and intrinsic viscosity (dilute-solution viscometry) were also studied. The pH of the medium was re-measured since the hydrolytic mechanism of polyester biodegradation is well known to be pH-dependent. The combined results therefore gave a physico-mechanical picture of the *in vitro* biodegradation profile.

## 5.2 Property Changes from Biodegradation

### 5.2.1 Weight Loss Profiles

An analytical balance was used to weigh all suture samples. After vacuum drying to constant weight, their % weight retention were calculated as follows:

$$\% \text{ Weight Retention} = \frac{W_f}{W_o} \times 100\ \%$$

where $W_o$ = initial weight of sample

$W_f$ = final weight of sample

The weights and % weight retention of MONOCRYL, MAXON, PDS II and PLCG samples are shown in Table 5.1 – Table 5.4. The corresponding weight loss profiles are plotted in Fig 5.2.

Table 5.1 Weights and %weight retentions of MONOCRYL sutures immersed in pH 7.40 phosphate buffer saline medium at 37.0 ± 1.0°C.

| Time (weeks) | Initial Weight ± 0.0001 (g) | Final Weight ± 0.0001 (g) | % Weight Retention ± 0.1 (%) |
|---|---|---|---|
| 1 | 0.1829 | 0.1815 | 99.2 |
| 2 | 0.1876 | 0.1834 | 97.8 |
| 3 | 0.1836 | 0.1698 | 92.5 |
| 4 | 0.1852 | 0.1550 | 83.7 |
| 5 | 0.1881 | 0.1387 | 73.7 |
| 6 | 0.1839 | 0.1251 | 68.0 |
| 7 | 0.1840 | 0.1142 | 62.1 |
| 8 | 0.1851 | 0.1058 | 57.2 |
| 9 | 0.1792 | 0.0974 | 54.4 |
| 10 | 0.1768 | 0.0925 | 52.3 |
| 12 | 0.1186 | 0.0502 | 42.3 |
| 14 | 0.1172 | 0.0449 | 38.3 |
| 16 | 0.1164 | 0.0364 | 31.3 |
| 18 | 0.1164 | 0.0317 | 27.2 |
| 20 | 0.1199 | 0.0348 | 29.0 |

Note : Due to shortage of MONOCRYL samples supplied the biodegradation of this suture was studied during 20 weeks period instead of 30 weeks as the other three type fibers.

Table 5.2 Weights and % weight retentions of MAXON sutures immersed in pH 7.40 phosphate buffer saline medium at 37.0 ± 1.0°C.

| Time (weeks) | Initial Weight ± 0.0001 (g) | Final Weight ± 0.0001 (g) | % Weight Retention ± 0.1 (%) |
|---|---|---|---|
| 1 | 0.1914 | 0.1906 | 99.6 |
| 2 | 0.1909 | 0.1909 | 100.0 |
| 3 | 0.1870 | 0.1866 | 99.8 |
| 4 | 0.1910 | 0.1906 | 99.8 |
| 5 | 0.1927 | 0.1914 | 99.3 |
| 6 | 0.1908 | 0.1859 | 97.4 |
| 7 | 0.1888 | 0.1833 | 97.1 |
| 8 | 0.1891 | 0.1825 | 96.5 |
| 9 | 0.1922 | 0.1806 | 94.0 |
| 10 | 0.1919 | 0.1725 | 89.9 |
| 12 | 0.1917 | 0.1509 | 78.7 |
| 14 | 0.1887 | 0.1405 | 74.4 |
| 16 | 0.1945 | 0.1366 | 70.2 |
| 18 | 0.1922 | 0.1294 | 67.3 |
| 20 | 0.1903 | 0.1116 | 58.6 |
| 22 | 0.1883 | 0.1008 | 53.5 |
| 24 | 0.1952 | 0.1026 | 52.6 |
| 26 | 0.1909 | 0.0926 | 48.5 |
| 28 | 0.1924 | 0.0896 | 46.6 |
| 30 | 0.1907 | 0.0889 | 46.6 |

Table 5.3 Weights and % weight retentions of PDS II sutures immersed in pH 7.40 phosphate buffer saline medium at 37.0 ± 1.0°C.

| Time (weeks) | Initial Weight ± 0.0001 (g) | Final Weight ± 0.0001 (g) | % Weight Retention ± 0.1 (%) |
|---|---|---|---|
| 1 | 0.1574 | 0.1573 | 99.9 |
| 2 | 0.1546 | 0.1537 | 99.4 |
| 3 | 0.1559 | 0.1521 | 97.6 |
| 4 | 0.1577 | 0.1570 | 99.6 |
| 5 | 0.1516 | 0.1515 | 99.9 |
| 6 | 0.1593 | 0.1563 | 98.1 |
| 7 | 0.1502 | 0.1476 | 98.3 |
| 8 | 0.1561 | 0.1550 | 99.3 |
| 9 | 0.1569 | 0.1549 | 98.7 |
| 10 | 0.1526 | 0.1478 | 96.9 |
| 12 | 0.1559 | 0.1453 | 93.2 |
| 14 | 0.1536 | 0.1385 | 90.2 |
| 16 | 0.1606 | 0.1369 | 85.2 |
| 18 | 0.1578 | 0.1240 | 78.6 |
| 20 | 0.1502 | 0.1095 | 72.9 |
| 22 | 0.1536 | 0.1034 | 67.3 |
| 24 | 0.1551 | 0.1014 | 65.4 |
| 26 | 0.1631 | 0.0994 | 60.9 |
| 28 | 0.1524 | 0.0716 | 47.0 |
| 30 | 0.1530 | 0.0765 | 50.0 |

Table 5.4 Weights and % weight retention of PLCG fibers immersed in pH 7.40 phosphate buffer saline medium at 37.0 ± 1.0°C.

| Time (weeks) | Initial Weight ± 0.0001 (g) | Final Weight ± 0.0001 (g) | % Weight Retention ± 0.1 (%) |
|---|---|---|---|
| 1 | 0.2051 | 0.2017 | 98.3 |
| 2 | 0.1898 | 0.1793 | 94.5 |
| 3 | 0.1752 | 0.1685 | 96.2 |
| 4 | 0.1887 | 0.1775 | 94.1 |
| 6 | 0.1845 | 0.1604 | 86.9 |
| 8 | 0.1919 | 0.1432 | 74.6 |
| 10 | 0.1930 | 0.1289 | 66.8 |
| 12 | 0.1927 | 0.1188 | 61.7 |
| 14 | 0.1989 | 0.1120 | 56.3 |
| 16 | 0.2066 | 0.1091 | 52.8 |
| 18 | 0.2009 | 0.1068 | 53.2 |
| 20 | 0.2042 | 0.1143 | 56.0 |

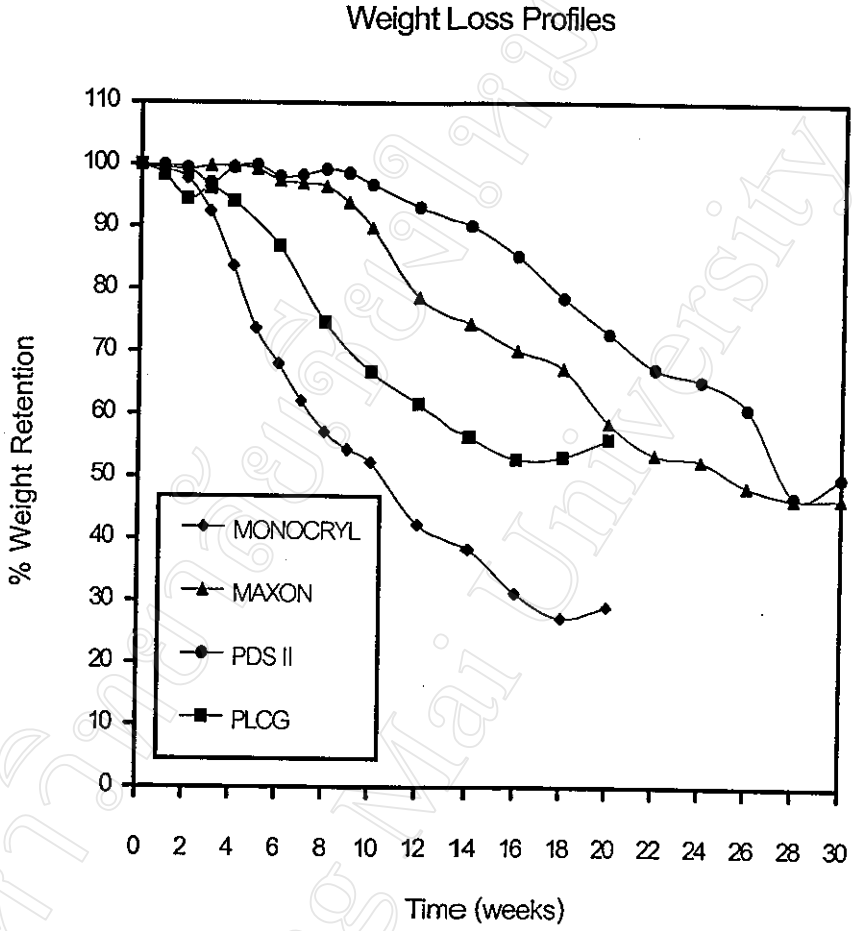



Fig. 5.2 Comparison of the weight loss profiles of the MONOCRYL, MAXON, PDS II and PLCG monofilament fibers during the period of the *in vitro* biodegradation experiments.

### 5.2.2 Tensile Strength Profiles

Complementary to the weight loss data is the tensile strength data. Dried fiber samples were tested by using the Universal Mechanical Testing Machine. Each fiber sample was tested at least 2 times and the tensile strength averaged to give the values in Table 5.5. The stress-extension curves during the *in vitro* biodegradation studies for the MONOCRYL, MAXON, PDS II and PLCG samples were shown in Fig. 5.3-5.6 respectively. The % tensile strength retention were calculated as follows:

$$\%\text{Tensile Strength Retention} = \frac{S_f}{S_o} \times 100$$

where;

$S_o$ = initial tensile strength of sample

$S_f$ = final tensile strength of sample

The tensile strength retention–time profiles for MONOCRYL, MAXON, PDS II and PLCG samples were shown in Fig. 5.7.

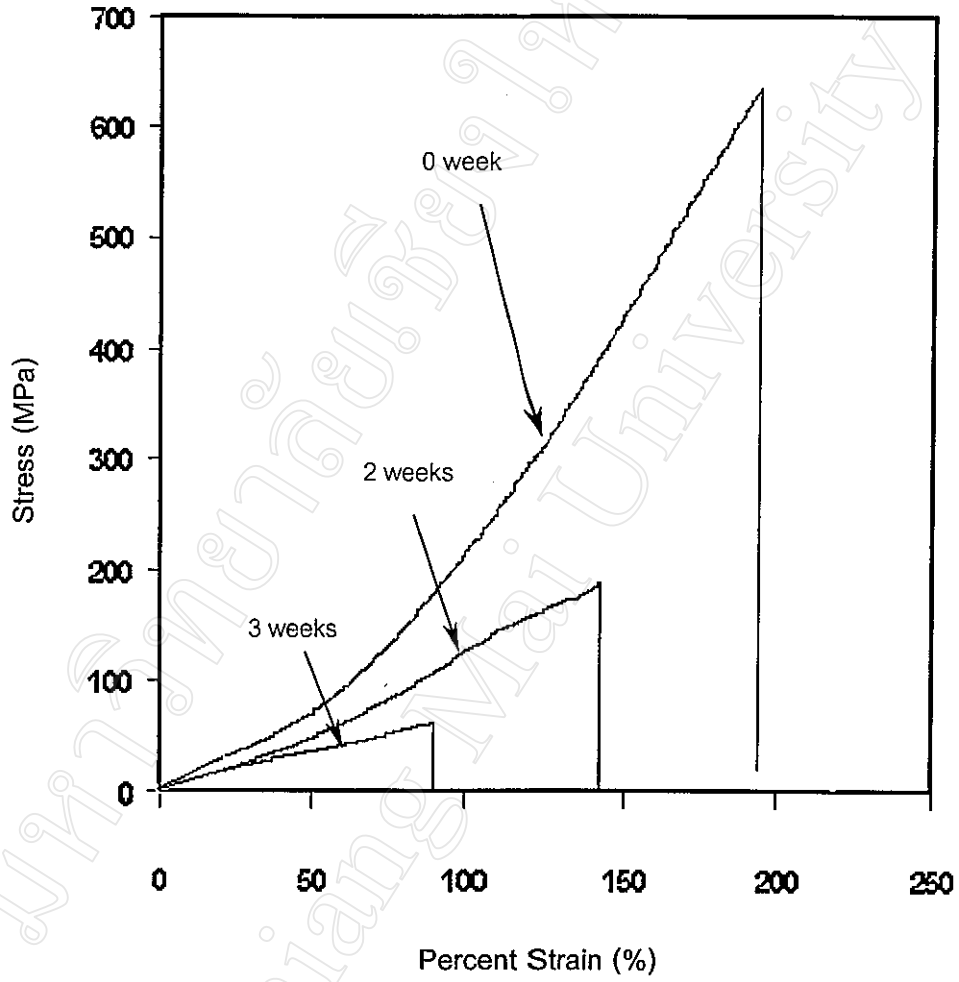



Fig. 5.3 Comparison of stress-strain curves of MONOCRYL suture during the period of the *in vitro* biodegradation experiments.

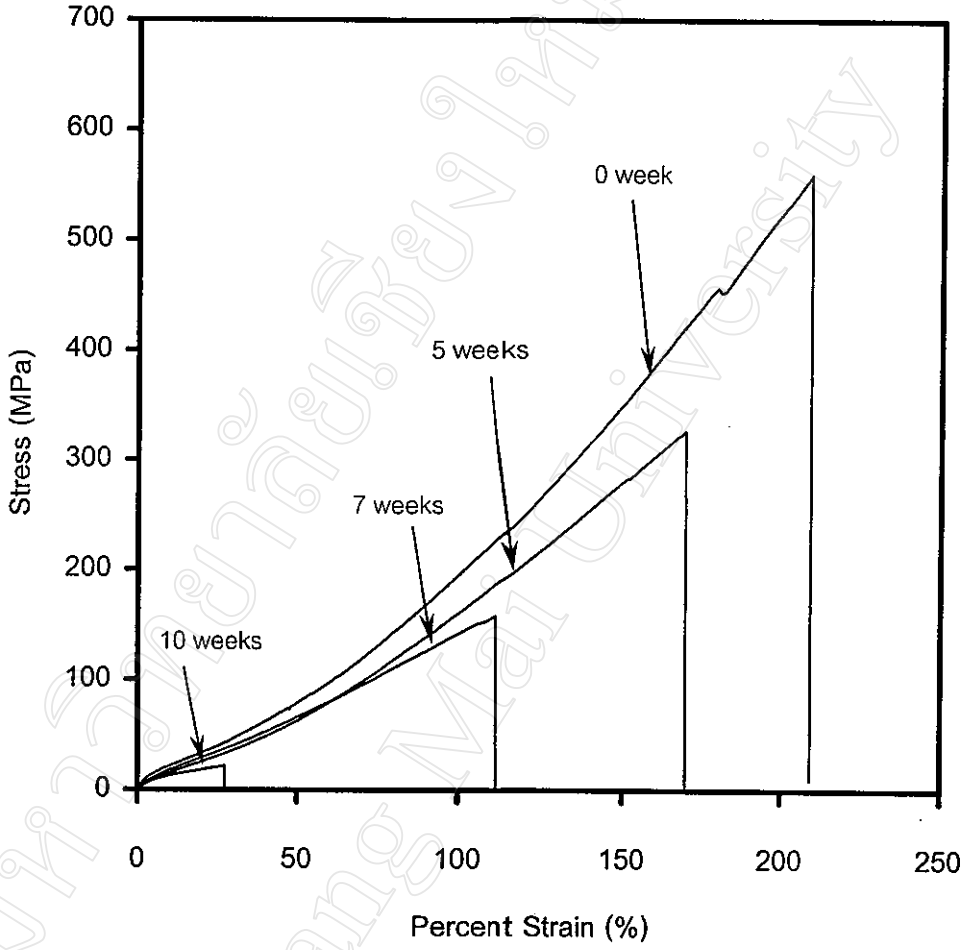



Fig. 5.4 Comparison of stress-strain curves of MAXON suture during the period of the *in vitro* biodegradation experiments.

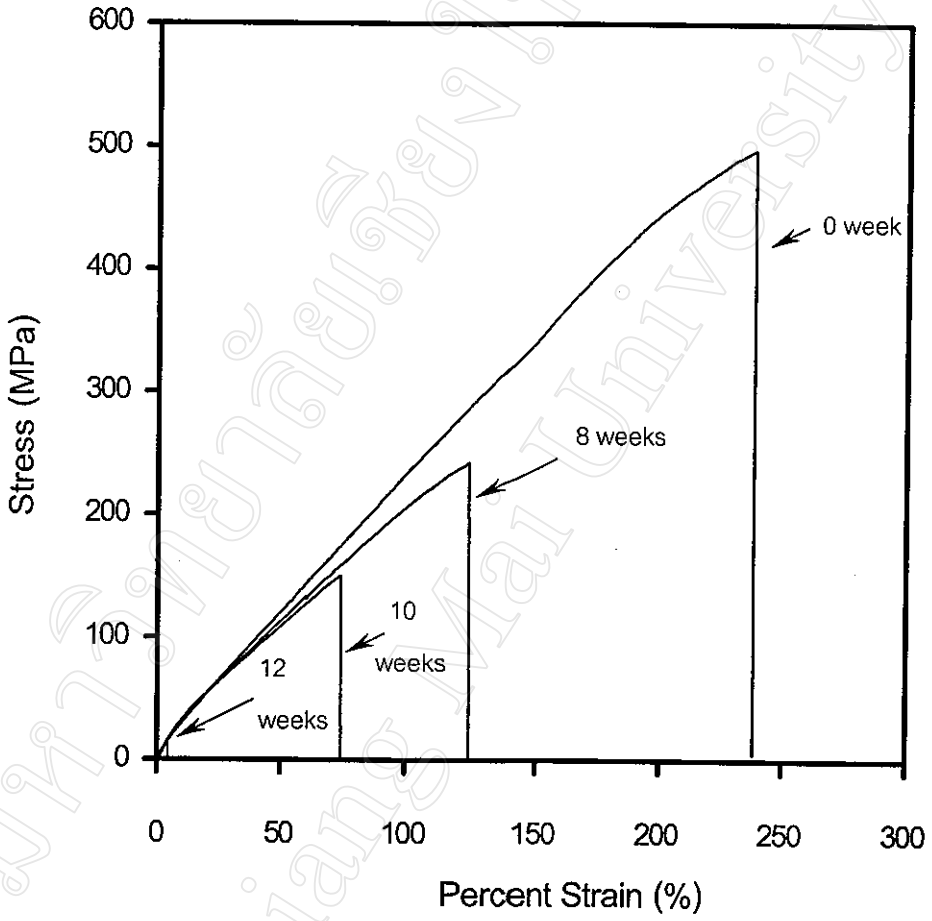



Fig. 5.5 Comparison of stress-strain curves of PDS II suture during the period of the *in vitro* biodegradation experiments.

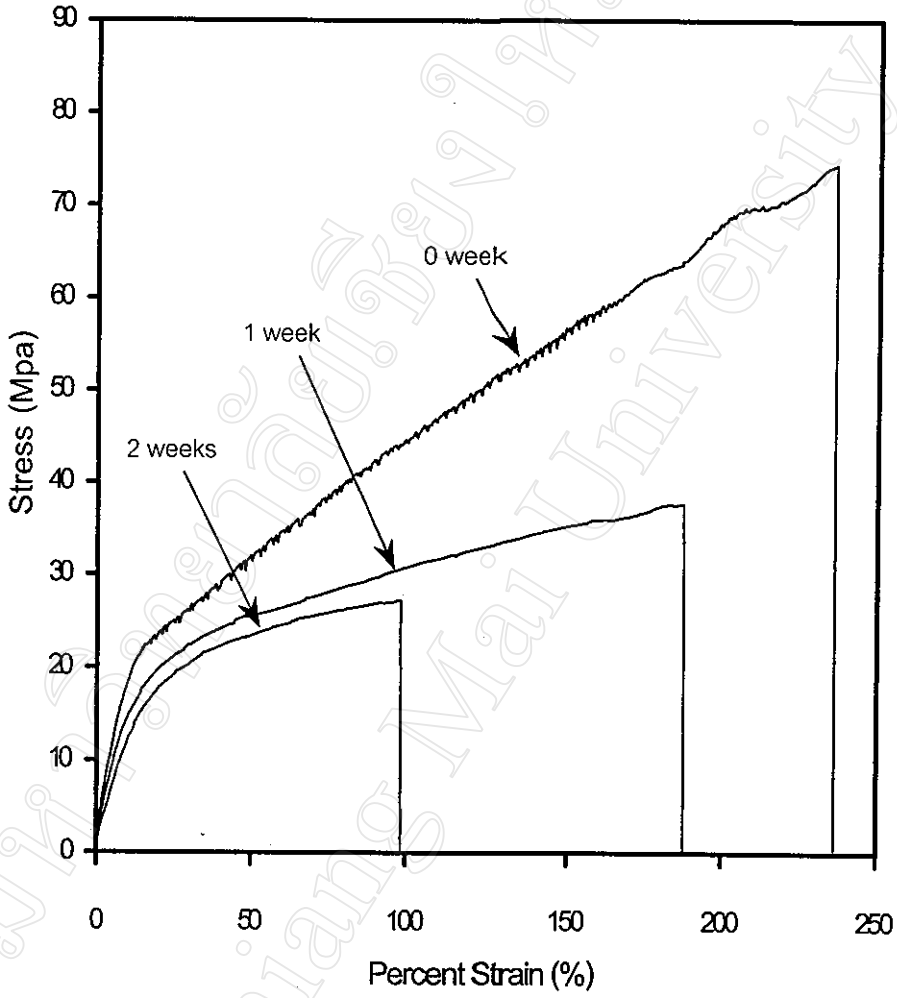



Fig. 5.6 Comparison of stress-strain curves of PLCG fiber during the period of the *in vitro* biodegradation experiments.

Table 5.5 Tensile strengths and % tensile strength retentions of MONOCRYL, MAXON, PDS II and PLCG monofilament fibers immersed in a pH 7.40 phosphate buffer saline medium at 37.0 ± 1.0°C.

| Time (Weeks) | Mean Tensile Strength or Mean Stress at Break (MPa) | | | | Tensile Strength Retention (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | MONOCRYL | MAXON | PDS II | PLCG | MONOCRYL | MAXON | PDS II | PLCG |
| 0 | 663.5 | 500.8 | 491.1 | 78.6 | 100.0 | 100.0 | 100.0 | 100.0 |
| 1 | 423.2 | 511.7 | 462.8 | 40.4 | 63.8 | 102.2 | 94.2 | 51.4 |
| 2 | 188.0 | 485.7 | 446.3 | 25.4 | 28.3 | 97.0 | 90.9 | 32.3 |
| 3 | 62.1 | 462.5 | 429.7 | | 9.4 | 92.4 | 87.5 | |
| 4 | | 390.6 | 407.8 | | | 78.0 | 83.0 | |
| 5 | | 323.0 | 373.1 | | | 64.5 | 76.0 | |
| 6 | | 230.9 | 376.6 | | | 46.1 | 76.7 | |
| 7 | | 167.4 | 337.3 | | | 33.4 | 68.7 | |
| 8 | | 98.8 | 247.6 | | | 19.7 | 50.4 | |
| 9 | | 47.7 | 183.1 | | | 9.5 | 37.3 | |
| 10 | | 22.3 | 159.1 | | | 4.4 | 32.4 | |
| 12 | | | 16.9 | | | | 3.4 | |
| 13-30 | | | | | | | | |

Note : After certain time intervals, the fibers studied disintegrated and broke into small pieces, whereupon they could not be tested.

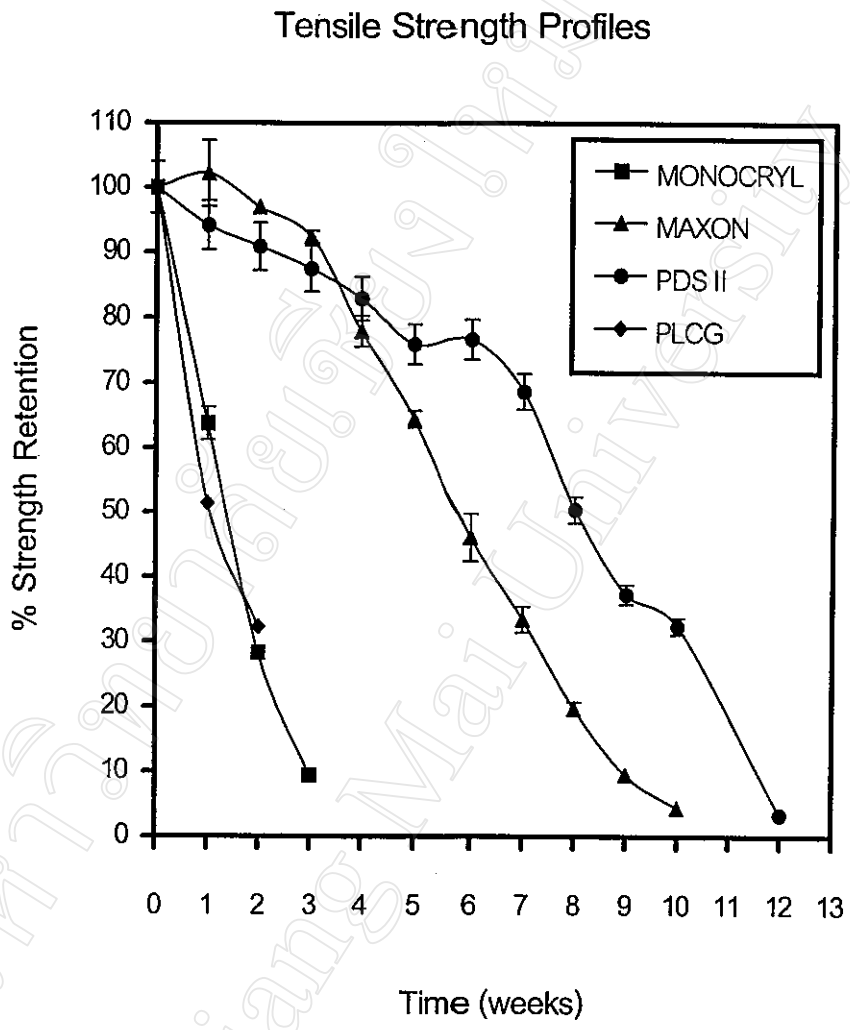



Fig. 5.7 Tensile strength retention-time profiles for MONOCRYL, MAXON, PDS II sutures and the PLCG monofilament fibers immersed in a phosphate buffer saline medium of pH 7.40 at 37.0 ± 1.0°C.

It was shown that amongst the commercial sutures, PDS II showed the slowest rate of decrease in tensile strength while MONOCRYL showed the fastest. PDS II and MAXON still remained 50 % of their original tensile strengths after 8 and 6 weeks respectively, while MONOCRYL lost of its strength after only 4 weeks. The tensile strength reduction rate of the PLCG fiber was comparable with MONOCRYL suture. Similarly, the PLCG and MONOCRYL showed faster weight loss than MAXON and PDS II (as shown in Fig. 5.2).

### 5.2.3 Melting Point and Heat of Fusion Profiles

For each DSC sample analysis, approximately 3–5 mg of sample were accurately weighed. The DSC thermograms during the *in vitro* biodegradation studies for MONOCRYL, MAXON, PDS II and PLCG samples were shown in Fig. 5.8-5.11 respectively.

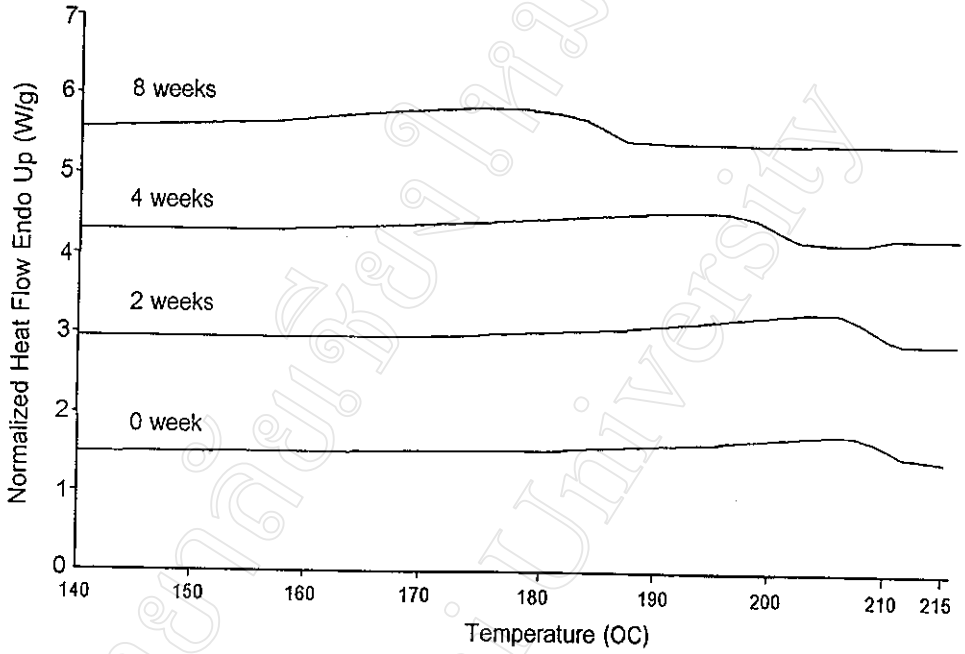



Fig. 5.8 Comparison of DSC thermograms of MONOCRYL sutures during the period of the *in vitro* biodegradation experiments.

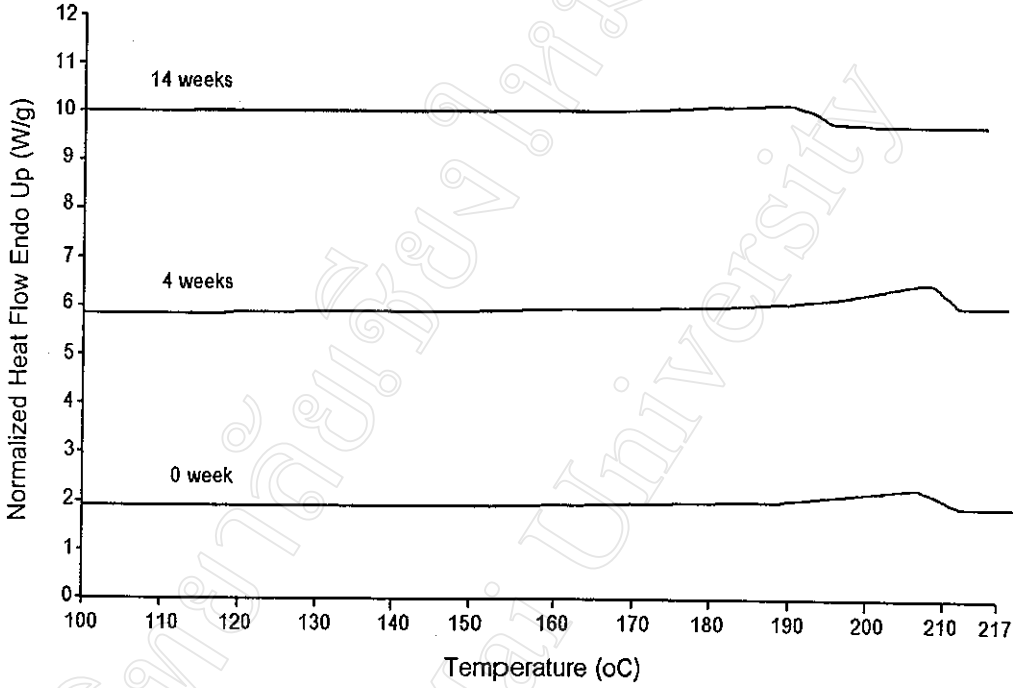



Fig. 5.9 Comparison of DSC thermograms of MAXON sutures during the period of the *in vitro* biodegradation experiments.

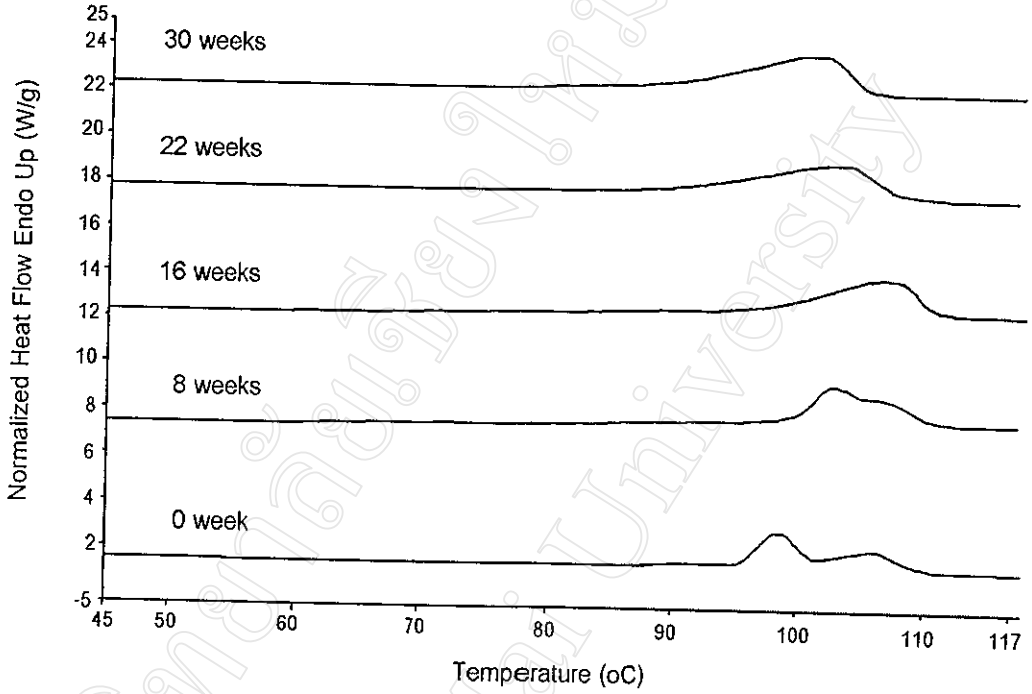



Fig. 5.10 Comparison of DSC thermograms of PDS II sutures during the period of the *in vitro* biodegradation experiments.

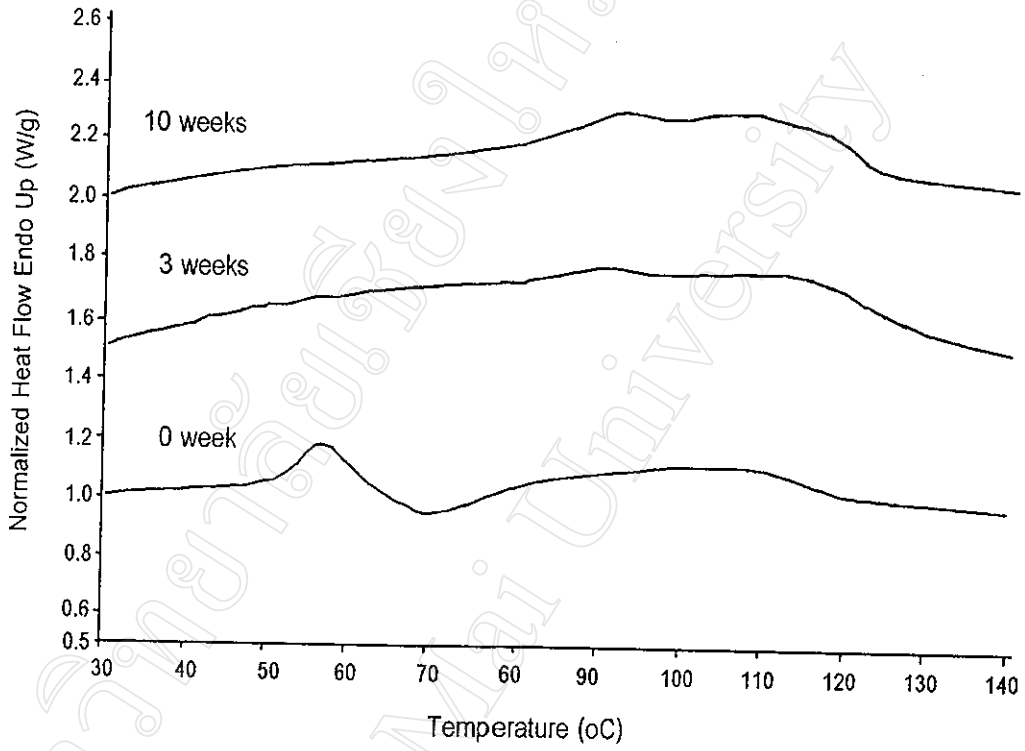



Fig. 5.11 Comparison of DSC thermograms of PLCG sutures during the period of the *in vitro* biodegradation experiments.

The DSC melting point and heat of fusion data are shown in Table 5.6 for each of the 4 samples. The results are also plotted graphically in Fig. 5.12 and 5.13.

Table 5.6 DSC melting peak data for the MONOCRYL, MAXON, PDS II and PLCG monofilament fibers immersed in pH 7.40 phosphate buffer saline medium at 37.0 ± 1.0°C.

| Time | MONOCRYL | | MAXON | | PDS II | | PLCG | |
|---|---|---|---|---|---|---|---|---|
| (weeks) | Melting Point (°C)* | Heat of Fusion (J/g) | Melting Poing (°C)* | Heat of Fusion (J/g) | Melting Point (°C)* | Heat of Fusion (J/g) | Melting Point (°C)* | Heat of Fusion (J/g) |
| 0 | 205.67 | 41.85 | 205.33 | 44.70 | 98.67 | 92.34 | 102.67 | 27.11** |
| 1 | 207.33 | 41.14 | 206.00 | 42.50 | 99.33 | 69.85 | 110.00 | 33.11 |
| 2 | 204.67 | 47.05 | 205.67 | 42.94 | 101.00 | 70.80 | 111.00 | 33.78 |
| 3 | 192.33 | 56.82 | 207.33 | 43.28 | 102.00 | 74.02 | - | - |
| 4 | 194.00 | 53.43 | 207.00 | 44.51 | 101.00 | 71.15 | 113.00 | 27.54 |
| 5 | 184.00 | 72.40 | 208.00 | 52.84 | 103.00 | 74.41 | - | - |
| 6 | 187.67 | 83.75 | 205.00 | 51.41 | 102.00 | 72.48 | 112.33 | 26.01 |
| 7 | 179.33 | 72.30 | 206.33 | 59.30 | 102.33 | 76.48 | - | - |
| 8 | 175.67 | 84.33 | 203.67 | 66.91 | 102.67 | 75.59 | 109.33 | 39.31 |
| 9 | 177.67 | 93.52 | 204.00 | 62.23 | 103.67 | 80.03 | - | - |
| 10 | | | 199.67 | 61.77 | 103.67 | 86.09 | 108.00 | 37.29 |
| 12 | | | 191.00 | 82.84 | 103.00 | 95.11 | 108.33 | 48.78 |
| 14 | | | 188.33 | 93.11 | 103.33 | 100.58 | 109.33 | 50.61 |
| 16 | | | 160.33 | 1.23 | 107.00 | 95.71 | 105.33 | 34.44 |
| 18 | | | 154.67 | 0.10 | 105.67 | 95.45 | 100.33 | 41.81 |
| 20 | | | 141.67 | 0.67 | 105.33 | 100.74 | 101.00 | 41.86 |
| 22 | | | | | 103.00 | 107.93 | | |
| 24 | | | | | 103.67 | 105.62 | | |
| 26 | | | | | 101.00 | 104.84 | | |
| 28 | | | | | 100.33 | 109.45 | | |
| 30 | | | | | 101.00 | 109.28 | | |

* taken at the melting peak maximium temperature; scanning rate = 10°C/min

** due to the processing operation, the $\Delta H_f$ of PLCG fiber increased

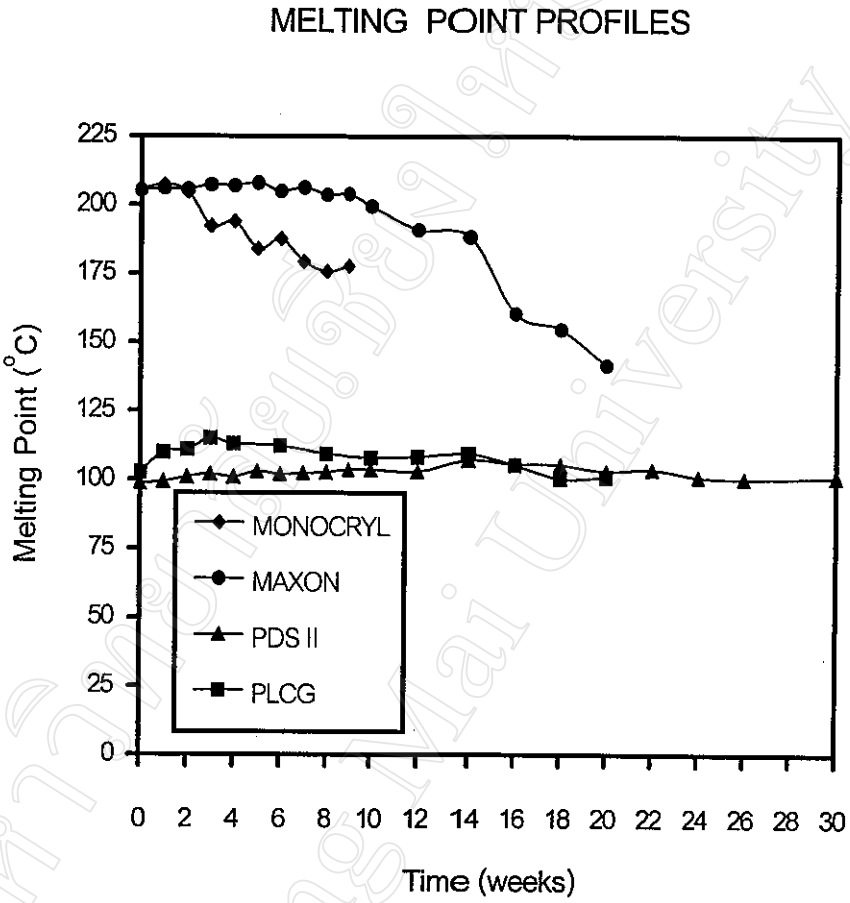



Fig. 5.12 Comparison of the melting point profiles of the MONOCRYL, MAXON, PDS II and PLCG monofilament fibers over the period of the *in vitro* biodegradation experiments.

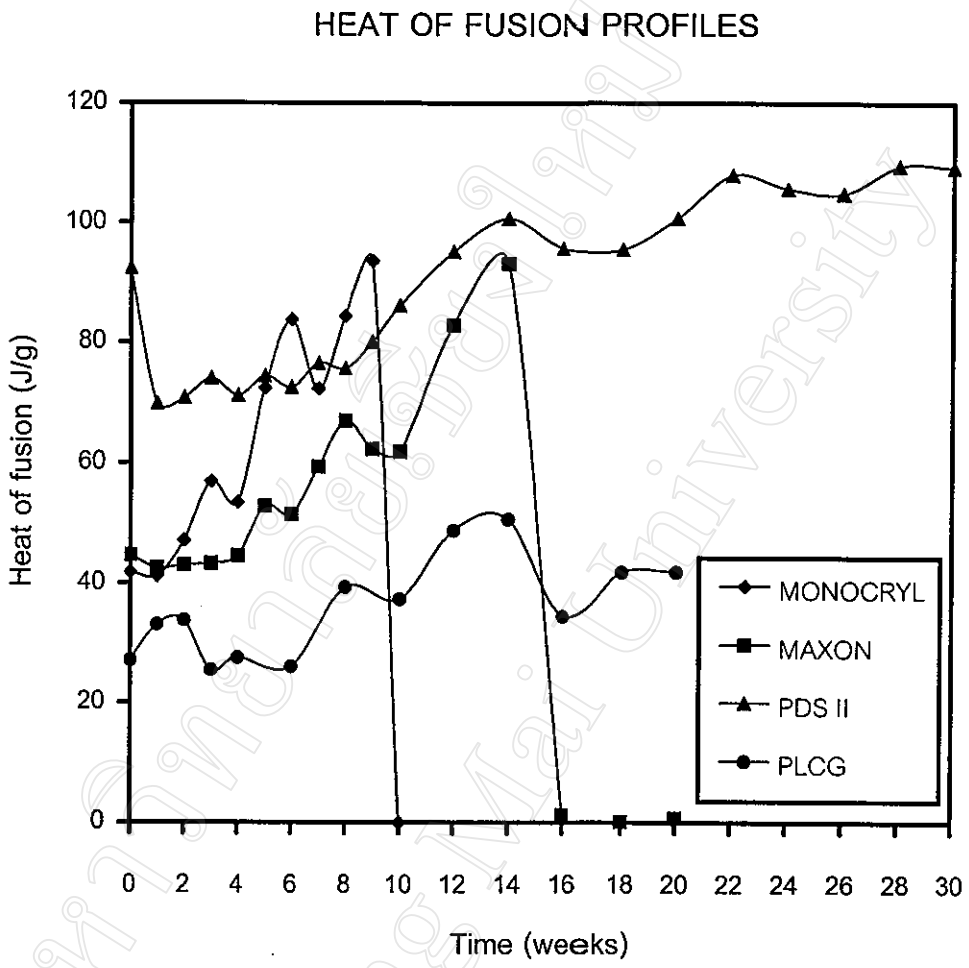



Fig. 5.13 Comparison of the heat of fusion profiles of the MONOCRYL, MAXON, PDS II and PLCG monofilament fibers over the period of the *in vitro* biodegradation experiments.

### 5.2.4 pH Stability of Phosphate Immersion Medium

The pH of the phosphate buffer saline immersion medium was monitored throughout the period of the experiment. It was found that the initially adjusted pH of 7.40 decreased, as shown in Table 5.7 and Fig. 5.14. No attempt was made to readjust the pH to 7.40 since it was of interest to note how the pH changed.

Table 5.7 Variation in pH of the phosphate buffer saline solutions containing the MONOCRYL, MAXON, PDS II and PLCG samples during the period of the biodegradation experiment.

| Time (Weeks) | pH of Phosphate Buffer Saline | | | |
|---|---|---|---|---|
| | MONOCRYL | MAXON | PDS II | PLCG |
| 0 | 7.40 | 7.40 | 7.40 | 7.40 |
| 1 | 7.15 | 7.40 | 7.40 | 6.75 |
| 2 | 6.83 | 7.30 | 7.30 | 7.20 |
| 3 | 6.00 | 7.29 | 7.30 | 6.73 |
| 4 | 5.60 | 7.38 | 7.36 | 7.10 |
| 5 | 4.73 | 7.33 | 7.30 | - |
| 6 | 4.70 | 7.25 | 7.40 | 6.65 |
| 7 | 3.35 | 7.18 | 7.40 | - |
| 8 | 3.23 | 6.90 | 7.40 | 5.50 |
| 9 | 3.29 | 6.29 | 7.19 | - |
| 10 | 3.11 | 6.02 | 7.20 | 4.95 |
| 12 | 3.05 | 5.40 | 6.63 | 4.48 |
| 14 | 3.05 | 3.79 | 6.72 | 3.93 |
| 16 | 3.15 | 3.45 | 6.30 | 3.90 |
| 18 | 3.09 | 3.40 | 5.70 | 3.95 |
| 20 | 3.00 | 3.48 | 5.87 | 4.05 |
| 22 | | 3.18 | 4.58 | |
| 24 | | 3.10 | 4.48 | |
| 26 | | 3.35 | 4.23 | |
| 28 | | 3.18 | 3.35 | |
| 30 | | 3.22 | 3.25 | |

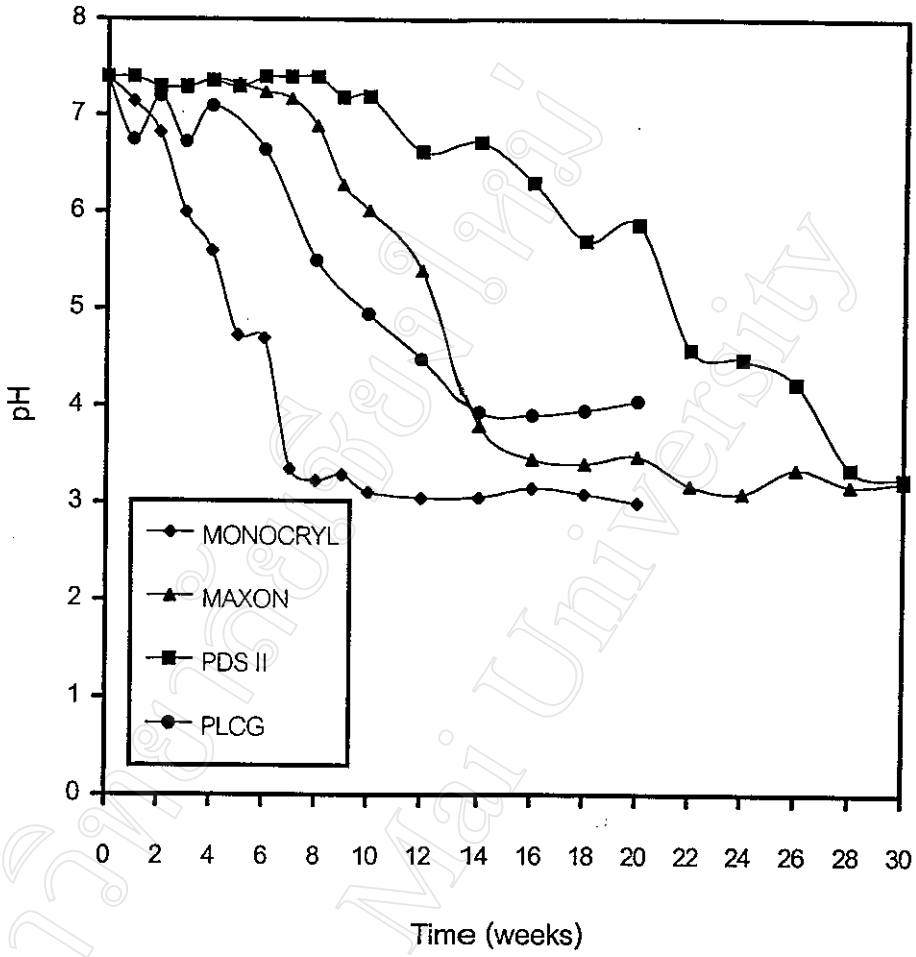



Fig. 5.14 Variation in pH of the phosphate buffer saline solution containing the MONOCRYL, MAXON, PDS II and PLCG samples during the period of the biodegradation experiments.

To verify that hydrolytic degradation was occurring, the pH of the buffer solutions were measured; pH values after each week of degradation for all samples are shown in Fig. 5.14. As it can be observed, pH decreases as the *in vitro* biodegradation time, indicating that the hydrolytic reaction taking place produces low molecular weight acid degradation products.

### 5.2.5 Intrinsic Viscosity

Approximately 0.27 % w/v PLCG solutions were accurately prepared using chloroform as solvent. Their flow-times were then determined at 30.0 ± 0.1°C using a Schott-Gerate micro-Ubbelohde viscometer (type No. 537 10, capillary size I). By using the One-Point Approximation Method, the intrinsic viscosities, $[\eta]$, were calculated from the Solomon-Ciuta Equation. Their values are given in Table 5.8 and the intrinsic viscosity retention calculated as follows:

$$\% \text{ Intrinsic Viscosity Retention} = \frac{[\eta]_f}{[\eta]_o} \times 100\%$$

where

$[\eta]_o$ = initial intrinsic viscosity of PLCG sample

$[\eta]_f$ = final intrinsic viscosity of PLCG sample

The intrinsic viscosity loss profiles were shown in Fig. 5.15.

**Table 5.8** Intrinsic viscosities, $[\eta]$, and their % retentions for the PLCG fibers immersed in the pH 7.40 phosphate buffer saline solution at 37.0 ± 0.1°C.

| Time (weeks) | $[\eta] \pm 0.01$ (dl/g) | % Retention of $[\eta]$ (± 0.1) |
|---|---|---|
| 0 | 0.76 | 100.0 |
| 1 | 0.44 | 57.9 |
| 2 | 0.06 | 7.4 |

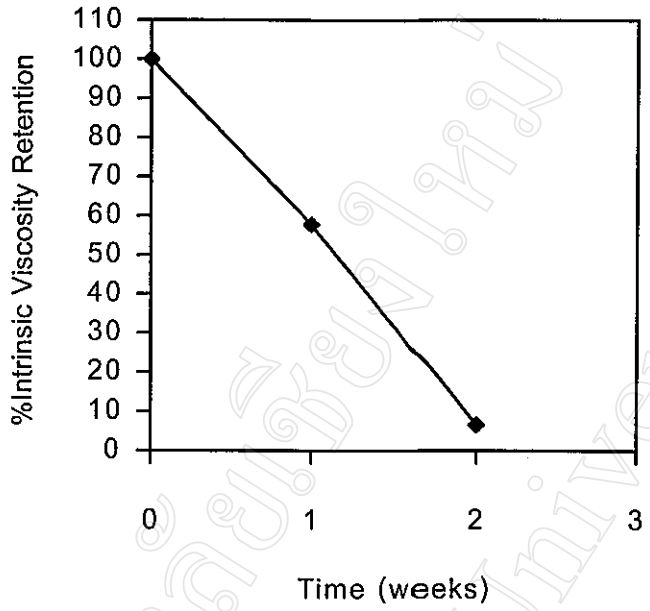
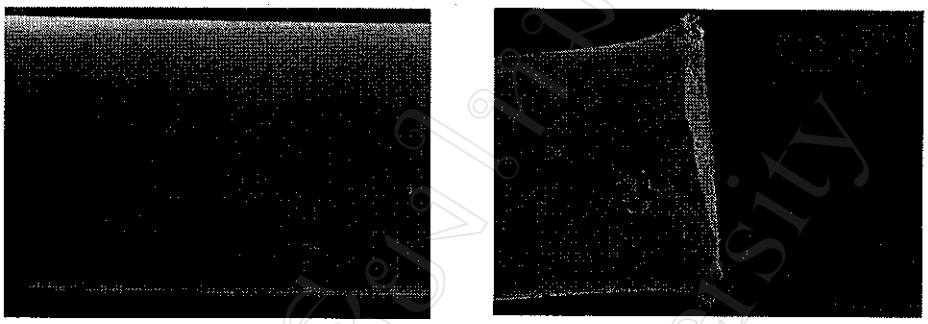



Fig. 5.15 The intrinsic viscosity loss profiles of PLCG fiber over the period of the biodegradation experiments.

### 5.2.6 Scanning Electron Microscopy

Surface analysis has assumed great importance in recent years in the field of material science. One of the most powerful techniques is scanning electron microscopy (SEM). SEM is of limited used in studying surface morphology but it provides useful information on surface topology with a resolution of about 100 Å

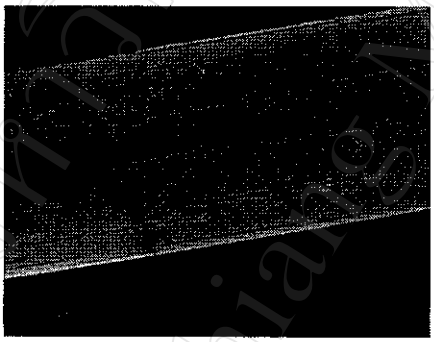
In this research project, SEM was used to follow the surface topology changes in the MONOCRYL, MAXON, PDS II and PLCG samples during the *in vitro* studies. The SEM micrographs obtained are illustrated in Fig. 5.16 – 5.19.



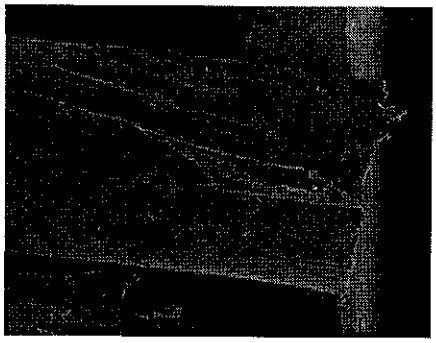
(a) 0 week (x 200)



(b) 0 week (x 180)

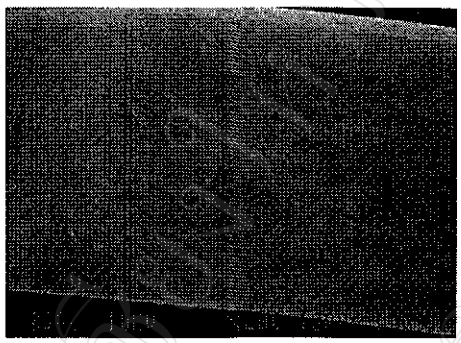


(c) 3 weeks (x 140)

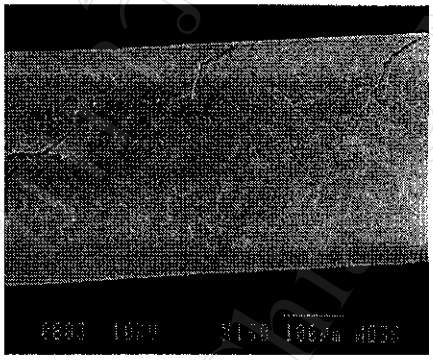


(d) 3 weeks (x 140)

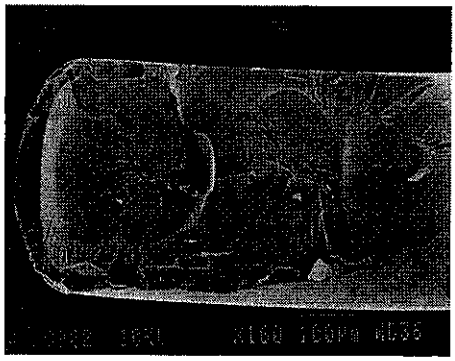
**Fig. 5.16** SEM micrographs of the MONOCRYL sutures after immersion in the pH 7.40 phosphate buffer at 37°C for various times from 0–3 weeks. (Magnification = x140-200)



(a) 0 week (x 200)

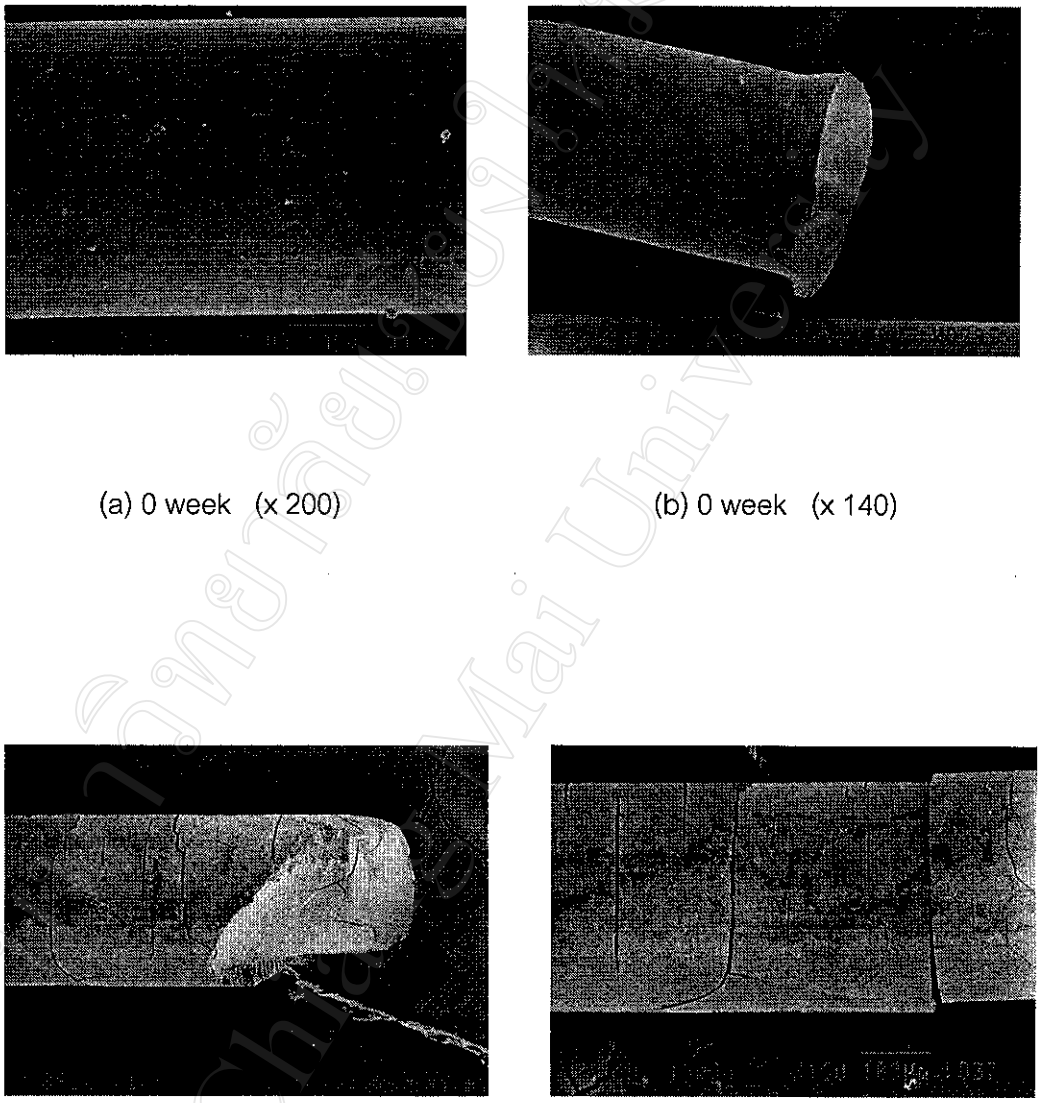


(b) 16 weeks (x 160)



(c) 16 weeks (x 160)

**Fig. 5.17** SEM micrographs of the MAXON sutures after immersion in the pH 7.40 phosphate buffer at 37°C for various times from 0–16 weeks. (Magnification = x160-200)



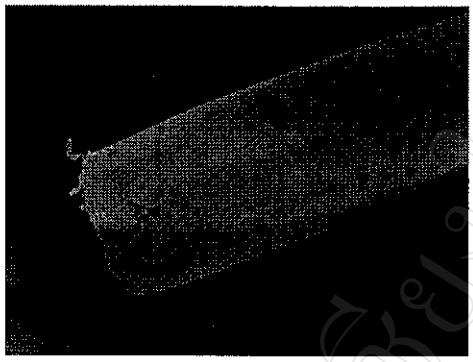
(a) 0 week (x 200)

(b) 0 week (x 140)

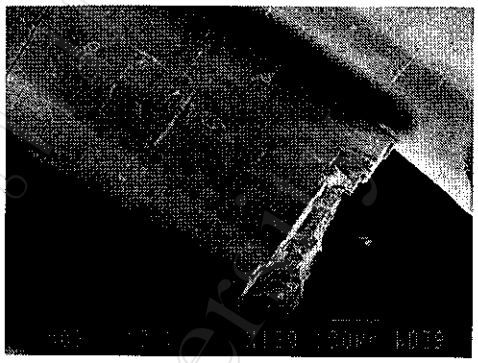
(c) 18 weeks (x 120)

(d) 18 weeks (x160)

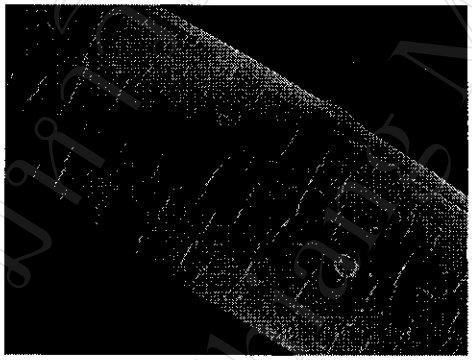
**Fig. 5.18** SEM micrographs of the PDS II sutures after immersion in the pH 7.40 phosphate buffer at 37°C for various times from 0–18 weeks. (Magnification = x120-200)



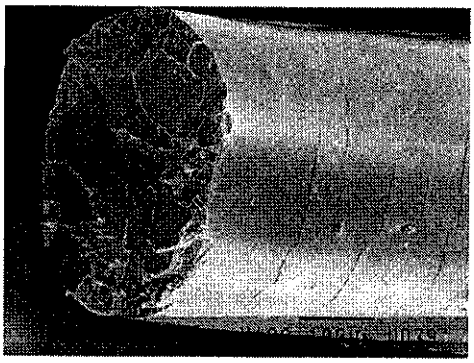
(a) 0 week (x 130)



(b) 4 weeks (x 120)



(c) 6 weeks (x 120)



(d) 6 weeks (x 180)

**Fig. 5.19** SEM micrographs of the PLCG sutures after immersion in the pH 7.40 phosphate buffer at 37°C for various times from 0–6 weeks. (Magnification = x120-180)

The micrographs in Fig. 5.16 – 5.19 showed that before 4 weeks no surface changes had occurred in the MAXON and PDS II samples while MONOCRYL and PLCG both exhibited surface cracks. For MONOCRYL, the cracks were longitudinal and started after 3 weeks, while for PLCG, MAXON and PDS II the cracks were transverse to the fiber axis and started after 4, 16 and 18 weeks respectively.