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# Effect of Ganciclovir on the Hydrolytic Degradation of Poly(lactide-co-glycolide) Microspheres

X. CHEN AND C. P. OOI\*

*Division of Bioengineering  
School of Chemical and Biomedical Engineering  
Nanyang Technological University  
639798 Singapore*

T. H. LIM

*Department of Ophthalmology  
Tan Tock Seng Hospital  
308433 Singapore*

**ABSTRACT:** Ganciclovir (GCV)-loaded poly(lactide-co-glycolide) (PLGA) microspheres,  $125 \pm 11 \mu\text{m}$  in diameter, are produced using the emulsification/solvent evaporation technique. The release rate of the drug is studied for 20 weeks in a phosphate-buffered solution of pH 7 at  $37^\circ\text{C}$ . The release of the drug shows a triphasic release pattern, i.e., an initial burst, a diffusive phase, and a second burst. The initial burst occurs within the first 2 days of immersion. After the burst, the release is by diffusion for up to 13 weeks, followed by another burst release, which signals the onset of bulk degradation of the PLGA polymer. The presence of GCV molecules decreases the hydrolytic rate of PLGA degradation. Gel permeation chromatography (GPC), differential scanning calorimetry (DSC), field emission scanning electron microscopy (FESEM), and ultraviolet (UV) spectroscopy are used to assess the hydrolytic degradation and drug release rate of the microspheres.

**KEY WORDS:** poly(lactide-co-glycolide), ganciclovir, microspheres, triphasic release, hydrolytic degradation.

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\*Author to whom correspondence should be addressed. E-mail: [ascpooi@ntu.edu.sg](mailto:ascpooi@ntu.edu.sg)

## INTRODUCTION

**Cytomegalovirus (CMV) retinitis** is the most common opportunistic ocular infection and the major cause of visual loss [1–3] in patients with acquired immune deficiency syndrome (AIDS). Ganciclovir (9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine) (GCV) is the most widely studied antiviral drug for the treatment of CMV infections. This drug prevents replication of the viral DNA but does not eliminate the virus from the tissue, hence long-term therapy is necessary to control the disease [4]. The available mode of treatments ranges from intravenous to implants for localized drug delivery. When administered by the intravenous route, GCV has a bone marrow-suppressive effect [5]. Local intravitreal therapy has been proposed as a safe and well-tolerated alternative route for the treatment of retinal infections. However, frequent intravitreal injections are required to maintain therapeutic levels of GCV due to the short half-life of the drug. These continuous administrations bring about the risk of retinal detachments, hemorrhages, or endophthalmitis [6]. Hence, a sustained release by a biodegradable device would decrease these risks and produce a longer therapeutic effect.

The family of aliphatic polyesters, such as poly(lactide), poly(glycolide), and their copolymers, poly(lactide-co-glycolide) (PLGA), has been by far the dominating choice for materials in degradable drug delivery systems [7]. Recently, microspheres of PLGA loaded with GCV were developed using the oil-in-oil emulsion technique [8,9]. However, the fluorosilicone (FSiO) and silicone (SiO) oils used in the preparation of the microspheres are nonbiodegradable and could cause intolerance problems especially in patients whose immune systems are already weakened by AIDS.

The purpose of this study was to obtain biodegradable PLGA microspheres loaded with GCV without FSiO and SiO. The technique used was the oil-in-water (O/W) single emulsion method. The microspheres were evaluated to determine their drug release profile. The effect of degradation on the morphology of the PLGA microspheres was also examined. Blank biodegradable PLGA microspheres (without GCV) were also prepared to act as control samples to the drug-loaded PLGA microspheres. In this study, the relationship between polymer matrix degradation and the drug release profile is drawn.

## EXPERIMENTAL

### Materials

Ganciclovir was obtained from Roche Products Limited (Welwyn Garden City, United Kingdom) in the form of Cymevene<sup>®</sup>, as capsules of white powder consisting of about 90% GCV and 10% povidone/K90, croscarmellose sodium, magnesium stearate, and purified water. The chemical name of GCV is 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]-methyl]guanine, with a molecular weight of 255.23. The  $pK_a$  (dissociation constants) for GCV are 2.2 and 9.4. Poly(DL-lactide-co-glycolide) 75:25 (PLGA) was obtained from Purac Far East and has an inherent viscosity of 0.94 dL/g in hexafluoroisopropanol. The molecular weight calculated by gel permeation chromatography (GPC) was 130,000 Da. Methylene chloride was bought from Merck Chemicals Ltd. (KGaA, Darmstadt, Germany) and poly(vinyl alcohol) (PVA), (87–89% hydrolyzed, weight-average molecular weight ( $M_w$ ) 13,000–23,000) was purchased from Aldrich Chemical Company Inc. (Milwaukee, USA). The degradation medium used was a phosphate-buffered solution of pH 7.00 at 20°C, obtained from Merck Chemicals Ltd. (KGaA, Darmstadt, Germany).

### Methods

#### *Preparation of Microspheres*

The GCV-loaded PLGA microspheres were prepared using the oil-in-water emulsion technique [10]. One hundred milligrams of GCV was added to a solution of 1000 mg PLGA in 10 mL methylene chloride and stirred continuously for 24 h with a magnetic stirrer. The polymer–GCV suspension was then added to a 100-mL solution of 1% PVA. An oil-in-water (O/W) emulsion was formed with the help of a homogenizer at a speed of 1000 rpm for 30 min. The organic solvent was allowed to evaporate under an aspirating hood at room temperature for 4 h, leaving behind solid microspheres. The microspheres were collected and washed three times with distilled water to remove any residual PVA. The microspheres were then dried under vacuum in a vacuum desiccator at room temperature for 24 h before they were used for drug-release assays and degradation tests. The blank microspheres were prepared using the same technique mentioned earlier, except that GCV was not added to the suspension.

### *Particle Size Measurement*

The microsphere size and distribution were measured using a particle analyzer (Fritsch Analysette 22 Compact, Germany). The measuring range was from 0.31 to 300.74  $\mu\text{m}$ , with the measurement duration of 3 scans. At first the microspheres were homogeneously dispersed in distilled water. They were then added dropwise into the analyzer. Particle size measurements were obtained when the microsphere concentration achieved 7% weight/volume.

### *Encapsulation Efficiency Study*

To find the true amount of GCV that was loaded into the microspheres during the fabrication process, 10 mg of the GCV-loaded microspheres were dissolved in 2 mL of methylene chloride. Twenty-five milliliters of deionized water was then added to the mixture and the contents were stirred overnight to allow the GCV to diffuse into the inorganic layer (water). The water phase was then retrieved from the bottle and tested for its GCV contents via a UV spectrophotometer.

### *Ganciclovir Release Studies*

Fifty milligrams of the GCV-loaded microspheres were immersed in 10 mL phosphate-buffered solution and kept at 37°C without agitation for various time periods. The buffered solution used was a phosphate-buffered saline solution of pH 7.00. At the end of each immersion period, 10 mL of the buffered solution was removed and measured spectrophotometrically at a wavelength of 252.4 nm on a SHIMADZU UV-2501 (PC) spectrophotometer, Australia. The measurements were repeated five times and the average and the standard deviation were calculated. The amount of drug was determined using a standard curve, developed in distilled water ( $m = 0.0515$ ,  $R^2 = 0.997$ ). An identical volume of fresh phosphate-buffered saline solution was added back into the container after each sample removal.

### *Degradation Studies*

Fifty milligrams of GCV-loaded microspheres were immersed in 10 mL phosphate-buffered solution and kept at 37°C without agitation for various time periods. At the end of each immersion period, the microspheres were removed and dried for 24 h in a vacuum desiccator at room temperature. The following methods were employed to assess the changes in the sample morphology with degradation: thermal analysis differential scanning calorimetry (DSC), field emission scanning electron microscopy (FESEM), and gel permeation chromatography (GPC). Control samples made of blank microspheres were also prepared

and tested for morphological changes at the end of each immersion period.

#### *Field Emission Scanning Electron Microscopy*

The surface morphology of the blank and GCV-loaded microspheres was observed using a JOEL JSM-6340F FESEM at 5.0 kV with an 8 mm working distance. The samples were first mounted onto metal stubs with the help of a double-sided tape, and coated with gold using a pulse plasma system (18 mA for 70 s) to prevent sample charging.

#### *Gel Permeation Chromatography*

The molecular weights of the degraded blank and GCV-loaded microspheres were determined by GPC (Agilent 1100 Series, Model No. G1311A). An Agilent's PLgel 5  $\mu\text{m}$  MIXED-C column with an Agilent's RI-254 refractive index detector was used. The mobile phase was a solvent mixture of 80:20 tetrahydrofuran (THF) and methylene chloride at a flow rate of 1 mL min<sup>-1</sup> at 35°C. The microspheres were first dissolved in the solvent mixture, filtered, and stored in 2-mL vials. The solution was then autoinjected into the column. The average molecular weights were calculated, using a series of polystyrene standards, ranging from 640 to 4,843,000.

#### *Differential Scanning Calorimetry*

DSC studies were performed using a modulated DSC (MDSC 2920, TA Instruments, USA). Approximately 5 mg of microspheres were weighed and sealed in hermetic aluminum pans. The samples were then heated from -20 to 250°C at a scan rate of 5°C/min in nitrogen atmosphere and their glass transition temperature ( $T_g$ ) and melting characteristics (melting temperature and heat of fusion) were recorded.

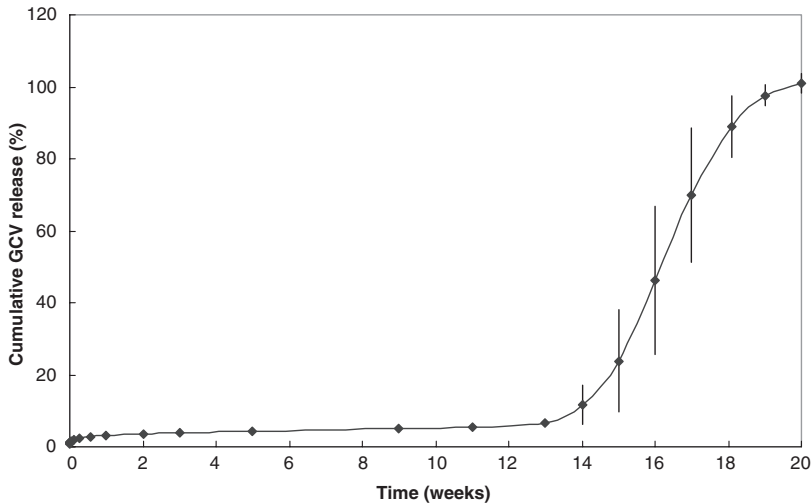
#### *pH Measurements*

The pH of the phosphate-buffered solution was measured using pH indicator strips obtained from Merck (KGaA, Germany) at various time periods to follow the acidity of the degrading medium with time.

## **RESULTS AND DISCUSSION**

### **Ganciclovir Release Studies**

The microspheres were of  $125 \pm 11 \mu\text{m}$  size containing 3763  $\mu\text{g}$  of GCV/50 mg of microspheres, which gave a theoretical encapsulation efficiency of 75.2%. This was comparable to the 84.79% encapsulation



**Figure 1.** Cumulative fractional release of GCV from microspheres of PLGA 75 : 25, I.V. 0.94 in phosphate-buffered solution (pH 7.0) at 37°C. The values are shown as mean  $\pm$  SD of  $n = 5$ .

efficiency obtained by Herrero-Vanrell et al., using the oil-in-oil emulsion technique [8,9]. GCV has a low solubility of 0.26 g/100 mL of water at 25°C, which may account for the high encapsulation efficiency obtained.

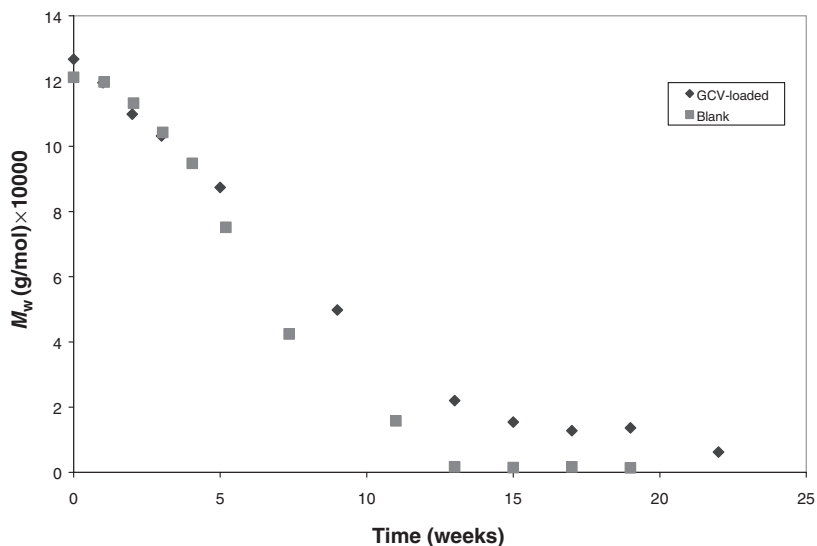
The cumulative fractional release of GCV from the microspheres ( $M_t/M_o$ ), where  $M_t$  is the weight of GCV in solution at time  $t$  and  $M_o$  is the incorporated weight of GCV in the microspheres, is plotted in Figure 1. The microspheres had a triphasic release pattern, i.e., an initial burst, a diffusive phase, and a second burst. The initial burst was small and occurred within the first 2 days of immersion. During the first hour after the immersion,  $24 \pm 1 \mu\text{g}$  of GCV was released and by the end of 2 days,  $86 \pm 2 \mu\text{g}$  or  $2.46 \pm 0.06\%$  of GCV was released. This could be due to the release of GCV deposited on the surface of the microspheres. After the initial burst and from week 1 to 13, the release was small but via diffusion-controlled mechanism. During this time, the microspheres released GCV constantly at a rate of  $1.4 \mu\text{g/day}$ . At the end of 13 weeks of immersion, only  $6.4 \pm 0.8\%$  GCV was released into the phosphate-buffered solution. The release rate was observed to increase from week 13 to 18, where the microspheres released GCV at a rate of  $80.5 \mu\text{g/day}$ . This second burst is related to the onset of bulk degradation of the PLGA polymer. After 20 weeks of immersion, the microspheres had released all the GCV into the phosphate-buffered solution.

## Degradation of the Microspheres

The degradation of PLGA involves chain scission of ester bond linkages in the polymer backbone by the hydrolytic attack of water molecules. Studies on the degradation of various poly(DL-lactic acid) (PDLA) and PLGA samples have shown a heterogeneous degradation mechanism [11–13]. The degradation products generated in the interior of the device autocatalytically accelerate the degradation process. This is due to an increased amount of carboxylic acid end groups, which are responsible for the faster degradation in the center of the device than at the surface. These studies were carried out on pure PLGA polymers without the presence of any drugs. Hence it would be interesting to observe the effect of drugs on the degradation profile of PLGA as the polymer is extensively used for drug delivery systems.

### *Gel Permeation Chromatography*

Figure 2 shows the decrease in the weight-average molecular weight,  $M_w$ , for both the GCV-loaded and the blank microspheres as a function of degradation time. It can be seen that the GCV-loaded microspheres degrade slower than the blank microspheres. Both the  $M_w$  decreased at

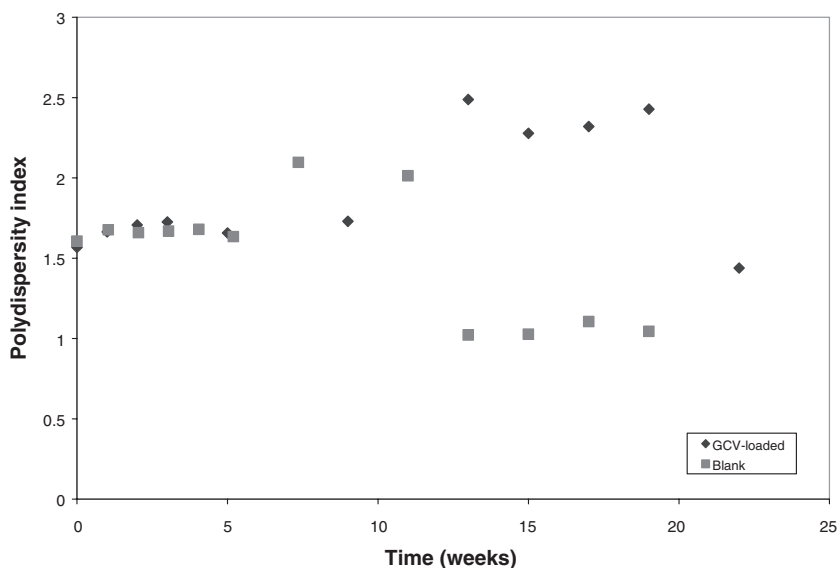


**Figure 2.** Weight-average molecular weight,  $M_w$ , of blank (■) and GCV-loaded (◆) PLGA microspheres as a function of degradation time.



the same rate in the first 4 weeks of degradation. After this, the  $M_w$  for the blank microspheres decreased at a much faster rate compared to the GCV-loaded microspheres. By 13 weeks of degradation, the  $M_w$  of the blank microspheres was only about 1.4% of its initial  $M_w$ , while the  $M_w$  for the GCV-loaded microspheres was 17.3% of its initial  $M_w$ . The presence of GCV inside the polymer matrix decreases the rate at which this chain scission occurs. This is probably due to the amino groups in GCV. The amino groups are basic in nature and can neutralize the carboxylic acid end groups of the degrading products, thus reducing any autocatalysis effect in the polymer matrix and effectively decreasing the rate of PLGA degradation.

The changes in the polydispersity index for both the blank and the GCV-loaded microspheres with respect to the degradation time are presented in Figure 3. Upon degradation, the peak in the chromatogram broadened and shifted to longer retention times. Eventually, however, the peak began to narrow at longer degradation times. The initial broadening of the polydispersity index indicates a mixture of high- and low-molecular weight fractions, while the subsequent narrowing thereafter, indicates a more uniform weight fraction. These changes may be attributed to the high probability of preferential breaking of long PLGA

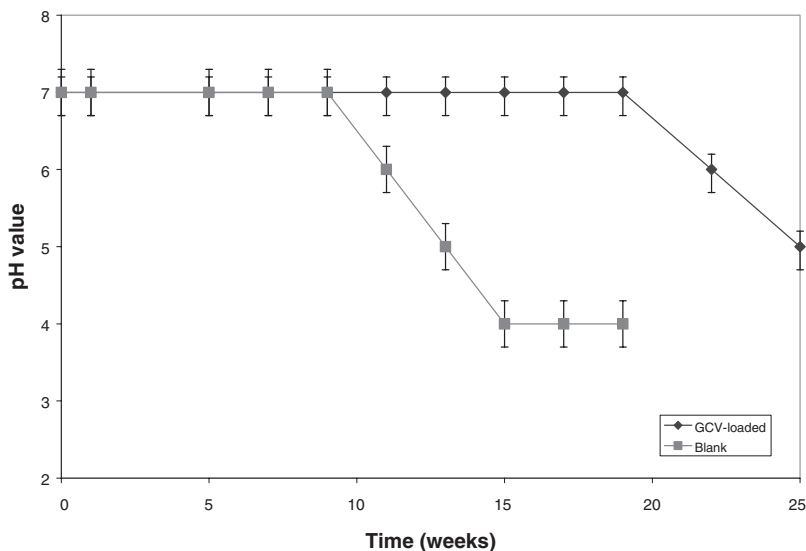


**Figure 3.** Changes in polydispersity index for blank (■) and GCV-loaded (◆) PLGA microspheres with respect to degradation time.

chains during the early stages of degradation compared to the shorter chains at the later stages of degradation [14]. This can be observed in the sharp reduction in  $M_w$  by  $\approx 67\%$  at short degradation times followed by a gentle decrease in  $M_w$  at long degradation times (Figure 2). In the case of the blank microspheres, the changes in the polydispersity index occurred much earlier compared with the GCV-loaded microspheres (Figure 3). This can be attributed to the faster rate of degradation of the blank microspheres.

### Changes in pH

Figure 4 shows the changes in the pH of the phosphate-buffered saline solution with immersion time for the blank and GCV-loaded PLGA microspheres. In the blank PLGA microspheres, the pH of the saline solution began to decrease after 9 weeks of immersion. After 19 weeks of immersion, the pH of the saline solution had dropped from pH 7.0 to 4.0. As for the GCV-loaded microspheres, the pH of the saline solution remained approximately constant at pH 7.0 even after 19 weeks of immersion. A drop in the pH was observed only after 22 weeks of immersion. The slower decrease in the pH for the GCV-loaded microspheres further supports the conclusion that the drug-loaded microspheres degraded slower than the blank microspheres.

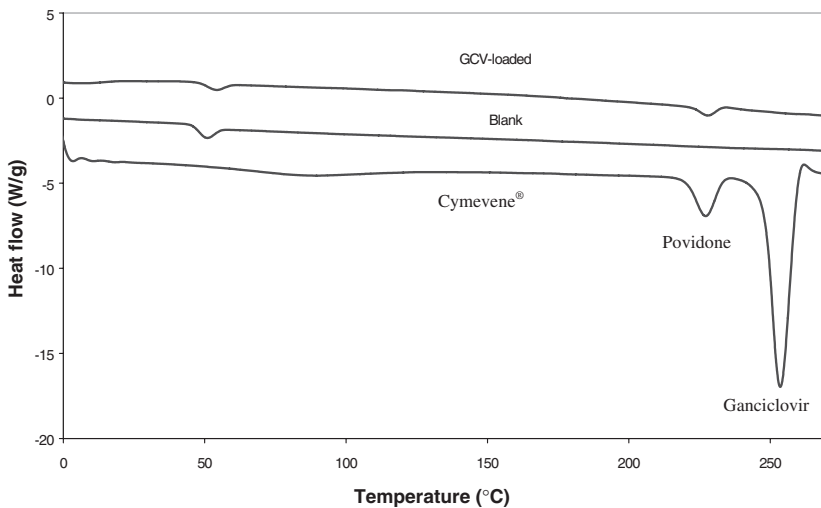


**Figure 4.** Changes in pH values of phosphate-buffered saline solution for blank (■) and GCV-loaded (◆) PLGA microspheres with respect to degradation time.

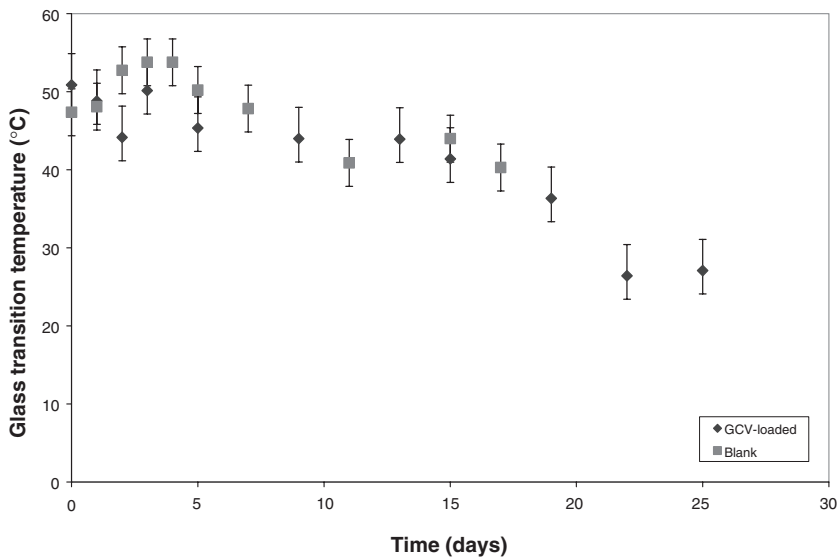
The relatively constant pH in the first 9 weeks of degradation for the blank PLGA microspheres (Figure 4) despite the drastic drop in the weight-average molecular weight (Figure 2) indicates that the degradation by-products are trapped inside the microspheres. Chain scission transforms the PLGA chains into oligomers and monomers, where the ester groups in the polymer chains are cleaved. These degradation products influence the pH of the surrounding medium as well as that inside the particles. The increase in the acidic concentration inside the polymer matrix encourages autocatalysis, causing degradation to proceed more rapidly in the center than at the surface. This type of degradation is known as heterogeneous mechanism and is also observed in matrix devices of PDLA, poly(L-lactide) (PLA), and PLGA [11,12].

#### *Differential Scanning Calorimetry*

Ganciclovir and the excipient, povidone melted at 254 and 232°C, respectively, are shown in Figure 5. The blank and GCV-loaded PLGA microspheres, amorphous in nature, with a  $T_g$  of  $49 \pm 4^\circ\text{C}$ , are also shown in Figure 5. The heating profile of the GCV-loaded microspheres displayed a melting peak at 227.8°C, which corresponded to the melting temperature of GCV. As povidone was present only in trace amounts in



**Figure 5.** Heating profiles of Cymevene<sup>®</sup>, blank microspheres, and GCV-loaded microspheres obtained from differential scanning calorimetry (DSC) at a heating rate of 10°C/min.



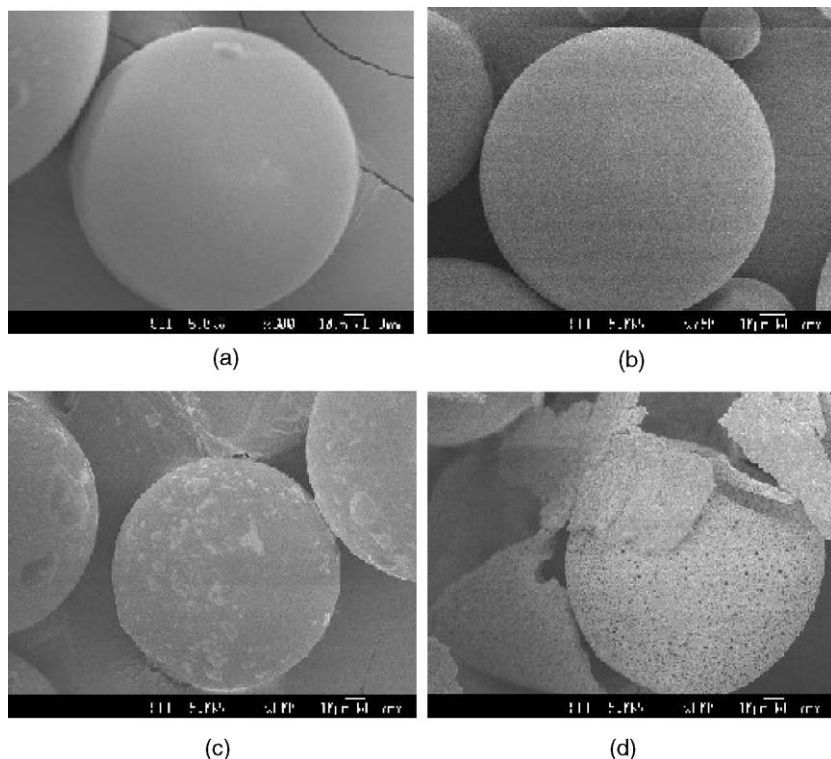
**Figure 6.** Glass transition temperature of degraded blank (■) and GCV-loaded (◆) microspheres as a function of *in vitro* hydrolysis time. A heating scan of 10°C/min was used.

the microspheres, its melting peak in the thermogram of GCV-loaded microspheres could not be detected.

A decrease in  $T_g$  was observed after 7 weeks of degradation for the blank microspheres and 15 weeks of degradation for the GCV-loaded microspheres, as illustrated in Figure 6. This decrease in  $T_g$  was attributed to the decrease in the molecular weight as obtained in Figure 2. This corresponded with the decrease in pH values of the degrading medium with degradation time (Figure 4).

#### Scanning Electron Microscopy

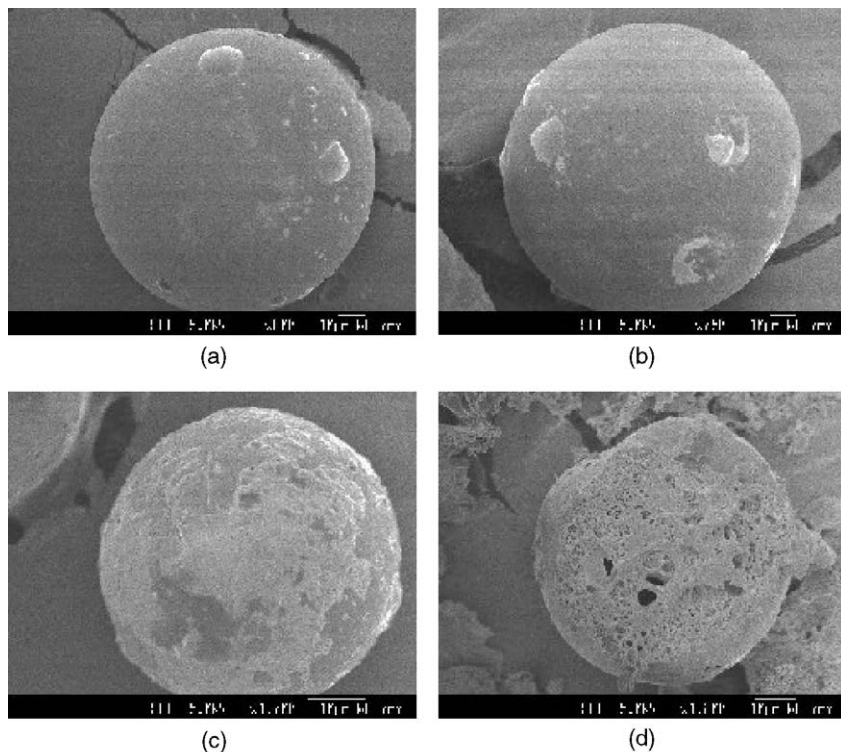
Scanning electron micrographs of the dry degraded blank and GCV-loaded microspheres are shown in Figures 7 and 8, respectively. The surfaces of the degraded blank and GCV-loaded microspheres were relatively smooth up to about 5 weeks of degradation. After 7 weeks of degradation, the blank microspheres showed blister-like formation on the surface and by the 11th week, pores were observed on the surface of the microspheres. By this time, the microspheres were brittle and fragmented easily. No scanning electron micrographs could be taken after 15 weeks of degradation as the sample became gel-like with further degradation.



**Figure 7.** Blank microspheres degraded in phosphate-buffered saline solution at 37°C at various times of degradation: (a) 1 week; (b) 5 weeks; (c) 7 weeks; and (d) 11 weeks.

As for the GCV-loaded microspheres, blister-like formation was observed on the surface after only 13 weeks of degradation. Pores were observed on the surface and throughout the microspheres after only 22 weeks of degradation.

Hydrolysis is the most important mode of degradation for PLGA. Water penetration occurred initially by diffusion of water molecules through either the bulk of the polymer or through voids in the material. Hydrolytic scission of polymer chains caused the average molecular weight of the polymer to decrease with time (Figure 2). Eventually, monomers or short oligomers were formed, which were sufficiently soluble in water for diffusion out of the matrix. The disappearance of monomer/oligomer from the matrix caused the pH of the surrounding medium (Figure 4) and  $T_g$  of the polymer (Figure 6) to decrease with time. As the small polymer fragments leave the matrix, additional



**Figure 8.** Ganciclovir-loaded microspheres degraded in phosphate-buffered saline solution at 37°C at various times of degradation: (a) 1 week; (b) 5 weeks; (c) 13 weeks; and (d) 22 weeks.

channels for water entry and diffusion are created and a microporous matrix is formed (Figure 7). This microporous matrix would eventually become spongy, with water-filled holes getting larger until the matrix was no longer mechanically stable. This mechanism of degradation observed in PLGA microspheres is termed bulk erosion.

The GCV-loaded microspheres degraded at a slower rate than the blank microspheres, as observed from the slower rate of decrease in the molecular weight (Figure 2), pH (Figure 4),  $T_g$  of the polymer (Figure 6), and changes in the polydispersity index (Figure 3). The basic nature of the amino groups in GCV neutralizes the carboxylic acid end groups of the degrading by-products, thus reducing the autocatalysis effect in the polymer matrix. This in turn effectively decreases the rate of the PLGA degradation.

### *Ganciclovir Release Mechanism*

The *in vitro* drug release study showed that the microspheres had a triphasic release pattern, i.e., an initial burst release, followed by a diffusive phase, and finally a second burst as illustrated in Figure 1. This release pattern is closely related to the degradation profile of the PLGA polymer. The initial burst was due to the rapid release of drugs that are loosely bounded or embedded in the surface. After the burst release, the diffusive phase, which lasted up to 13 weeks, could be from the release of drugs in the water channels in the matrix, which is diffusion controlled as suggested by Yasukawa et al. [15]. During this time, the molecular weight of the polymer continued to decrease (Figure 2), while the  $T_g$  of the polymer (Figure 6) and pH of the degrading medium (Figure 4) remained unchanged. Even though chain scission was taking place in the polymer matrix, no significant loss of material was observed. The second burst release of GCV was observed after 13 weeks of degradation in conjunction with the appearance of blister-like formation on the surface of the particles (Figure 8). Advanced degradation/erosion of the polymer matrix had created a microporous matrix, which provided additional pathways for diffusion of drugs trapped in the polymer matrix and hence the large burst of GCV observed after 13 weeks of degradation. The matrix was also no longer mechanically stable by this time.

## CONCLUSIONS

Biodegradable PLGA microspheres without FSiO and SiO and with a high loading of GCV (75%) were successfully prepared using the oil-in-water (O/W) single emulsion method described earlier. The release profile of GCV from the microspheres had a triphasic pattern, which followed closely the degradation profile of the polymer. The small initial burst was due to the rapid release of drugs deposited on the surface and the large final burst happened when advanced degradation of the polymer matrix created a microporous matrix, corresponding to the onset of bulk degradation of the PLGA polymer. The presence of drug molecules often influences the rate of water penetration, thus affecting the rate of hydrolytic degradation. In the case of GCV-loaded microspheres, GCV decreased the rate of PLGA degradation. This was believed to be due to the basic nature of the amino groups in GCV, which neutralizes the acidic by-products of PLGA degradation.

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