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ScienceDirect
International Journal of Pharmaceutics 338 (2007) 133–141
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Effect of lactide/glycolide ratio on the *in vitro* release of ganciclovir and its lipophilic prodrug (GCV-monobutyrate) from PLGA microspheres

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Received 16 October 2006; received in revised form 2 January 2007; accepted 27 January 2007
Available online 2 February 2007

Abstract

Purpose: The aim of this study is to investigate the roles of the lactide/glycolide ratio and prodrug derivatization on the *in vitro* release of ganciclovir and its lipophilic prodrug (GCV-monobutyrate) from PLGA microspheres for the treatment of cytomegalovirus (CMV) retinitis.
Method: Two grades of PLGA, a higher lactide content PLGA 6535 [D,L-lactide:glycolide = 65:35, MW 45,000–75,000 Da] and lower lactide content PLGA 5050 [D,L-lactide:glycolide = 50:50, MW 45,000–75,000 Da] were employed to prepare GCV loaded microspheres. The effect of lipophilic prodrug derivatization was investigated by converting GCV to GCV-monobutyrate (GCVMB). Microspheres were prepared by the oil-in-oil (O/O) solvent evaporation method and characterized *in vitro*, by studying their surface/internal morphology, entrapment efficiency, particle size, drug release, true density and glass transition temperature. *In vitro* release data were analyzed by a model equation to estimate various parameters of the drug release curves.
Results: The O/O solvent evaporation method generated a high drug payload of up to 91%. Higher entrapment efficiencies were observed in the case of hydrophilic drug (GCV) relative to the lipophilic prodrug (GCVMB). Loosely bound or surface adsorbed drug/prodrug molecules may have resulted in the very short period (about 6 h) of the initial burst phase in all types of microspheres. GCV loaded microspheres utilized more time to release 50% (T_{50} value) of entrapped drug than GCVMB microspheres. T_{50} values estimated for GCVMB were shorter than those for GCV from microspheres with similar lactide/glycolide ratios. Lactide content in PLGA did not significantly alter GCVMB release relative to GCV release. The proposed model equation effectively estimated the drug release parameters ($R^2 > 0.98$) with all drug/prodrug-PLGA combinations. SEM pictures have revealed that although both GCV and GCVMB microspheres were spherical but internal morphology was different, with former having uniform and dense whereas later have porous structures. Corroborating with internal morphologies, results revealed that true densities of GCV microspheres were relatively greater than corresponding GCVMB microspheres.
Conclusion: The proposed method of preparation yields higher efficiency of drug entrapment for the hydrophilic drug. Prodrug entrapment into microspheres could result in longer residence time at the site of administration due to multiple processes involved in drug release at infected tissue. These processes include release from microspheres and enzymatic conversion of the prodrug to parent drug.

Keywords: Cytomegalovirus retinitis; Controlled release; Ganciclovir; GCV-monobutyrate; PLGA microspheres; Drug/prodrug

1. Introduction

Human cytomegalovirus (HCMV) retinitis is a serious sight threatening disease affecting immuno-compromised individuals, such as acquired immunodeficiency syndrome patients (Holland et al., 1983) with low CD4 cell counts (<50 cells/μl) (Hoover et al., 1996). Ganciclovir (GCV), a 2′-deoxyguanosine analog, was the first FDA approved drug with significant activity against HCMV. Virustatic properties of GCV require continuous maintenance therapy in addition to the induction regimen to prevent disease relapse (Drew, 1988). Owing to poor oral bioavailability, daily infusion of GCV is necessary. However, systemic toxicity (Faulds and Heel, 1990) and poor ocular drug permeation (Kuppermann et al., 1993) with such infusions have prompted the development of local ganciclovir therapy, mostly through direct intravitreal administration (Henry et al., 1987) and to some extent non-biodegradable implants (al-Yousuf et al., 2000; Martin et al., 1994). Maintaining the

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doi:10.1016/j.ijpharm.2007.01.038

GCV concentration above minimum inhibitory concentration (MIC) requires twice weekly intravitreal injections (due to rapid vitreal elimination ($t_{1/2}$ = 7–10 h)). Such frequent intravitreal injections may result in endophthalmitis, retinal detachment and patient non-compliance (Arevalo et al., 2005; Henry et al., 1987; Kuppermann et al., 1993).

Sustained drug delivery to the retina/vitreal space is still in a developmental stage. Sustained/modified drug delivery strategies via PLGA polymers as implants, microspheres, and nanoparticles have gained wide acceptance. In a previous study, effects of PLGA molecular weight and polymer blending on the *in vitro* GCV release from microspheres prepared by aqueous solvent evaporation method were reported. GCV release was prolonged in polymers with higher molecular weight and lactide content relative to short polymers with lower lactide levels. Increasing the matrix content of the shorter polymer also resulted in greater entrapments and faster release. Results from the study indicate that release of GCV is associated with a lag time that is dependent on the matrix composition. Long lag period may cause lowering of GCV levels leading to therapeutic failure. These earlier reports have described an investigation of strategies to overcome such lag phase without the incorporation of any additives to the formulation (Duvvuri et al., 2005, 2006).

Lipophilic prodrug strategy has been widely applied to various hydrophilic therapeutic agents to improve their ocular bioavailability (Hughes et al., 1993; Narurkar and Mitra, 1988, 1989), including GCV. GCV-monobutyrate, a lipophilic prodrug of GCV (partition coefficient (log *P*) −0.30 versus −1.55 for GCV) (Dias et al., 2002), may enhance its permeability into ocular cells thereby raising intracellular concentrations. The prodrug also reverts back into the parent drug in the vitreous humor at a slow rate resulting in sustained maintenance of GCV levels above MIC.

This study unites the concept of sustained prodrug release from polymeric microspheres with controlled bioconversion at the target site. GCVMB microspheres may not only sustain the release of the prodrug but also in turn slow the rate of degradation into the parent drug. GCV levels may thus be maintained for prolonged periods of time in the vitreous.

In this study, the effect of LA/GA ratios on the *in vitro* release kinetics of GCV and its lipophilic prodrug, GCVMB from PLGA microspheres are investigated. Results presented in this report may aid in better understanding of the role of PLGA polymers in GCV entrapment and release. Encapsulation of GCV prodrug into microspheres has never been attempted. *In vitro* release kinetics of GCV prodrugs may provide a newer strategy for further development of formulations where the active drug release can be controlled by several mechanisms, i.e. polymer hydration, degradation and prodrug reversion. In this study, a non-aqueous method of solvent evaporation has been employed to obtain higher drug entrapments along with lower particle sizes. Effects of polymer composition and prodrug derivatization on drug release, i.e. by altering lactide/glycolide ratios are also investigated. To this extent, two polymers, one with equal ratios of lactide and glycolide, i.e. PLGA 5050 [D,L-lactide:glycolide = 50:50, MW 45,000–75,000 Da] and another with higher lactide content, i.e. PLGA 6535 [D,L-lactide:glycolide = 65:35, MW 45,000–75,000 Da], are employed. Additionally, the effect of prodrug derivatization on GCV release from PLGA microspheres is studied with GCV and its lipophilic prodrug, GCVMB. A mathematical empirical equation is employed to fit the drug release data and to estimate the parameters that govern the shape of the release profile.

2. Materials and methods

2.1. Materials

GCV was a generous gift from Hoffman La Roche (Nutley, NJ). GCV-monobutyrate was prepared and characterized in our laboratory (Gao and Mitra, 2000). PLGA polymer, i.e. PLGA 5050 (D,L-lactide:glycolide = 50:50, MW 45,000–75,000 Da) and PLGA 6535 (D,L-lactide:glycolide = 65:35, MW 45,000–75,000 Da) were procured from Sigma Chemicals (St. Louis, MO). Span 80 was a product of ATLAS Chemical Industries, Inc. (Wilmington, DE). Light mineral oil, acetonitrile (ACN) and hexanes were obtained from Fischer Scientific (New Brunswick, NJ).

2.2. Methods

2.2.1. Preparation of microspheres

GCV and GCVMB microspheres were prepared by a non-aqueous method using solvent evaporation technique. GCV and GCVMB (20 mg) were suspended in 0.5 ml of ACN and sonicated for 15 min in a bath sonicator (50 Hz/60 Hz; 125 W). To this suspension, PLGA (200 mg) was added and further sonicated for 30 min with occasional vortexing to obtain complete dissolution of the polymer in the organic phase. Simultaneously, 0.4% (w/v) solution of SPAN 80 in light mineral oil was prepared. A primary emulsion was obtained by adding 2.5 ml of oil solution to drug–polymer–ACN mixture and vortexing the resultant. This emulsion was then added slowly, drop-wise to rest of the 0.4% SPAN 80 + light mineral oil solution (150 ml) and stirred at a constant speed (250–300 rpm) for 3 h at room temperature for evaporation of the organic solvent. The formed microspheres were then filtered and washed twice with hexane to remove any residual oil. Microspheres were first air-dried overnight and then vacuum dried for 12 h to remove any traces of hexane. Finally, the solid microparticles were stored over anhydrous $CaSO_4$ at 4 °C.

2.2.2. Drug entrapment

Microspheres (5 mg) were dissolved in 5 ml of methylene chloride by sonication for 30 min. GCV and GCVMB were extracted from the organic phase by three portions of 7 ml distilled de-ionized water. Samples were subsequently analyzed by a HPLC method described in Section 2.2.6. Studies were conducted from two batches (n = 4/batch).

2.2.3. Microsphere size, surface/internal morphology and true density

Surface and internal morphology was studied by scanning electron microscopy (SEM) (FEG ESEM XL 30, FEI, Hillsboro, OR). Microspheres and broken microspheres were attached to double-sided tape, spray-coated with gold–palladium at 0.6 kV, and then examined under the electron microscope. Internal morphology was examined by breaking the particles in the liquid nitrogen using pestle mortar. Sizes were also measured from the SEM pictures. At least 200 particles were counted for each batch.

Surface morphology and particle sizes of GCV and GCVMB were also studied using SEM. The methods used to analyze these compounds were similar to those used to analyze the microspheres.

True volume and density of the microspheres were measured by helium pycnometry (Micromeritics Analytical Services, Norcross, GA).

2.2.4. Differential scanning calorimetry (DSC)

Glass transition temperatures of the polymers and drug/prodrug loaded microspheres were measured with a Thermal Analysis Q1000 differential scanning calorimeter (Thermal Analysis Instruments, New Castle, DE). A total of 10–15 mg samples crimped into aluminum pans were subjected to a heat/cool/heat cycle between −40 and 100 °C. Heating and cooling rates were 10 °C/min and a steady stream of nitrogen gas was supplied at 50 ml/min. Glass transition temperatures (T_g) were calculated from the second heating cycle by Universal Analysis software supplied by the instrument manufacturer.

2.2.5. In vitro drug release

Microspheres (10 mg) were placed in 10 ml isotonic phosphate buffer saline containing 0.025% (w/v) sodium azide to avoid microbial growth and placed in a shaker bath at 37 °C with a constant agitation of 60 oscillations/min. One milliliter samples were withdrawn at appropriate time intervals and replaced with equal volumes of fresh buffer. Samples were analyzed by the HPLC method described below. Studies were conducted in quadruplicates from two batches. Cumulative release data were fitted to equations described under the Theory section by nonlinear curve fitting with SCIENTIST® program (Micromath, St. Louis, MO).

2.2.6. HPLC analysis

A high-performance liquid chromatography system (Waters 600 pump; Waters, Milford, MA), equipped with a fluorescence detector (HP1100, Hewlett Packard, Waldbronn, Germany) and a reversed-phase C12 column (4 μm, 250 mm × 4.6 mm, Synergy-max, Phenomenex, Torrance, CA), was employed for GCV and GCVMB analysis. Samples were analyzed with an isocratic method comprised of a mobile phase containing 15 mM phosphate buffer (pH 2.5) and 2.5% acetonitrile pumped at a flow rate of 1 ml/min. Separation of GCV and GCVMB was achieved through a gradient method with 15 mM phosphate buffer (pH 2.5): acetonitrile in proportions of 99.8:0.2 as phase A and 1:1 as phase B at a flow rate of 1 ml/min. All samples were analyzed at an excitation wavelength of 265 nm and at an emission wavelength of 380 nm. The limit of quantification was 50 ng/ml for GCV and 100 ng/ml for GCVMB (Dias et al., 2002).

2.2.7. Data analysis

All experiments were carried out with four samples taken from two different batches unless otherwise specified. Data are represented as mean ± standard error of mean (S.E.M.). Statistical significance was determined with ANOVA and Student's *t*-test at $p < 0.05$.

2.2.8. Theory

Drug release, exemplified by ganciclovir, from PLGA microspheres may occur in three distinct phases (Herrero-Vanrell et al., 2000). Surface-bound and poorly encapsulated drug may diffuse through the pores and cracks in the polymeric matrix resulting in initial (first) burst phase, i.e. phase I. Distribution of drug in the matrix and the polymer packing may govern the extent and duration of drug release in this phase. Following initial phase, release tends to become very slow or minimal which is mark of phase II. Inability of the drug to diffuse out of PLGA matrix in phase II and may be attributed to the binding of drug to the polymer as in case of peptides (Niwa et al., 1994; Bodmer et al., 1992). The length of phase II can be critical in maintaining antiviral drug levels above MIC. Longer phase II duration can result in failure to maintain drug levels above MIC in the retina. Final phase (III) of rapid drug release is generally attributed to faster drug diffusion from the eroding matrix. Hydrolysis of the ester bonds in PLGA molecule results in decrease in the molecular weight of the polymer. This consequently widens the gaps in the matrix thereby making it easier for drug molecules to diffuse into the dissolution medium. Drug release rate in this phase also termed as 'second burst' is determined primarily by the rate of matrix hydrolysis and the drug's ability to diffuse through the generated spaces. Onset of phase III is reported to be associated with rapid increase in water uptake by the matrix and a sudden mass loss (Bodmer et al., 1992).

A triphasic empirical equation (Eq. (1)) was fit to the data and was found to satisfactorily describe the drug release parameters from the microspheres in our previous studies (Duvvuri et al., 2006).

$$F = A(1 - \exp(-K_1 T)) + \frac{B}{1 + \exp(-K_2(T - T_{50}))} \quad (1)$$

where F is the fraction of entrapped drug released, A the percent release of total drug released during phase I, K_1 the rate constant of drug release during phase I due to diffusion, B the percent of total drug released during phase III due to polymer degradation, K_2 the rate constant of drug release during phase III and T_{50} is the time taken to release 50% of entrapped drug.

The current method for preparation of microspheres produced a very rapid initial burst release phase or phase I that would result in a very high K_1 thereby reducing the first exponential in Eq. (1) to infinitesimally small values. Such small values can be ignored without a major effect on the fitting process. Thus, Eq. (1) was reduced to Eq. (2) which was employed to fit the drug

Table 1
Entrapment efficiency, particle size, glass transition temperature and true density of GCV and GCVMB loaded PLGA microspheres

Microspheres	Entrapment efficiency[a] (%)	Particle size[b] (μm)	T_g[c] (°C)	True density (g/cm^3)
(a) GCV loaded microspheres				
PLGA 5050	88.7 ± 0.86	75.9 ± 1.6	33.5	1.324
PLGA 6535	91.3 ± 0.54	85.8 ± 1.5	37.1	1.301
(b) GCV-monobutyrate loaded microspheres				
PLGA 5050	81.7 ± 0.25	65.9 ± 1.0	35.0	1.275
PLGA 6535	85.5 ± 0.67	69.7 ± 1.1	37.8	1.274

[a] Values are expressed as mean ± S.E.M. of at least three samples each from two batches for percent entrapment efficiency studies.
[b] Values are expressed as mean ± S.E.M.
[c] T_g's for pure polymer PLGA 5050 and PLGA 6535 are 42.13 and 44.42 °C, respectively.

release data:

$$F = A + \frac{B}{1 + \exp(-K_2(T - T_{50}))}. \quad (2)$$

3. Results

Non-aqueous method of preparation of microspheres resulted in very high entrapments of 82–91% of both GCV as well as its lipophilic prodrug. Approximately 70% of microspheres were within the range of 30–110 μm in size and were small enough to pass though a 25 G needle (Table 1); moreover, the particles were spherical. Drug entrapment efficiencies were marginally higher for the hydrophilic drug, i.e. GCV relative to its lipophilic prodrug GCVMB for both the PLGA microspheres. Drug entrapments in the case of PLGA 6535 were higher for both GCV and GCVMB (Table 1). This trend was consistent with the amount of drug entrapped per milligram of the microspheres, i.e. PLGA 5050 (GCVMB: 79.75 ± 0.55 μg/mg) < PLGA 6535 (GCVMB: 83.93 ± 0.78 μg/mg) < PLGA 5050 (GCV: 85.49 ± 0.87 μg/mg) < PLGA 6535 (GCV: 88.28 ± 0.48 μg/mg).

Surface morphology studies performed with the aid of scanning electron microscope revealed that GCV loaded microspheres probably contained adsorbed particles adhered to the surface (Fig. 1), which was not observed with an aqueous method of preparation (Duvvuri et al., 2006). Surface texture is found to be rough for all the microspheres batches of GCV and GCVMB. GCV crystals can be observed on the surface but no such crystals of GCVMB were found on its microspheres, although GCVMB microspheres have a consistently rough texture (Fig. 2). SEM pictures of broken microspheres revealed that GCV microspheres have a compact smooth internal morphology, whereas the GCVMB has a rough and porous internal structure. Micrographs of pure drug shows that GCV crystals are rectangular in shape ranging from 6 to 400 μm. Whereas the GCVMB crystals are irregular in shape, however the size distribution is comparatively narrow, i.e. 10–130 μm (Fig. 3). The

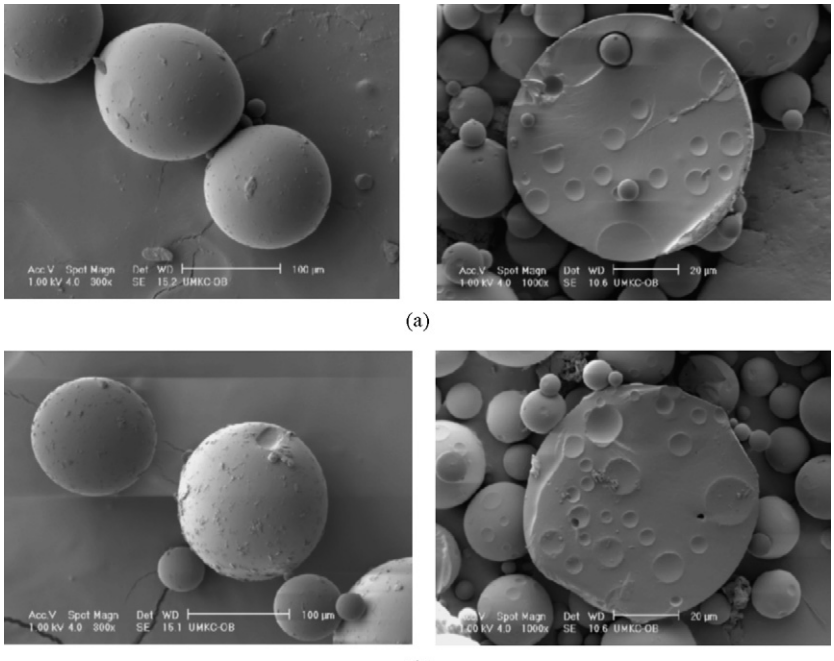

Fig. 1. Scanning electron microscope (surface and internal) photographs of GCV loaded microspheres prepared from (a) PLGA 5050 and (b) PLGA 6535.

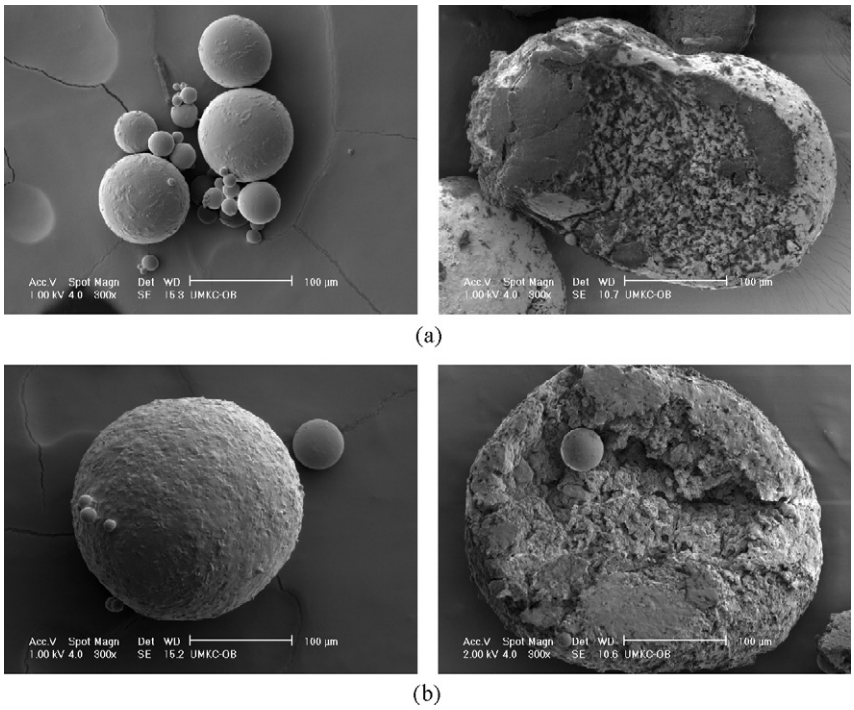

Fig. 2. Scanning electron microscope (surface and internal) photographs of GCV-monobutyrate loaded microspheres prepared from (a) PLGA 5050 and (b) PLGA 6535.

average particle size of GCV and GCVMB were 52.70 μm (standard deviation of 71.55 μm) and 55.62 μm (standard deviation of 22.60 μm), respectively. Smaller crystals of the prodrug could be the possible reason for the smaller size of GCVMB microspheres. On the contrary, wide size distribution (6–400 μm) of GCV crystals might have resulted in greater average particle size of the corresponding microspheres relative to GCVMB.

The data tabulated in Table 1 show that GCV loaded in PLGA 5050 has the highest true density (1.342 g/cm^3) followed by GCV loaded in PLGA 6535 (1.301 g/cm^3). Similar trend was observed with GCVMB loaded microspheres formulated from PLGA 5050 (1.275 g/cm^3) and PLGA 6535 (1.274 g/cm^3). These true density values reveal that GCV particles have compact packing compared to GCVMB and the density follows the order: GCV 5050 > GCV 6535 > GCVMB 5050 > GCVMB 6535.

T_g's obtained from the DSC studies did not show much difference (Table 1). Although there was a difference of 1.5 °C with PLGA 5050 microspheres containing GCV (33.47 °C) and GCVMB (34.98 °C) and 0.6 °C with PLGA 6535 microspheres (GCV: 37.07 °C and GCVMB: 37.77 °C), the difference was not significant enough to produce such variation in release profiles observed with the two agents. Any observed difference in the T_g's between the two polymers was the inherent property of the polymer because of its lactide glycolide ratio. It was observed that higher lactide content results in rising T_g (PLGA 6535: 44.42 °C and PLGA 5050: 42.13 °C).

Microparticles appeared to generate triphasic release profiles *in vitro* with a rapid release in phase I (within 6 h). Such rapid or burst releases were observed in all types of microspheres and the rate of release was so high it was futile to estimate the release parameters in this phase due to the paucity of adequate points on the curve in the initial phase (Figs. 4 and 5). Due to this burst effect, all release profiles were found to fit Eq. (2), which is a derived form of Eq. (1). R^2 values for all the linear release profiles were greater than 0.98. Estimated release parameters are listed in Tables 2a and 2b for GCV and GCVMB, respectively. The total percentage of entrapped drug released in phase I estimated by parameter A was found to be higher for GCV relative to that of GCVMB. GCV entrapped in PLGA 5050 generated the highest initial drug release among all the microspheres followed by GCV entrapped PLGA 6535 microspheres. Amount of initial release for GCVMB was significantly lower and showed a reverse trend than what was observed with GCV. Moreover, the initial amount released from PLGA 6535 for GCVMB was higher than that from PLGA 5050 microspheres.

Table 2a
Drug release parameters estimated from non-linear regression fit of GCV release data from PLGA microspheres fitted to Eq. (2)

Microspheres	A (%)	B (%)	K_2 (day^{-1})	T_{50} (days)
PLGA 5050	17.5 ± 0.32	77.7 ± 0.34	0.31 ± 0.10	20.1 ± 0.06
PLGA 6535	14.8 ± 0.52	83.2 ± 0.55	0.38 ± 0.02	39.9 ± 0.24

Table 2b
Drug release parameters estimated from non-linear regression fit of GCV-monobutyrate release data from PLGA microspheres fitted to Eq. (2)

Microspheres	A (%)	B (%)	K_2 (day^{-1})	T_{50} (days)
PLGA 5050	8.34 ± 0.18	90.35 ± 0.28	1.62 ± 0.03	4.07 ± 0.02
PLGA 6535	7.77 ± 0.30	91.89 ± 0.47	1.08 ± 0.05	3.23 ± 0.04

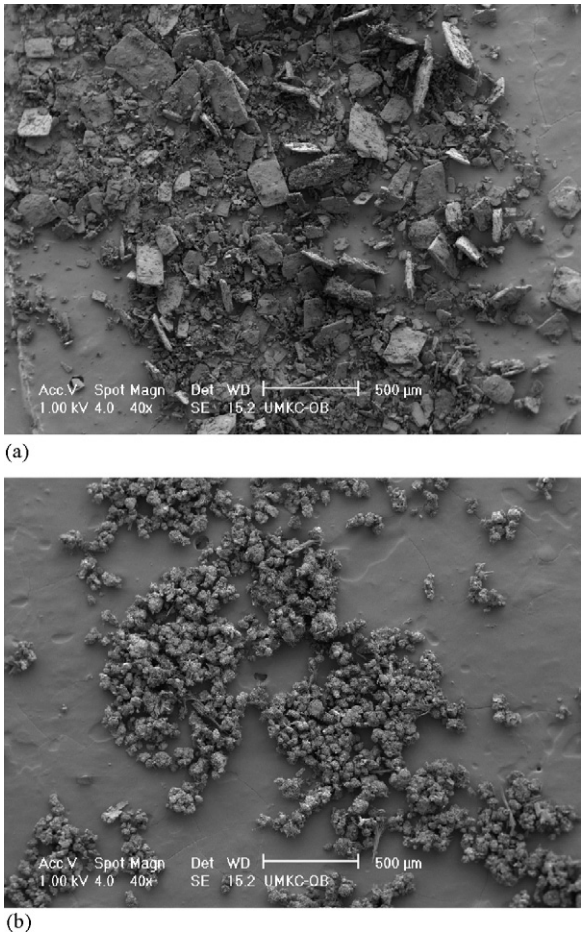

Fig. 3. Scanning electron microscope photographs of (a) GCV and (b) GCV-monobutyrate crystals.

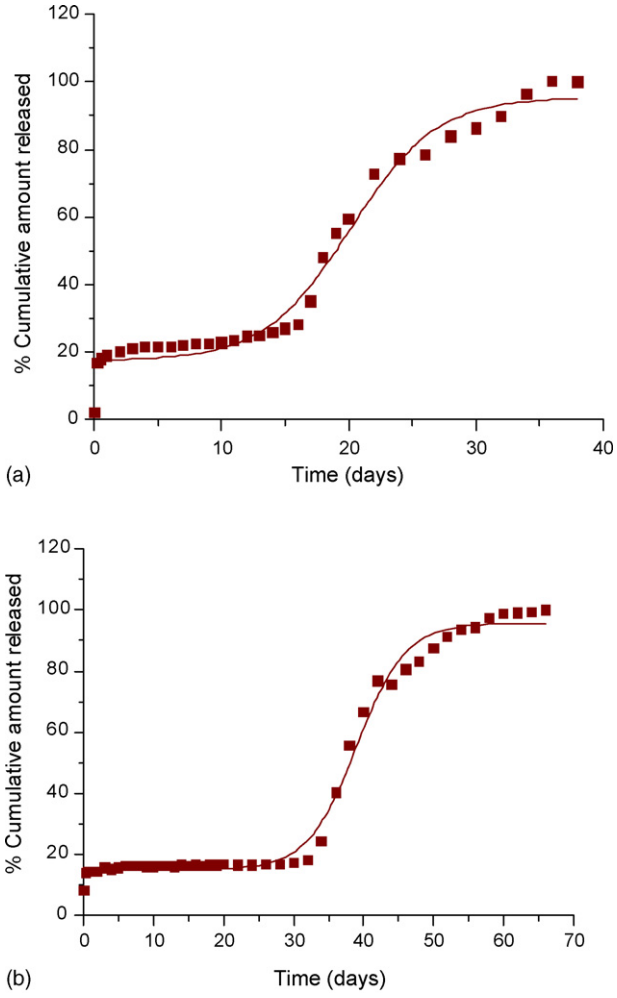

Fig. 4. *In vitro* release of GCV from (a) PLGA 5050 and (b) PLGA 6535 microspheres. Lines drawn represent least square fit of the data to Eq. (2) by non-linear regression analysis ($n = 4$/batch).

T_{50} (time taken to release 50% of entrapped drug) is an index of phase II duration. Results revealed that T_{50} values were higher in the case of GCV loaded microspheres. T_{50}, i.e. time taken to release 50% of entrapped drug, was higher in the case of GCV loaded microspheres. The values here were directly proportional to the lactide content of the polymer, i.e. 20.1 and 39.9 days for PLGA 5050 and PLGA 6535, respectively. T_{50} values estimated for the GCVMB were considerably smaller than those of corresponding polymers entrapping GCV. Moreover, with GCVMB loaded microspheres, T_{50} value for PLGA 6535 (3.23 days) was slightly lower than that of PLGA 5050 (4 days).

B values summarized in Tables 2a and 2b for both GCV and GCVMB microspheres revealed that most of the drug release occurred in phase III. Release in phase III accounts for 77–83% of entrapped GCV and 90–91% of GCVMB. Rate constants during phase III (K_2) were significantly lower (3–4-fold) for GCV relative to GCVMB.

From the previous studies, PLGA polymers with higher lactide content degrade at a slower rate as compared to polymer with lower lactide content (Wu and Wang, 2001). Similar results were observed with GCV release from PLGA 6535 relative to PLGA 5050. However, faster release of GCVMB from PLGA microspheres cannot be explained only by polymer degradation. Higher inherent diffusivity of GCVMB due to its lipophilicity relative to GCV could result in such rapid release rates.

4. Discussion

Controlled drug delivery systems have been developed for ganciclovir. These systems include micro and nano-spheres, liposomes and non-biodegradable implants. The microspheres are one of the important delivery systems that are easy to prepare and administer. PLGA, a polymer approved by the FDA for use in humans, is extensively studied for microspheres based drug delivery because of its biodegradable nature. PLGA based microspheres have been used in the past to deliver GCV to the posterior segment of eye.

One other system that can be used for sustained delivery includes the use of prodrugs in sustaining the release of ganciclovir in the vitreous humor. Lipophilic ester prodrugs of GCV have an added advantage of better permeability across the cell membrane. Intracellularly these compounds are acted upon by the enzymes and cleaved to generate parent drug. Among the

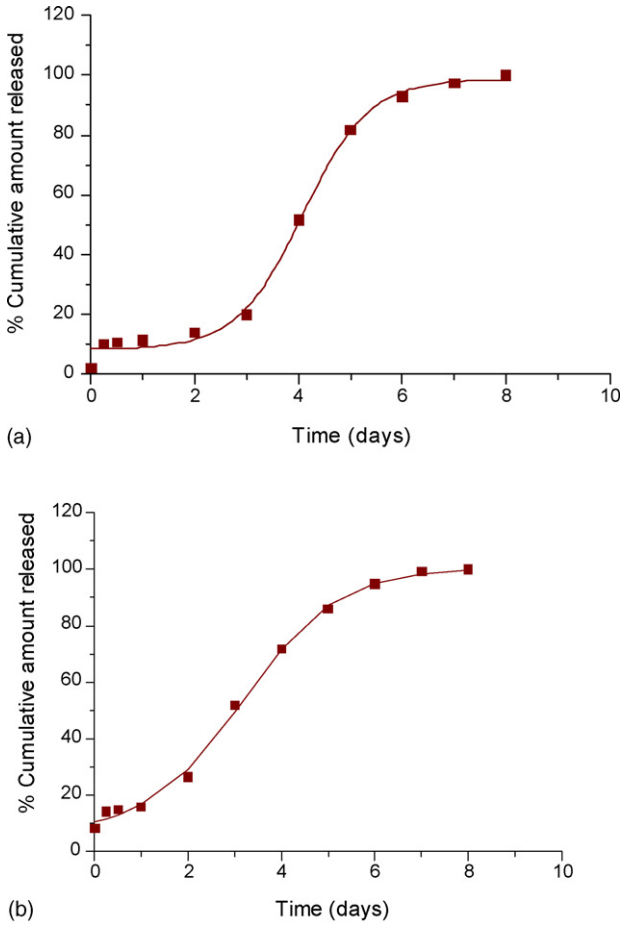

Fig. 5. *In vitro* release of GCV-monobutyrate from PLGA 5050 (a) and PLGA 6535 (b) microspheres. Lines drawn represent least square fit of the data to Eq. (2) by non-linear regression analysis ($n = 4$/batch).

various acyl ester prodrugs of GCV studied, GCVMB emerged as the ideal candidate for ocular delivery due to its strong prodrug characteristics for antiviral activity among the other lipophilic ester prodrugs of GCV (Dias et al., 2002). GCVMB molecular properties strike a good balance of water solubility and lipophilicity that is required for formulation and optimum drug permeation. Even though this prodrug is an ideal substitute for GCV, it suffers from rapid elimination from the vitreous (Macha and Mitra, 2002). The elimination of prodrug from the vitreous corresponds to its conversion into the parent drug plus accumulation into the retina. Moreover, being a lipophilic prodrug of GCV, GCVMB has better permeability into the retina requiring relatively lower concentration to be maintained in the vitreous. Previous study from our lab has revealed that rate of conversion of this prodrug into the parent drug was much higher in the retina (61.42×10^{-4} min^{-1} mg^{-1}) than in the vitreous (2.31×10^{-4} min^{-1} mg^{-1}) which might result in toxicity if administered as intravitreal bolus injection (Dias et al., 2002). Thus, entrapment of GCVMB in biodegradable microspheres may result in its sustained release and accumulation of optimal therapeutic concentration in the retina.

In the current study, it was observed that the release of both GCV and its prodrug during phase I was very rapid. Such instantaneous release could be attributed to the non-aqueous solvent evaporation method which resulted in deposition of drug particles onto the microspheres surface. Scanning electron micrographs clearly reveal free ganciclovir crystals on the surface of microspheres. Molecules adhered to the surface of these microspheres will dissolve or diffuse rapidly into the aqueous media causing the initial burst effect. The equation was therefore modified to predict the amounts of drug released in phase I. This modified equation (Eq. (2)) was used to determine drug-release parameters. No such faster initial release was observed with aqueous method of microsphere preparation, moreover, no GCV crystals were seen on the surface of such microspheres (Duvvuri et al., 2006).

Glass transition temperatures (T_g) for GCV and GCVMB loaded microspheres were considerably lower than the pure polymers (Table 1). A decrease in T_g could be a result of plasticizing effect of entrapped drug, traces of oil or hexane and residual acetonitrile present in microsphere matrix. It is also possible that smaller PLGA polymer fragments were generated during the sonication process and might have decreased the T_g (Reich, 1998). PLGA 6535 polymer, due to its higher lactide content has higher T_g than PLGA 5050. T_g's for GCVMB loaded microspheres were slightly higher than the corresponding microspheres loaded with GCV. Such elevation in T_g albeit small might be due to greater association forces between drug and polymer molecules. Higher T_g is expected to slow down drug diffusion through the polymer matrix. GCV release from PLGA microspheres revealed that higher T_g values mean slower release. However, the opposite is true for the lipophilic prodrug, GCVMB, in which higher values mean faster release.

T_{50} is an index of duration of phase II, i.e. time taken to release 50% drug. Following the lag time or minimal drug release, the second burst release indicated by phase III is mainly attributed to polymer degradation and may cause the release of the residual drug. Bodmer et al. (1992) reported that BSA release from PLGA matrices phase III, was initiated once the polymer molecular weight was reduced to 10,000 Da. Thus, if the release of drug (GCV) is mainly due to polymer degradation then higher molecular weight and greater lactide content would proportionally increase the phase II, i.e. T_{50}. On the contrary, drug release due to diffusion through the matrix may result in faster release rates independent of polymer degradation (Le Corre et al., 1997). Therefore, a polymer degradation independent drug (GCVMB) release may reduce the length of phase II. Another plausible explanation for faster release of compounds could be their distribution in the polymer matrix. During the micellization process (initial stage of microsphere formation), GCV will go deep into the PLGA (GA phase) droplet as external phase is oily (liquid paraffin) and GCV is hydrophilic. Therefore GCV will be at core with PLGA all around it. Therefore it takes longer to come out and delays the release. GCVMB is more lipophilic than GCV; therefore, it is dispersed more in the outer layers of the droplet relative to GCV. This results in formation of pore channels towards the core, through which drug leaches out rapidly. These micelles eventually precipitate as solid microspheres due to the evaporation of the organic phase. It is clear

from the internal micrographs of the GCV and GCVMB microspheres that the former has smooth internal morphology perhaps may also be due to the easy compacting of overlapping crystals. Although the initial burst release is high, compact packing seems produce a slower release of GCV. On the contrary, due to non-compactness of GCVMB crystals in the microsphere, the morphology is characterized as sparse and fluffy. Even though the initial release is slow subsequent hydration causes release of the prodrug due to faster diffusion through the pores formed and that are already present. Moreover, because of the less dense internal structure of GCVMB and almost same size distribution relative to GCV microspheres, it could be concluded that more number of microspheres were have formed per milligram of PLGA in the case of GCVMB than in GCV. This indicates more surface area would have been exposed to the dissolution media in case of the prodrug relative to the drug. True particle densities of the microspheres measured by helium pycnometry further bolster the results concluded from SEM pictures of internal morphologies. Comparatively less dense particles resulted form GCVMB than GCV. This may also explain the faster drug release of GCVMB relative to GCV loaded microspheres. Further, it was observed that use of lipophilic polymer results in higher drug entrapments; therefore PLGA 6535 produced better entrapment of both compounds. The T_{50} value of GCV loaded in PLGA 6535 polymer based microspheres is the highest, followed by GCV entrapped in PLGA 5050. The lowest T_{50} value was observed for GCVMB loaded PLGA 6535 microspheres.

The total percentage of entrapped drug release in phase III estimated by B is the total remaining drug in the microspheres released after initial phase I and subsequent phase II, K_2 determines the rate of release of drug from microspheres in phase III (Tables 2a and 2b). The B values were higher for the GCVMB (∼90%) than for the GCV (∼80%), suggesting that most drug release occurred in phase III, albeit with different mechanisms. Interestingly, hydrophilic drug release rate in phase III, i.e. K_2 values, were found to be smaller for PLGA 5050 and vice versa for lipophilic drug.

In conclusion, GCV and its lipophilic prodrug encapsulated microspheres were prepared and evaluated. Effects of polymer composition and drug lipophilicity on release were delineated. Parameters were determined employing an empirical equation. Prodrug entrapped microspheres have demonstrated release profiles with minimal phase II. Prodrug entrapment into microspheres could result in longer residence time at the site of administration due to multiple processes involved in drug release at infected tissue. These processes include release from microspheres and enzymatic conversion of the prodrug to parent drug.

Acknowledgements

The authors thank Dr. Vladimir Dusevich, School of Dentistry, for providing us with scanning electron microscope. This work was supported by the National Institutes of Health grants R01 EY 09171-12 and R01 EY 10659-10.

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