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Development of a novel formulation containing poly(D,L-lactide-co-glycolide) microspheres dispersed in PLGA–PEG–PLGA gel for sustained delivery of ganciclovir

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Abstract

The purpose of this work is to develop empirical equations for describing the in vitro ganciclovir (GCV) release from PLGA microspheres and also to develop and characterize a formulation containing GCV loaded PLGA microspheres dispersed in thermogelling PLGA–PEG–PLGA polymer gel. Effect of polymer chain length and polymer blending on GCV entrapment and release from PLGA microspheres is also examined. PLGA microspheres of GCV were prepared from two polymers PLGA 6535 (D,L-lactide : glycolide :: 65 : 35, Mw = 45,000–75,000 Da) and Resomer RG 502H (D,L-lactide : glycolide :: 50 : 50, Mw = 8000 Da) and a 3 : 1 mixture. PLGA–PEG–PLGA polymer was synthesized and characterized. In vitro GCV release studies were conducted with microspheres and microspheres dispersed in 23% w/v PLGA–PEG–PLGA solution. Polymer blended microspheres entrap more GCV (72.67 ± 2.49%) than both PLGA 6535 (51.37 ± 2.7%) and Resomer RG 502H (47.13 ± 1.13%) microspheres. In vitro drug release data was fit to sigmoid equations and release parameters were estimated by nonlinear regression analysis. These equations effectively describe three different phases in GCV release from PLGA microspheres, initial diffusion, matrix hydration and degradation. The amount of drug release during the initial phase decreased for the blend microspheres indicating efficient packing between the PLGA 6535 and Resomer RG 502H in the microsphere matrix. Moreover, upon dispersion into the polymer gel, the rate of drug release during initial diffusion phases slowed relative to microspheres alone. In conclusion, this study reports the development of PLGA microspheres with high payloads and their PLGA–PEG–PLGA gel based formulations. Drug release equations have been developed that effectively describe the triphasic GCV release.
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Keywords: Cytomegalovirus retinitis; Ganciclovir; Thermogelling polymers; Microspheres; Drug release equations

1. Introduction

Cytomegalovirus (CMV) retinitis is a sight threatening inflammation of the retina caused by human

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cytomegalovirus (HCMV). CMV retinitis occurs in terminally ill acquired immune deficiency syndrome (AIDS) patients [1]. Pathogenesis involves invasion of sensitive neural tissue of the retina affecting vision [2]. Ganciclovir (GCV) is the first FDA approved drug in the treatment of HCMV retinitis. Due to poor oral bioavailability of the drug, it is administered as daily IV infusion, which increase the risk of infections at the site of administration and could precipitate systemic toxicity [3]. To overcome these drawbacks, local therapy with GCV was initiated by direct intravitreal injections [4,5]. The major problem with the intravitreal therapy of GCV is its short vitreal elimination half-life (7–10 h) that necessitates frequent injections of high doses [6]. Such high frequency of intravitreal injections could lead to retinal detachment, endophthalmitis and also patient discomfort [7–9]. Development of formulations for sustained delivery of GCV can decrease the frequency of intravitreal injections in a manner that is safe and nontoxic to the eye.

Various formulation strategies, such as microspheres, liposomes and implants, etc,. have been employed to deliver drugs in a controlled fashion to the retina and vitreous [10–13]. The major impediment towards widespread use of these formulations is their potential for retinal toxicity due to their composition. Although microspheres and nanospheres deliver drugs in a controlled manner to the posterior segment following intravitreal injection, retinal irritation and vision obstruction may occur due to particle movement in the vitreous. Retinal implants result in sustained drug levels over 4–6 months [13]. However, non-biodegradable nature of the implant requires its removal and subsequent replacement upon drug exhaustion.

A major requirement for a successful sustained release microsphere based formulation is constant and controlled delivery of the entrapped drug. As shown in Fig. 1, GCV release could involve long phases of inactivity (Phase II) where drug levels at the target site may fall below therapeutic levels leading to possible development of drug resistance. Drug release enhancing agents have been added to obtain constant release of acyclovir from PLGA microspheres [14]. Similar agents may be incorporated into PLGA microspheres of GCV. Instead of employing release enhancing agents, drug release rates from
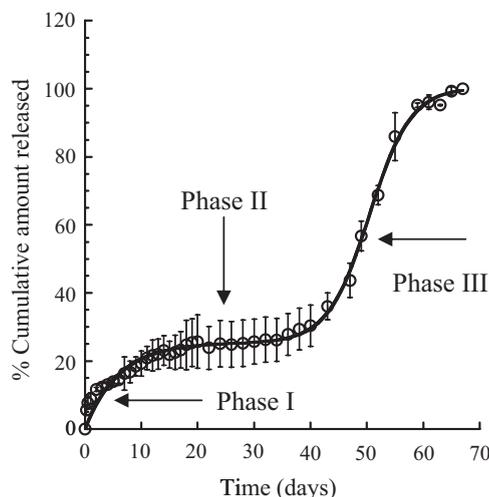

Fig. 1. In vitro release of GCV from PLGA 6535 microspheres. Lines drawn represent least squares fit of the data to Eq. (3) by nonlinear regression analysis ($n = 3$/batch).

PLGA microspheres can be affected by employing polymer mixtures in microsphere preparation with different molecular weights and compositions. A small molecular weight hydrophilic PLGA polymer could be used along with a higher molecular weight polymer to alter the drug release characteristics from microspheres.

Delivery of PLGA microspheres at the target site by an injection is another obstacle towards development of a successful microsphere based formulation. Due to their lipophilic nature, PLGA microspheres may agglomerate in aqueous vehicles making it difficult to pass through a needle. Particle agglomeration can be prevented by the addition of surfactant or by the use of a viscous vehicle. A viscous aqueous based vehicle prepared from biocompatible and biodegradable polymer that can prevent particle migration at the site of administration would be ideal for in vivo administration of PLGA microspheres.

Block co-polymers containing polyethyleneglycol (PEG) and PLGA blocks are gaining importance in controlled release due to their thermogelling and biocompatible properties [15,16]. The triblock co-polymer, PLGA–PEG–PLGA is one of the most studied polymer and was first synthesized by Zentner et al. [15]. This polymer gels at 32 °C and can deliver drugs and proteins in a controlled manner. The biocompatible nature of the polymer renders it suitable for a wide variety of applications including controlled ret-

inal drug delivery. This report describes the development of a novel formulation containing GCV loaded PLGA microspheres suspended in PLGA–PEG–PLGA gel suitable for continuous delivery for a period of 4–5 weeks.

In this study, PLGA loaded microspheres of GCV from two different PLGA grades, 1) PLGA 6535 (D,L-lactide : glycolide :: 65 : 35, Mw = 45,000–75,000 Da) and 2) Resomer RG 502H (D,L-lactide : glycolide :: 50 : 50, Mw = 8000 Da), are prepared and studied. Microspheres were also prepared from a PLGA 6535 : Resomer RG 502H :: 3:1 polymer blend to study the effect of polymer blending on GCV entrapment and release. The microspheres were then suspended in PLGA–PEG–PLGA gel and release parameters were studied. These studies show the utility of these delivery systems for controlled release of GCV over a 4- to 5-week time period.

2. Materials and methods

2.1. Materials

GCV was a generous gift from Hoffman La Roche (Nutley, NJ). PLGA 6535 (D,L-lactide : glycolide :: 65 : 35, Mw = 45,000–75, 000 Da), polyvinylalcohol (30,000–70,000 Da) (PVA), stannous 2-ethylhexanoate and polyethylene glycol 1450 (PEG were purchased from Sigma Chemicals (St Louis, MO). Resomer RG 502H (D,L-lactide : glycolide :: 50 : 50, Mw = 8000 Da) was obtained from Boehringer Ingelheim (Germany). Methylene chloride was procured from Fischer Scientific (New Brunswick, NJ). Lactide and glycolide monomers for synthesis of PLGA–PEG–PLGA polymer were generously donated by Purac America (IL, USA) and were used without further purification.

2.2. Methods

2.2.1. Preparation and characterization of microspheres

2.2.1.1. Preparation of microspheres. GCV microspheres were prepared by solvent evaporation method. GCV (20 mg) was suspended in 0.5 ml of methylene chloride and sonicated for 30 min. Then 200 mg of polymer was added and further sonicated for 30 min with occasional vortexing to cause complete dissolution of the polymer in the organic phase. A primary emulsion was prepared by adding 2.5 ml of 2.5% PVA solution. This emulsion was then added slowly to 2.5% PVA solution (150 ml) drop-wise and stirred at a constant speed (250–300 rpm) for 3 h at room temperature for complete evaporation of the organic solvent. The formed microspheres were then filtered and washed with distilled de-ionized water and air dried for complete removal of any traces of methylene chloride. Finally, the microspheres were stored over anhydrous $CaSO_4$ at 4 °C. Microspheres were prepared from PLGA 6535, Resomer RG 502H and PLGA 6535 : Resomer RG 502H :: 3 : 1 blend.

2.2.1.2. Entrapment efficiency. Microspheres (5 mg) were dissolved in 5 ml of methylene chloride by sonication for 30 min. GCV was then extracted from the organic phase by three portions of 7 ml distilled de-ionized water. Samples were subsequently analyzed by a method described in HPLC analysis section. Studies were conducted from two batches ($n = 3$/batch).

2.2.1.3. Microsphere size, surface morphology and true density. Surface morphology was studied by scanning electron microscopy (SEM) (FEG ESEM XL 30, FEI, Hillsboro, OR). Microsphere particles were attached to a double-sided tape, spray-coated with gold–palladium at 0.6 kV, and then examined under the electron microscope.

Size was measured microscopically with the aid of a stage, an eye piece micrometer, and camera (Carl Zeiss, Germany). At least 200 particles were counted for each batch. True volume and density of the microparticles were measured by helium pycnometry (Micromeritics Analytical Services, Norcross, GA).

2.2.1.4. Differential scanning calorimetry (DSC). Glass transition temperatures of the polymers and the drug loaded microspheres were measured with a Thermal Analysis Q1000 differential scanning calorimeter (Thermal Analysis Instruments, New Castle, DE). 10 to 15 mg samples crimped into aluminum pans were subjected to a heat/cool/heat cycle between −40 °C 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.

2.2.1.5. In vitro ganciclovir release.

Microspheres (10 mg) were placed in 10 ml of 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. 1 ml samples were withdrawn at appropriate time intervals and replaced with equal volumes of fresh buffer. Samples were analyzed by the described HPLC method. Studies were conducted in tripilcates from two batches except for PLGA 6535 microspheres where studies were conducted from a single batch.

Cumulative release data was fitted to equations described under Theory section by nonlinear curve fitting with SCIENTIST® program (Micromath, St Louis, MO). Best-fit models were selected based on F-test, R^2 values, residual analysis, parameter % CV and weighted sum of squares of errors.

2.2.2. Preparation and characterization of gel

2.2.2.1. Synthesis of gel.

PLGA–PEG–PLGA gel was synthesized according to a previously published method [15]. Briefly, PEG was dried under vacuum at 155 °C in a round bottom flask for 3 h. Vacuum was released under nitrogen and the monomers, D,L-lactide (19.9 g) and glycolide (5.7 g), were added and allowed to melt at the same temperature for 30 min and mixed with constant stirring. At the end of 30 min, stannous (2-ethylhexanoate) was added and the reaction was carried out for 8 h at 150 °C under an atmosphere of nitrogen. After 8 h, vacuum was applied to the reaction mixture for 30 min to remove any un-reacted monomers. Crude polymer was dissolved in ice cold water (5–8 °C). After complete dissolution, the polymer solution was heated (80 °C) to precipitate the polymer and remove water-soluble impurities. Precipitated polymer was separated by decanting and the procedure was repeated three times for complete removal of impurities. Finally, the polymer was freeze-dried for complete removal of water and was stored at −20 °C until further use.

Polymeric gel was characterized by procedures followed by Zentner et al. [15]. Number average molecular weight and lactide to glycolide ratios were determined by end group analysis by ^{1}H NMR spectra in $CDCl_3$ (Bruker 250 MHz). Weight average molecular weight was determined by gel permeation chromatography with a Waters® Styragel HR4E column (7.8 × 300 mm) at 30 °C using Waters® 410 Diffraction Refractometer. Mobile phase (dimethylformamide) was delivered by a Waters® 515 HPLC pump. Standard curve was generated by PEG standards in the size range of 100–15 000 Da (Polysciences, PA).

2.2.2.2. Thermogelling properties of gel.

Thermal gelling properties of the gel were studied by tube inversion method [17]. Aqueous solutions of the gel (10%, 15%, 20%, 25%, 35% w/v) were prepared in distilled de-ionized water and 1 ml of each of the solution was transferred to screw capped borosilicate glass test tubes (16 × 125 mm). The tubes were then transferred into water bath and heated from 15 to 65 °C. At each 1 °C interval, tubes were inverted to check the flow properties. Solutions were considered to be in the gel state if no flow was observed for 30 s following tube inversion.

2.2.2.3. Drug release from gel and formulation.

Microspheres (10 mg) were dispersed in 200 μl of 23% w/v polymer solution (referred as formulation from here onwards) in flat bottom screw cap vials and allowed to gel by immersing in water bath at 37 °C for 5 min. After gelling, 2.5 ml of buffer was added and release was initiated. 1 ml samples were withdrawn at appropriate time points and replaced with equal volumes of fresh buffer. Same procedure was followed for studying release of GCV from gel. Samples were analyzed by an HPLC method described below. Cumulative release data was fitted to equations described under Theory section by nonlinear regression analysis with SCIENTIST® program (Micromath, St Louis, MO). Best-fit models were selected based on F-test, R^2 values, residual analysis, % CV and weighted sum of squares of errors.

2.2.3. HPLC analytical method

HPLC system (Waters® 600 pump; Waters® Corp, Milford, MA), equipped with a fluorescence detector (HP1100, Hewlett Packard, Germany) and a reversed phase C_{12} column (4 μ, 250 × 4.6 mm, Synergy-max,

Phenomenex, Torrance, CA) was employed for GCV quantification. 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. All samples were analyzed at an excitation wavelength of 265 nm and emission wavelength of 380 nm. Limit of quantification was 50 ng/ml for GCV.

2.2.4. Data analysis

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

2.2.5. Theory

2.2.5.1. Drug release equations. Various equations have been employed to describe the release of entrapped drugs from microspheres [18–20]. Sigmoid equations have been applied previously to characterize drug release from PLGA microspheres [20]. A simple sigmoidal equation does not completely describe the different phases in a typical release profile. In order to render the equation more applicable towards drug release patterns, modifications were made which fit the experimental data better than the simple sigmoidal equation. The equations used to fit the data are

$$F = B/(1 + \text{Exp}(-K_2*(T - T_{50}))) \tag{1}$$

$$F = A*T + B/(1 + \text{Exp}(-K_2*(T - T_{50}))) \tag{2}$$

$$F = A*(1 - \text{Exp}(-K_1*T))$$
$$\quad + B/(1 + \text{Exp}(-K_2*(T - T_{50}))) \tag{3}$$

where F = fraction of entrapped drug released; A = percent of total drug released during Phase I; K_1 = rate of drug release during Phase I due to diffusion; B = percent of total drug released during Phase III; K_2 = rate of drug release during Phase III due to polymer degradation; T_{50} = time taken to release 50% of entrapped drug.

Eq. (1) is the simple sigmoid equation that describes drug release profiles from microspheres with an initial lag followed by drug release due to matrix degradation. However, this equation fails to account for the drug released during Phase I. The term $A*T$ was included in Eq. (2) to account for zero-order drug release during Phase I and $A*(1 - \text{Exp}(-K_1*T))$ was used in Eq. (3) for explaining first-order exponential release.

Data was fit to all the equations by nonlinear regression analysis with Scientist® program. Best fitting model was chosen based on sum of squares of errors, R^2 value, % CV and residual analysis. Eq. (3) was found to fit the drug release from microspheres better than Eqs. (1) and (2).

3. Results

3.1. Microspheres

The current method employed for preparation of PLGA microspheres of GCV involves minimal use (0.5 ml) of organic solvent. Moreover, percent drug entrapment values ranged from 47% to 72% of the initially added amount. Drug entrapment increased from 47.13% with Resomer RG 502H microspheres to 51.37% with PLGA 6535 microspheres. However, polymer blend microspheres resulted in maximum entrapment efficiency at 72.67% (Table 1). Amount of GCV entrapped/mg of microspheres followed a similar trend as the percent entrapment efficiencies i.e. Resomer RG 502H (50.93 ± 0.78 µg/mg) < PLGA 6535 (56.42 ± 3.27 µg/mg) < PLGA 6535:Resomer RG 502H :: 3 : 1 microspheres (77.39 ± 1.73 µg/mg). Such high entrapment in case of polymer blend could be due to more efficient packing amongst the large and low molecular weight polymers which could result in better GCV entrapment than the individual polymers. The current procedure also results in repro-

Table 1
Values are expressed as mean $\pm$ S.E.M. of at least 3 samples each from 2 batches for percent entrapment efficiency studies

Microsphere	Entrapment efficiency (%)	Particle size (µm)
PLGA 6535	51.37 ± 2.70	229.1 ± 9.42
PLGA 6535:Resomer RG 502H :: 3 : 1	$72.67 \pm 2.49*$	240.0 ± 10.9
Resomer RG 502H	47.13 ± 1.13	220.0 ± 6.23

* Represents significant difference at $p < 0.05$.
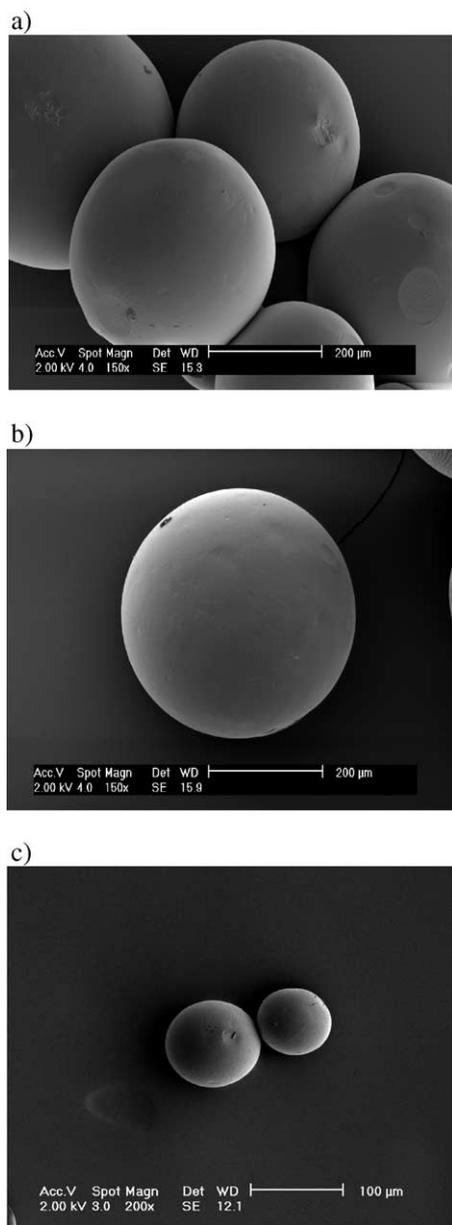
a)

b)

c)

Fig. 2. Scanning electron microscope photographs of GCV loaded microspheres prepared from (a) PLGA 6535, (b) PLGA 6535 : Resomer RG 502H ∷ 3 : 1, and (c) Resomer RG 502H.

ducible results with minimum batch variations in entrapment efficiencies.

Resomer RG 502H microspheres exhibit the highest true density (1.357 g/cm³) and PLGA 6535 microspheres the least (0.99 g/cm³). The blend microspheres

exhibit an intermediate true particle density (1.189 g/cm³). Such a lowering trend in particle true density implies better packing and lesser free volume in the order Resomer RG 502H microspheres > blend microspheres > 6535 microspheres. The particle size of all the batches of microspheres was close and no significant difference was observed among the three groups of microspheres (Table 1). Scanning electron microscopy revealed a smooth and uniform texture for all the microspheres prepared by the solvent evaporation procedure (Fig. 2).

DSC thermograms were processed and T_g values were calculated for both the polymers and microspheres. PLGA 6535 polymer has a T_g value of 44.8 °C whereas Resomer RG 502H exhibits a T_g value of 41.4 °C. However, GCV-loaded microspheres prepared from individual polymers exhibited considerably lower T_g values (PLGA 6535 microspheres: 32.1 °C; Resomer RG 502H microspheres: 24.9 °C) and the blend microspheres exhibit a single T_g at 32.9 °C, close to PLGA 6535 microspheres.

In vitro drug release studies indicate triphasic drug release from PLGA microspheres. It is evident from Figs. 1 and 3 that GCV release from PLGA 6535,
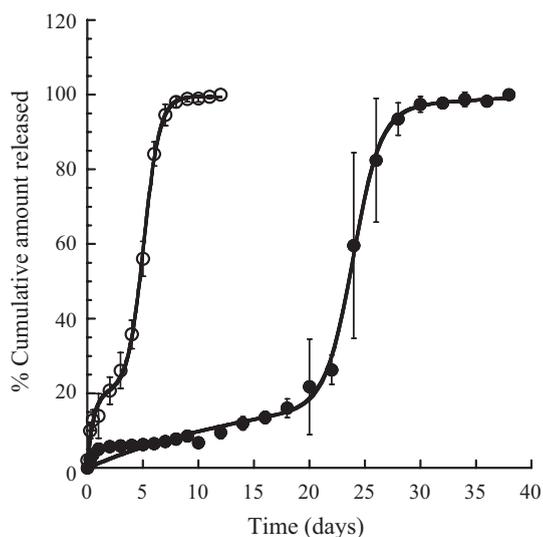

Fig. 3. In vitro release of GCV from PLGA 6535 : Resomer RG 502H ∷ 3 : 1 and Resomer RG 502H microspheres. (●) GCV release from PLGA 6535 : Resomer RG 502H ∷ 3 : 1 microspheres and (○) Resomer RG 502H microspheres. Lines drawn represent least squares fit of the data to Eq. (3) by nonlinear regression analysis (n = 3–4/batch).

Resomer RG 502H and blend microspheres follow the triphasic release pattern in vitro. All the release profiles were found to fit Eq. (3) better than Eqs. (1) and (2). The R^2 values for all the fits were greater than 0.99. Moreover, F-test, % CV and residual analysis indicate Eq. (3) as the best-fit model to the experimentally generated data. Estimated release parameters are listed in Table 2. The total amounts of drug released during Phase I (A) for all the batches were found to be about 21–25% of the total entrapped drug. However, the release rate constants decreased from 0.191 ± 0.040 day^{-1} for PLGA 6535 to 0.0735 ± 0.015 day^{-1} for polymer blend microspheres. Resomer RG 502H exhibited the highest rate constant (2.05 ± 0.28 day^{-1}). Moreover, drug release rate during the Phase I ($A*K_1$) also followed a similar trend i.e., release rates were highest for Resomer RG 502H microspheres (43.7% day^{-1}), followed by PLGA 6535 (4.83% day^{-1}) and lowest rates were observed for PLGA 6535: Resomer RG 502H blend microspheres (1.83% day^{-1}). Such a decrease in both K_1 and $A*K_1$ values for the blend microspheres could be due to the efficient packing between the large and low molecular weight polymers in the microsphere matrix.

Table 2

Drug release parameters estimated from nonlinear regression fit of GCV release data from PLGA microspheres to Eq. (3) during Phases I, II and III

a) Phase I

Microspheres	A (% rel)	K_1 (day^{-1})	$A*K_1$ (% rel day^{-1})
PLGA 6535	25.45 ± 4.42	0.191 ± 0.040	4.83
PLGA 6535: Resomer RG 502H :: 3 : 1	22.56 ± 3.50	0.0735 ± 0.015*	1.64
Resomer RG 502H	21.31 ± 1.31	2.05 ± 0.28*	43.7

b) Phases II and III

Microspheres	B (%)	K_2 (day^{-1})	T_{50} (days)
PLGA 6535	75.50 ± 5.86	0.27 ± 0.028*	50.56 ± 0.66*
PLGA 6535: Resomer RG 502H :: 3 : 1	80.86 ± 1.23	1.14 ± 0.19	24.22 ± 0.56*
Resomer RG 502H	78.25 ± 1.28	1.46 ± 0.084	5.090 ± 0.053*

* Represents significant difference at $p < 0.05$.

The B values in Table 2b show no significant differences indicating equal amounts of drug release during phase (Phase III) accounting for 75–85% of the entrapped drug. Rate constant of GCV release during Phase III (K_2) is significantly lower for PLGA 6535 (0.27 ± 0.028 day^{-1}) relative to PLGA 6535 : Resomer RG 502H :: 3 : 1 polymer blend (1.14 ± 0.19 day^{-1}) and Resomer RG 502H (1.46 ± 0.084 day^{-1}). Previous studies have shown that PLGA polymers with higher lactide content degrade at slower rates as compared to polymers with lower lactide content. Similar observations were noted where high molecular weight PLGA molecules degraded at slower rates than the lower molecular weight PLGA molecules. As PLGA 6535 has higher lactide content and molecular weight than Resomer RG 502H, it can release GCV at a relatively slower rate than Resomer RG 502H microspheres during Phase III. However, the blend microspheres release GCV at a rate similar to Resomer RG 502H microspheres (1.14 day^{-1} vs. 1.46 day^{-1}). This observation indicates that drug release during Phase III from the blend microspheres is controlled by degradation of the Resomer RG 502H component in the matrix rather than by the PLGA 6535 component.

T_{50}, time taken to release 50% of entrapped drug, is an index of matrix hydration time i.e. approximate duration of Phase II. T_{50} values in Table 2b appear to indicate longer hydration times for the higher molecular weight PLGA 6535 microspheres (50.56 ± 0.66 days) and shorter durations for lower molecular weight Resomer RG 502H microspheres (5.09 ± 0.05 days). Blend microspheres exhibit an intermediate hydration time (24.22 ± 0.56 days), due to the presence of Resomer RG 502H in the matrix.

3.2. Gel formulation

PLGA–PEG–PLGA triblock copolymer was obtained in good yields. Number average molecular weight (M_n^a) calculated from NMR spectra by end group analysis was found out to be 4203 Da. Weight average molecular weight (M_w^b) as determined by gel permeation chromatography was 4759 Da and corresponding number average molecular weight (M_n^b) was 4348 Da. The synthesized polymer had a polydispersity index (M_w^b/M_n^b) of 1.09.

Phase transition studies reveal that 20–25% w/v aqueous solution of the polymer convert to gel phase

at 32 °C and remain in gel state till 60 °C (Fig. 4). Thus a 23% w/v solution of gel was employed for further studies. GCV release studies from the gel indicate that polymer structure may not control GCV release in vitro (data not shown). Total drug (350 μg) was released within 3–6 h suggesting minimal control of the gel over GCV diffusion into the release medium. However, with microspheres dispersed in gel, GCV release could be controlled by the polymer makeup of microspheres suspended in the gel. GCV release studies with micro- spheres suspended in PLGA–PEG–PLGA solutions were performed for Resomer RG 502H and PLGA 6535:Resomer RG 502H :: 3:1 blend microspheres only. PLGA 6535 microspheres were excluded from these studies since their in vitro release characteristics exhibit a relatively long Phase II with very little or no drug release from these particles. Formulations from such microspheres could result in subtherapeutic drug levels and possible development of drug resistance.

Drug release studies with PLGA microspheres sus- pended in PLGA–PEG–PLGA gel reveal a release retarding effect during the Phase I and no effect on Phases II and III of drug release from microspheres. Data fitting to the proposed models reveal that PLGA 6535:Resomer RG 502H :: 3:1 blend microspheres dispersed in gel follow an initial zero-order release (Eq. (2)) for the first 20 days (Fig. 5) as compared to the exponential release pattern observed with PLGA 6535:Resomer RG 502H :: 3:1 blend micro- spheres alone. Although the exponential model still fits the drug release data from Resomer RG 502H microspheres dispersed in gel, the rate constant (K_1) for drug release during Phase I reduced from
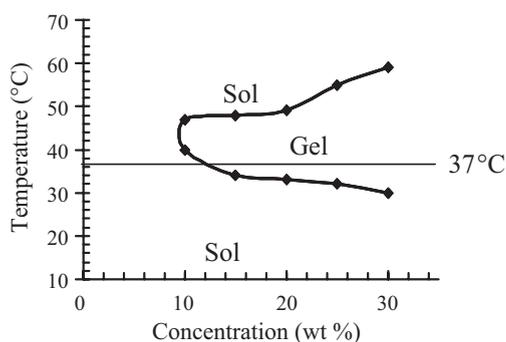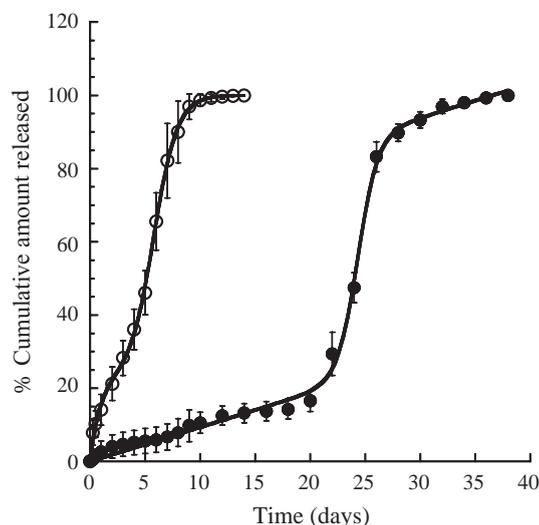

Fig. 5. In vitro release of GCV from PLGA 6535:Resomer RG 502H :: 3:1 and Resomer RG 502H microspheres-based formula- tions. (●) GCV release from PLGA 6535:Resomer RG 502H :: 3:1 formulation and (○) represent Resomer RG 502H formulation. Lines drawn represent least squares fit of the data to Eq. (3) (Resomer RG 502H) and Eq. (2) (PLGA 6535:Resomer RG 502H :: 3:1) by nonlinear regression analysis ($n = 3–4$/batch).

2.05 ± 0.28 day^{-1} to 1.10 ± 0.31 day^{-1}. No signifi- cant differences were observed between B, K_2 and T_{50} values between the microspheres and formulations suggesting little or no effect of gel on Phases II and III (Table 3).

4. Discussion

Drug release equations that can describe various phases of GCV release from PLGA microspheres have been developed and the effects of polymer blend- ing on entrapment and release of GCV from PLGA microspheres investigated. Entrapment efficiency, par- ticle size and surface morphology studies show that the physical characteristics of the GCV microspheres are similar to acyclovir, a structural analogue of GCV, reported by other investigators [14]. Moreover, poly- mer blending increases the amount of GCV entrapped in the PLGA microspheres (Table 1). We hypothesize that such an increase could be due to the closer packing between the large molecular weight PLGA 6535 (45,000–55,000 Da) and the small molecular weight Resomer RG 502H (8000 Da). A probable reason for

Fig. 4. Effect of polymer concentration on transition temperature of aqueous solutions of PLGA–PEG–PLGA.

Table 3
Effect of microsphere dispersion in PLGA–PEG–PLGA gel on GCV release from PLGA 6535 : Resomer RG 502H :: 3 : 1 and Resomer RG 502H microspheres

	A (%)	K_1 (day^{-1})	B (%)	K_2 (day^{-1})	T_{50} (days)
a) PLGA 6535 : Resomer RG 502H :: 3 : 1 microspheres					
PLGA 6535 : Resomer RG 502H :: 3 : 1 formulation[a]	0.934 ± 0.084	ND	65.81 ± 2.74	1.14 ± 0.068	24.04 ± 0.073
PLGA 6535 : Resomer RG 502H :: 3 : 1 microsphere	22.56 ± 3.50	0.073 ± 0.015	80.86 ± 1.23	1.14 ± 0.19	24.22 ± 0.56
b) Resomer RG 502H microspheres					
Resomer RG 502H formulation	28.04 ± 5.35	1.10 ± 0.31	73.06 ± 5.40	1.12 ± 0.20	5.88 ± 0.17
Resomer RG 502H microsphere	21.31 ± 1.31	$2.05 \pm 0.28*$	78.25 ± 1.28	1.46 ± 0.084	5.09 ± 0.05

[a] Data fits to Eq. (2).

 * Represents significant difference at $p < 0.05$.

low entrapments by aqueous based solvent evaporation technique could be due to the slow leakage of GCV into the dispersion medium during the microsphere hardening phase. During this stage, the polymer matrix is not efficiently packed due to the presence of organic phase and GCV can easily diffuse through the spaces between the polymer chains into the dispersion medium. However, in blend microspheres, the low molecular weight polymers, due to their smaller size, can fit within the spaces of large molecular weight polymer chains and reduce leakage causing greater entrapment efficiencies. Moreover, the amounts of GCV entrapped/mg of microsphere are high for all the microspheres, with the maximum in PLGA 6535 : Resomer RG 502H :: 3 : 1 blend (77.39 μg/mg).

True particle densities of the microspheres measured by helium pycnometry also reinforce the phenomenon of better packing in blend microspheres. Resomer RG 502H molecules being smaller in size (Mw = 8000 Da), tend to pack better in the microsphere matrix relative to the large PLGA 6535 molecules (Mw = 45,000–55,000 Da). Resomer RG 502H microspheres were the most dense among all three types of microspheres (1.357 g/cm^3). They are followed by blend microspheres (1.189 g/cm^3) with PLGA 6535 microspheres (0.99 g/cm^3) being the least dense. A better packing of the matrix results in greater matrix mass per unit volume, density. Therefore, blend microspheres with intermediate particle density appear to produce better polymer packing between PLGA 6535 and Resomer RG 502H molecules relative to PLGA 6535 alone.

Glass transition temperatures (T_g) for GCV loaded microspheres were considerably lower relative to pure polymers. A decrease in T_g could be due to the plasticizing effect of the entrapped drug or traces of water present in the microsphere matrix. PLGA 6535 microspheres exhibited a higher T_g (32.1 °C) relative to Resomer RG 502H microspheres (24.9 °C). Increase in T_g with polymer molecular weight has been reported previously [21]. The blend microspheres exhibited a single inflection in DSC thermograms revealing the miscible nature of the polymers. However, T_g of the blend microspheres was 32.9 °C, outside the range of 24.9 °C to 32.1 °C. Therefore, blend microspheres require temperatures similar to PLGA 6535 for transitioning into rubbery state from glassy state despite the presence of a lower T_g polymer in the matrix. Such relatively higher than expected T_g for blend microspheres suggests stronger binding between PLGA 6535 and Resomer RG 502H molecules in the microsphere matrices.

In vitro drug release profiles from the PLGA microspheres of GCV fit to Eq. (3) better than Eqs. (1) and (2) (R^2 values greater than 0.99 for Eq. (3)). The release parameters estimated (Table 2) by non-linear regression of the data reveal that as the molecular weight is ascended, rate of release from Phase I decreases (4.83% day^{-1} for PLGA 6535 vs. 43.7% day^{-1} for Resomer RG 502H). This could be due to the greater hydrophilicity and rapid diffusion of GCV through Resomer RG 502H relative to PLGA 6535. However, the microspheres prepared from polymer blend exhibit a significantly slower initial release rate (1.64% day^{-1}). The release rate constants for Phase I (K_1) decrease for the blend microspheres as compared to individual PLGA 6535 and Resomer RG 502H microspheres. The rate of GCV release during Phase I is dependent on the spaces in the microsphere matrix through which GCV slowly diffuses into the

dissolution medium. Low rates of GCV release during Phase I suggest lesser number of spaces in the microsphere matrix for the blend microsphere. Thus, the hypothesis that PLGA polymers of disparate molecular weights tend to pack more efficiently is consistent with entrapment, densitometry, DSC and in vitro drug release results.

As discussed previously T_{50} indicates a lag time or an index of matrix hydration time. A greater T_{50} value means longer hydration time and vice versa. In vitro degradation studies with PLGA have shown that as lactide content and molecular weight increase, initiation time for the polymer degradation is longer [22]. As increase in lactide content and molecular weight of PLGA polymers increase the lipophilicity of the molecule, longer hydration periods are required for such molecules before the onset of bulk degradation. Consecutively, PLGA 6535 microspheres may require the longest time to hydrate, Resomer RG 502H microspheres the least and the blend microspheres a value between the two. Results in Table 2b suggest that matrix hydration times decreased with lower molecular weight microspheres. PLGA 6535 microsphere has T_{50} of 50.56 ± 0.66 days whereas Resomer RG 502H microspheres exhibited a T_{50} of 5.09 ± 0.053 days. The blend microspheres exhibited an intermediate T_{50} of 24.22 ± 0.56 days. Resomer RG 502H probably renders the matrix more hydrophilic than PLGA 6535 alone.

Drug release during Phase III is governed by both the rate of matrix bulk degradation and GCV diffusion. In vitro polymer degradation data reported by Wu and Wang [22] suggests that drug degradation diminishes with elevation in lactide content of the PLGA polymer. PLGA 6535 appears to degrade at a slower rate relative to Resomer RG 502H. K_2 values in Table 2b follow the reported trend. During the degradation phase (Phase III), rate constant of drug release is lowest for PLGA 6535 (0.27 ± 0.028 day^{-1}) and highest for Resomer RG 502H (1.46 ± 0.084 day^{-1}) with the intermediate release rate for blend microspheres (1.14 ± 0.19 day^{-1}). The rate constant for blend microspheres is not significantly different from Resomer RG 502H microspheres. As Resomer RG 502H degrades at a faster rate than PLGA 6535, in blend microspheres, it could create spaces in the matrix through which GCV is released into the dissolution medium faster relative to PLGA 6535. Thus

drug release during Phase III is primarily governed by Resomer RG 502H content in the blend microspheres.

After complete characterization of GCV release from microspheres, effect of microsphere dispersion into PLGA–PEG–PLGA solution was investigated. These studies show that the gel is not effective for sustained delivery of GCV for durations longer than 2–4 h. Therefore, drug loaded microspheres suspended in a gel can be an effective alternative. Release studies with microspheres suspended in the PLGA–PEG–PLGA gel indicate that the gel structure probably controls GCV release during Phase I (Table 3). However, the duration and extent of phases II and III are not affected by dispersion into the gel. Drug release rate constant during Phase I (K_1) for individual Resomer RG 502H microspheres is higher than the value obtained for same microspheres suspended in gel (Table 3b). This observation indicates that the gel retards GCV diffusion into the release buffer. Such decrease in drug release rate constants could be due to diffusional resistance of the gel due to its semisolid nature or association of the PLGA blocks in the triblock gel with the PLGA microspheres effectively increasing the thickness of diffusion layer for GCV before it is released into the aqueous buffer. Further studies are required to elucidate the exact mechanism involved in retarding GCV release from PLGA microspheres suspended in PLGA–PEG–PLGA gel. The blend microspheres exhibited a linear zero-order release behavior for 20 days from the gel as compared to exponential release in the absence of gel (Fig. 5). Therefore, suspension of microspheres inside such gels could effectively slow down the initial burst phase and may result in more controlled release patterns. Therapeutic amounts of GCV can be delivered from 5 mg of PLGA 6535 : Resomer RG 502H :: 3 : 1 microspheres suspended in 100 μl of polymer solution and its release can be controlled over 4–5 weeks.

Ocular administration of microspheres suspended in PLGA–PEG–PLGA gel can result in instantaneous gelation of formulation at the site of administration resulting in minimal particle migration from the site of administration with no vision interference. Entrapped in the gel structure, the microspheres will be held in place at the site of administration and release drug continuously over prolonged periods in a controlled manner. Moreover, the gel matrix may also offer valuable protection to the microspheres from enzy-

matic and cellular degradation following in vivo administration.

5. Conclusion

Model equations have been developed that can effectively describe all the three phases of GCV release from PLGA microspheres, in vitro. Mechanism of GCV release from PLGA microspheres has been investigated in two PLGA grades and utility of polymer blending strategy demonstrated. A novel formulation containing GCV loaded PLGA microspheres dispersed in PLGA–PEG–PLGA gel has been prepared and investigated for its utility in drug delivery. The formulation can be used to deliver GCV to the vitreous and retina/choroid following intravitreal administration continuously over 4–5 weeks. Following completion of drug release, the formulation can be re-administered by simple injection without the need for implant removal because of its biodegradable nature. These studies also suggest that sustained delivery formulations of GCV can be tailor made by polymer blending strategy.

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