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Research Paper

Effect of Polymer Blending on the Release of Ganciclovir from PLGA Microspheres

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Purpose. The aim of the study is to investigate the effect of polymer blending on entrapment and release of ganciclovir (GCV) from poly(D,L-lactide-co-glycolide) (PLGA) microspheres using a set of empirical equations.

Methods. Two grades of PLGA, PLGA 7525 [D,L-lactide:glycolide(75:25), MW 90,000–126,000 Da] and Resomer RG 502H [D,L-lactide:glycolide(50:50), MW 8000 Da], were employed in the preparation of PLGA microspheres. Five sets of microsphere batches were prepared with two pure polymers and their 1:3, 1:1, and 3:1 blends. Drug entrapment, surface morphology, particle size analysis, drug release, and differential scanning calorimetric studies were performed. *In vitro* drug-release data were fitted to a set of empirical sigmoidal equations by nonlinear regression analysis that could effectively predict various parameters that characterize both diffusion and degradation cum diffusion-controlled release phases of GCV.

Results. Entrapment efficiencies of GCV ranged from 47 to 73%. Higher amounts of GCV were entrapped in polymer blend microspheres relative to individual polymers. Triphasic GCV release profiles were observed, which consisted of both diffusion and degradation cum diffusion-controlled phases. *In vitro* GCV release was shortest for Resomer RG 502H microsphere (10 days) and longest for PLGA 7525 microspheres (90 days). Upon blending, the duration of release gradually decreased as the content of Resomer RG 502H in the matrix was raised. Equations effectively estimated the drug-release rate constants during both the phases with high R^2 values (>0.990). GCV release was slower from the blend microsphere during the initial diffusion phase. Majority of entrapped drug (70–95%) was released during the matrix degradation cum diffusion phase.

Conclusions. Drug entrapment and release parameters estimated by the equations indicate more efficient matrix packing between PLGA 7525 and Resomer RG 502H in polymer-blended microspheres. The overall duration of drug release diminishes with rising content of Resomer RG 502H in the matrix. Differential scanning calorimetry studies indicate stronger binding between the polymers in the PLGA 7525/Resomer RG 502H:: 3:1 blend. Polymer blending can effectively alter drug-release rates of controlled delivery systems in the absence of any additives.

KEY WORDS: controlled release; drug-release equations; ganciclovir; polymer blending; triphasic drug release.

INTRODUCTION

Controlled delivery of drugs via PLGA polymers as implants, microspheres, and nanoparticles has gained wide acceptance. Availability of a variety of PLGA polymers makes it easy for pharmaceutical scientists to custom-develop a sustained release formulation based on the disease state and the drug indicated. An ideal controlled release formulation should release the entrapped drugs in a continuous manner over desired time periods. Drug release occurs from PLGA microspheres because of drug diffusion from both the intact and degrading microsphere matrix (1–3). However, the extent of drug release during each phase and time lag between the two phases could vary considerably among the PLGA polymers possibly because of differences in their lactide/glycolide ratios and molecular weights.

In vitro release of protein/peptide and hydrophilic drugs from PLGA matrices is known to occur in two or three phases (3,4). An initial phase (phase I) consists of drug release from microspheres because of diffusion from surface-bound and poorly encapsulated drug. A relatively slow-releasing second phase (phase II) is generally attributed to binding of drug molecules to the polymer in case of peptides (3,5). Rapid drug release during the third phase is commonly attributed to the faster drug diffusion from the eroding matrix. All three phases are usually not evident for lipophilic drugs, as these molecules can diffuse efficiently across the intact polymer resulting in the loss of phase II. However, the second phase becomes evident for hydrophilic drugs that exhibit limited or no diffusivity across the PLGA matrix. The

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Table I. Composition of Microspheres

Name	GCV content (mg)	PLGA 7525 (mg)	Resomer RG 502H (mg)
MS 1	20	200	–
MS 2	20	150	50
MS 3	20	100	100
MS 4	20	50	150
MS 5	20	–	200

GCV = Ganciclovir; PLGA = poly(D,L-lactide-co-glycolide).

length of phase II can be critical in case of antimicrobial and antiviral drugs, where maintenance of drug levels above minimal inhibitory concentration (MIC) during a dosing interval is very critical for disease management.

Release-modifying agents such as ethylene glycols, isopropyl myristate, and Tweens have been incorporated into microspheres to induce constant release of a hydrophilic drug such as acyclovir from microspheres (6,7). For microspheres intended for local implantation, addition of such agents could result in potential tissue sensitivity. A strategy that can successfully be adopted to modulate drug release without the need for additives is polymer blending. Blending of two PLGA polymers with different molecular weights and lactide/glycolide ratio has been previously studied for modifying drug release (4,5,8–10). Despite some success of this approach, a thorough investigation into the effect of polymer blending on entrapment and release of hydrophilic drug from microspheres has never been attempted. Ganciclovir (GCV) is a hydrophilic molecule indicated in the treatment of cytomegalovirus retinitis. It is a nucleoside analog, structurally similar to acyclovir.

The aim of this project is to study the release of GCV, a model drug, from microspheres prepared by blending two PLGA molecules, PLGA 7525 [D,L-lactide:glycolide (75:25), MW 90,000–126,000 Da] and Resomer RG 502H [D,L-lactide:glycolide (50:50), MW 8000 Da], in various ratios. Microspheres are prepared from three different blends, i.e., PLGA 7525/Resomer RG 502H: : 1:3, 1:1, and 3:1. Components of each batch are given in Table I. Nomenclature given in the table is followed in the rest of the article. Drug-release studies have been performed, and data were fitted to a set of empirical equations that effectively characterize all three phases simultaneously. These equations could provide better understanding of various mechanisms involved in GCV release from PLGA microspheres. Such mathematical modeling can also delineate the effect of polymer blending on drug release.

MATERIALS AND METHODS

Materials

Ganciclovir was a generous gift from Hoffman La Roche (Nutley, NJ, USA). PLGA polymers, i.e., PLGA 7525 [D,L-lactide:glycolide (75:25), MW 90,000–126,000 Da] and polyvinyl alcohol (PVA, 30,000–70,000 Da), were purchased from Sigma Chemicals (St. Louis, MO, USA). Resomer RG 502H [D,L-lactide:glycolide (50:50), MW 8000 Da] was obtained from Boehringer Ingelheim (Ingelheim, Germany). All other solvents and chemicals were procured from Fischer Scientific (USA) and were used without further purification.

Methods

Preparation and Characterization of Microspheres

Preparation of Microspheres. Ganciclovir microspheres were prepared by solvent evaporation method. GCV (20 mg) was suspended in 0.5 ml of methylene chloride and sonicated for 30 min in a bath sonicator (50/60 Hz; 125 W). After sonication, 200-mg polymer was added, and the mixture was further sonicated for 30 min with occasional vortexing to insure 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 primary emulsion was then added slowly to 2.5% PVA solution (150 ml) dropwise with continuous stirring at a constant speed (250–300 rpm) for 3 h at room temperature for complete evaporation of the organic solvent. The resulting microspheres were then filtered and washed with distilled deionized water and air-dried for minimizing residual methylene chloride. Finally, the microspheres were stored over anhydrous $CaSO_4$ at 4°C. Microspheres were prepared from PLGA 7525, Resomer RG 502H, and PLGA 7525/Resomer RG 502H: :3:1; 1:1 and 1:3 polymer blends.

Entrapment Efficiency. Accurately weighed samples (5 mg) of microspheres 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 deionized water. Samples were subsequently analyzed by a method described in "HPLC Method of Analysis". Studies were conducted in triplicates from two batches.

Microsphere Size and Surface Morphology. Surface morphology of the microspheres was studied with scanning electron microscopy (SEM; FEG ESEM XL 30, FEI, Hillsboro, OR, USA). Microspheres were attached to a double-sided tape and were spray-coated with gold palladium at 0.6 kV prior to inspection under electron microscope.

Size of microspheres was measured microscopically (Carl Zeiss, Germany) with the aid of a stage and an eye piece micrometer. Size of at least 200 particles was measured from each batch.

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 were placed in a shaker at 37°C with a constant agitation of 60 oscillations/min. One-milliliter samples were withdrawn at appropriate time intervals and were replaced with equal volumes of fresh release buffer. Samples were analyzed by a high-performance liquid chromatography (HPLC) method as described below. Studies were conducted in triplicate from two batches except for PLGA 7525 microspheres, where studies were conducted from a single batch.

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

Differential Scanning Calorimetry. Glass transition temperatures of the polymers and drug-loaded microspheres were measured with a Thermal Analysis Q1000 differential scanning calorimeter (Thermal Analysis Instruments, New Castle, DE, USA). Samples of 10–15 mg 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.

HPLC Method of Analysis

High-performance liquid chromatography system (Waters 600 pump; Waters, Milford, MA, USA), equipped with a fluorescence detector (HP1100, Hewlett Packard, Waldbronn, Germany) and a reversed-phase C_{12} column (4 μm, 250 × 4.6 mm, Synergy-max, Phenomenex, Torrance, CA, USA), 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 at an emission wavelength of 380 nm. Limit of quantification was 50 ng/ml for GCV.

Data Analysis

All experiments were carried out with three samples taken from two different batches unless specified. Data are presented as mean ± standard error of the mean. Statistical significance was determined by ANOVA and Student's t test at $p < 0.05$.

Theory

Ganciclovir release from PLGA microspheres occurs in three distinct phases (11). Phase I is the initial diffusion phase where release from PLGA microspheres occurs by slow diffusion of the drug from the microsphere matrix. Such diffusion occurs from both surface-bound drug and poorly encapsulated drug through the cracks and pores in the polymeric matrix. The extent and duration of this period may depend on the drug distribution in the matrix and the efficiency polymer packing during the microsphere-hardening stage. This phase is followed by a very slow and minimal release phase (phase II). Water uptake studies by Bodmer *et al.* (3) reveal a sudden increase of water content in PLGA matrices following a period of "modest" water uptake. Such rapid water uptake was associated with sudden mass loss and fragmentation of the matrix, which resulted in the rapid release of encapsulated protein (phase III). Moreover, occurrence of phase II could also be a result of the inability of the drug to diffuse out of the PLGA matrix until the

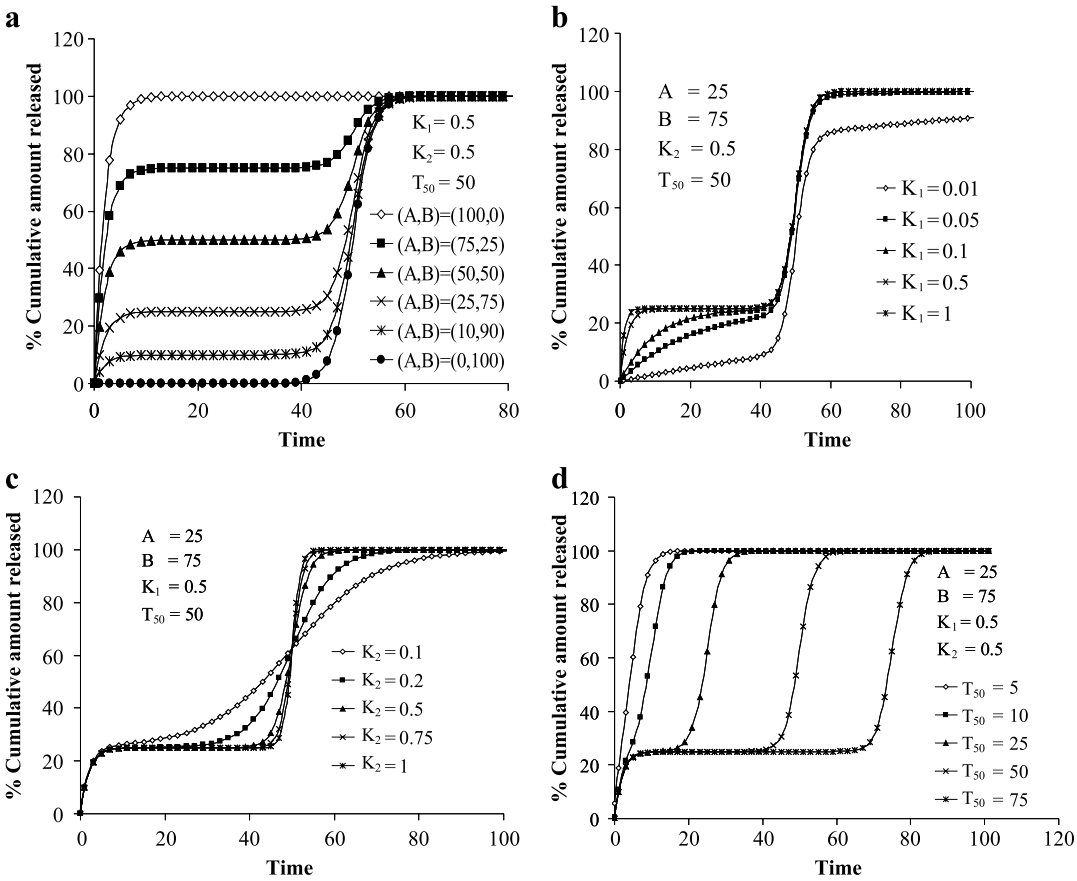


Fig. 1. Effect of A and B (a), K_1 (b), K_2 (c), and T_{50} (d) on drug release profiles simulated from Eq. (3).

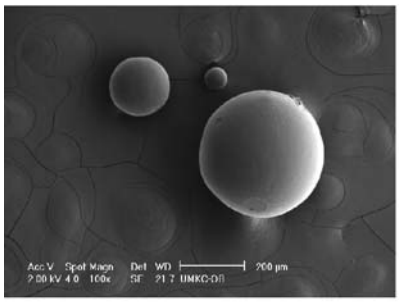
Table II. GCV Entrapment and Particle Size

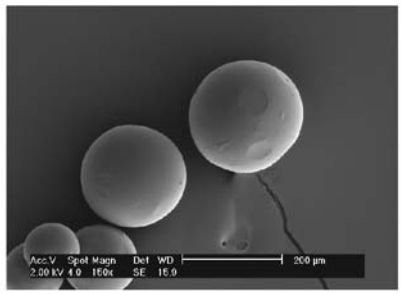
Microsphere	Percentage of entrapment efficiency	Particle size (μm)
PLGA 7525 (MS 1)	51.21±1.36[a]	158.29±6.63
PLGA 7525/Resomer RG 502H: :3:1 (MS 2)	72.54±2.92[a]	178.12±7.85
PLGA 7525/Resomer RG 502H: :1:1 (MS 3)	62.47±2.49	168±6.14
PLGA 7525/Resomer RG 502H: :1:3 (MS 4)	73.26±2.36	197.85±8.68
Resomer RG 502H (MS 5)	47.13±1.13	220.01±6.23

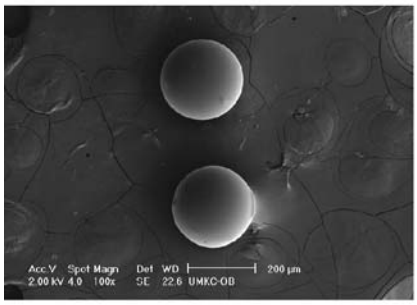
Values are expressed as mean ± SEM (n = 3/batch).
[a] Represents significant difference at $p < 0.05$.

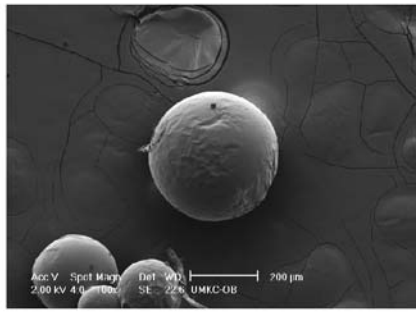
molecular weight of the polymer has decreased to a certain extent because of *in vitro* hydrolysis. Drug release during phase III is primarily determined by the rate of drug diffusion from the matrix because of degradation. As the ester bonds in the PLGA molecule hydrolyze, molecular weight of the polymer descends, which widens the gaps in the matrix, making it easier for drug molecules to diffuse into the dissolution medium. The rate of drug release is determined primarily by the matrix hydrolysis rate and the ability of the drug to diffuse through the generated spaces. PLGA molecules with higher lactide content degrade at a slower rate than lower lactide content polymers (12). Thus, microspheres with higher lactide content may release GCV at a slower rate relative to PLGA molecules with lower lactide content. Onset of phase III is reported to be associated with rapid increase in water uptake by the matrix and a sudden mass loss (3).

Various equations have been employed to describe the release of entrapped drugs from microspheres (13–15). Modeling *in vitro* cumulative drug-release profiles on a mechanistic basis has been reported by various researchers (16–18). However, an empirical approach is adopted in this report where equations are developed on the basis of the cumulative drug-release profiles without any mechanistic basis. The use of such equations could result in easy comparison of duration and extent of each of the phases among various formulations. Such comparisons are important for delineating the exact effect of both drug- and polymer-linked parameters on overall drug release.


PLGA 7525


PLGA 7525: Resomer 502H:: 3:1


PLGA 7525: Resomer 502H:: 1:1


PLGA 7525: Resomer 502H:: 1:3

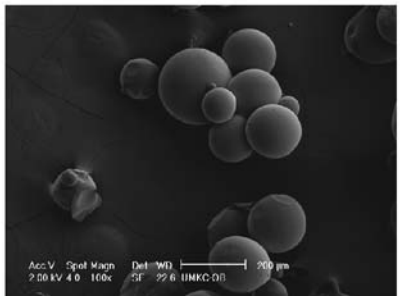

Resomer 502H

Fig. 2. Scanning electron microscope photographs of microspheres.

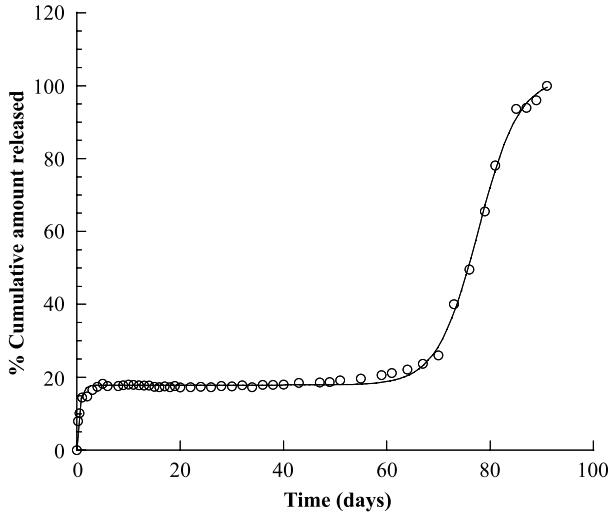


Fig. 3. Cumulative amount released *vs.* time profile of ganciclovir (GCV) from poly(D,L-lactide-co-glycolide) (PLGA) 7525 (MS 1) microspheres. Lines drawn represent nonlinear regression fit of the data to Eq. (3) by least-squares error method.

Sigmoidal equations have been applied previously to characterize drug release from PLGA microspheres (15). A simple sigmoidal equation does not completely describe the different phases in a typical release profile. To model the observed drug-release patterns, we modified the equation to fit the experimental data better than the simple sigmoidal equation. The equations used to fit the data are

$$F = B/\{1 + \exp[-K_2*(T - T_{50})]\} \quad (1)$$

$$F = A + B/\{1 + \exp[-K_2*(T - T_{50})]\} \quad (2)$$

$$F = A[1 - \exp(-K_1*T)] + B/\{1 + \exp[-K_2*(T - T_{50})]\} \quad (3)$$

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

Equation (1) is the simple sigmoid equation previously employed to first describe drug release from microspheres. However, this equation fails to effectively describe drug release during phase I. The term A was included in Eq. (2) to account for the burst release during phase I, and $A[1 - \exp(-K_1T)]$ was added in Eq. (3) to describe exponential drug release during phase I.

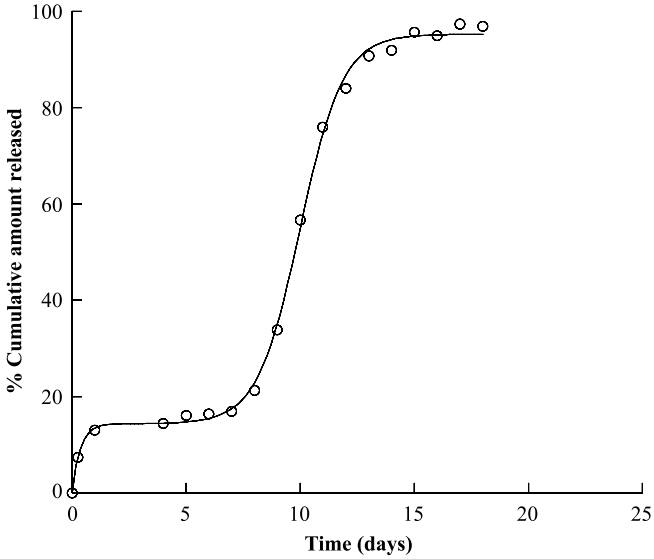


Fig. 5. Cumulative amount released *vs.* time profile of GCV from PLGA 7525/Resomer RG 502H: :1:1 (MS 3) microspheres. Lines drawn represent nonlinear regression fit of the data to Eq. (3) by least-squares error method.

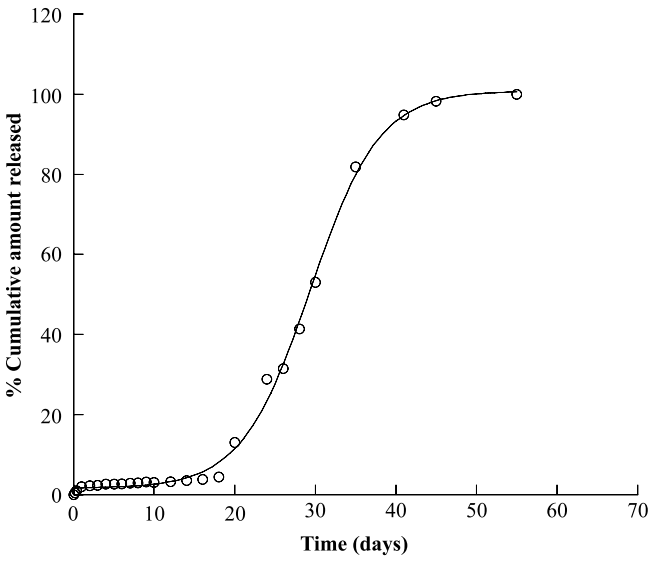


Fig. 4. Cumulative amount released *vs.* time profile of GCV from PLGA 7525/Resomer RG 502H: :3:1 (MS 2) microspheres. Lines drawn represent nonlinear regression fit of the data to Eq. (2) by least-squares error method.

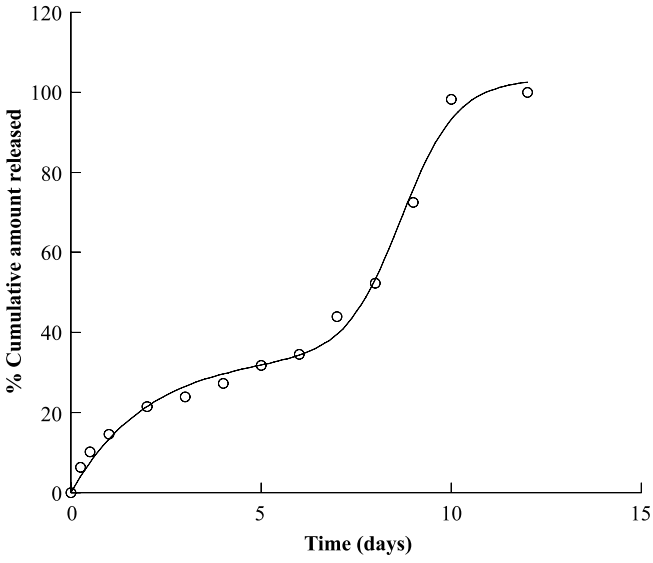


Fig. 6. Cumulative amount released *vs.* time profile of GCV from PLGA 7525/Resomer RG 502H: :1:3 (MS 4) microspheres. Lines drawn represent nonlinear regression fit of the data to Eq. (3) by least-squares error method.

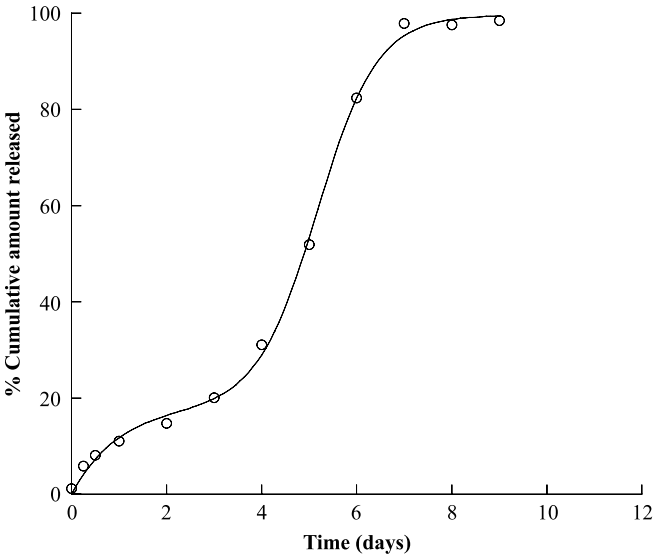


Fig. 7. Cumulative amount released *vs.* time profile of GCV from Resomer RG 502H (MS 5) microspheres. Lines drawn represent nonlinear regression fit of the data to Eq. (3) by least-squares error method.

In Eq. (3), A denotes the percentage of entrapped amount released during phase I, and K_1 is the release rate constant. The reciprocal of K_1 is an index of phase I duration. The product (AK_1) is the drug-release rate during phase I. B accounts for the percentage of entrapped drug released during phase III, and K_2 is the rate constant associated with drug release because of matrix degradation cum drug diffusion. T_{50}, i.e., time taken to release 50% of the entrapped drug, is an index of phase II duration. As the value of T_{50} rises, time taken for the onset of phase III also becomes longer. To delineate the effect of each parameter on overall drug diffusion from microspheres, drug-release profiles were simulated (Fig. 1). Simulation experiments reveal that equation-predicted profiles vary in an ordered manner with the values of the individual parameters. As the value of A was ascended from 0 to 100, the extent of phase I in Fig. 1a became extended proportionally. Increments in values of K_1 and K_2 resulted in greater drug release during phases I and III, respectively (Fig. 1b and c). Similar behavior was also observed with T_{50} (Fig. 1d). These simulations clearly suggest the utility of the proposed equations in characterizing drug release from PLGA microspheres.

RESULTS

Poly(D,L-lactide-co-glycolide) microspheres containing GCV were prepared by solvent evaporation method. The major advantage of the method is that a very low amount (0.5 ml) of methylene chloride is required for the preparation of microspheres. Drug entrapment amounts are tabulated in Table II. Entrapment efficiencies were least for MS 5 (47.13%) and highest for MS 4 (73.26%). It is interesting to note that the percent entrapment values for all the polymer-blended batches (MS 2, MS 3, and MS 4) are significantly higher than both the parent polymer batches (MS 1 and MS 5). Mean particle sizes of the microspheres are in the range of 150–225 μm (Table II). No significant differences were observed with particle sizes and size ranges of all the batches. About 60–70% of the particles in all batches lie in the size range of 100–300 μm. Mean particle sizes of the microspheres prepared from polymer blends lie in between the mean particle sizes of microspheres prepared from the parent polymers (MS 1 and MS 5). PLGA microspheres of GCV with such particle sizes were previously evaluated *in vivo* for their ability in controlling disease progression (19). Surface morphology points toward the formation of microspheres with near perfect spherical nature and uniform texture (Fig. 2).

In vitro drug-release studies with all the batches reveal the triphasic release profiles with varying durations of phases I, II, and III. *In vitro* drug-release data were fitted to Eq. (3) for all microsphere batches except MS 2 for which Eq. (2) generated better correlation than Eqs. (1) and (3) (Figs. 3–7). Equation (1) was inadequate in describing all the drug-release profiles. Release parameters were estimated by fitting the experimental data to the equations by nonlinear regression analysis. R^2 values for all the proposed models were greater than 0.990. Release parameters for phase I (A and K_1) have been summarized in Table III and phase II (T_{50}) and phase III (B and K_2) in Table IV.

Release parameters for phase I are A (percentage of entrapped drug released during phase I) and K_1 (rate constant of drug release). As previously discussed, the product AK_1 is the rate of drug released during the initial diffusion phase (phase I). Drug-release rate constant K_1 was 1.42 ± 0.17 day^{-1} for MS 1 and 2.05 ± 0.28 day^{-1} for MS 5. K_1 values for all the blend microspheres (MS 3 and MS 4) were smaller than K_1 values for microspheres prepared from parent polymers (Table III). MS 2 did not exhibit any significant phase I such that K_1 can be estimated. Among the blend microspheres, K_1 values follow a descending trend with increase in content of smaller molecular weight polymer in the microsphere matrix. This result is translated into longer durations of phase I because of the possible increase in hydrophilicity of the matrix. The value of A (percentage of entrapped amount released) decreased from 23.93% for MS

Table III. Release Parameters for Phase I Estimated by Nonlinear Regression Fit of the Data to Eqs. (2) and (3)

Microsphere	A (% rel)	K_1 (day^{-1})	AK_1 (% rel/day)
PLGA 7525 (MS 1)	23.13±3.81[a]	1.42±0.17	32.88
PLGA 7525/Resomer RG 502H: :3:1[b] (MS 2)	3.75±0.76[a]	ND	ND
PLGA 7525/Resomer RG 502H: :1:1 (MS 3)	10.12±1.91	1.1±0.25	11.13
PLGA 7525/Resomer RG 502H: :1:3 (MS 4)	28.77±1.95	0.70±0.17	20.09
Resomer RG 502H (MS 5)	21.31±1.31	2.05±0.28	43.68

Values are represented as mean ± SEM (n = 3–4/batch).
[a] Represents significant difference at $p < 0.05$.
[b] Modified sigmoid equation (Eq. 2) fits the data, whereas others are fit to dual-release equation (Eq. 3).

Table IV. Release Parameters for Phases II and III Estimated By Nonlinear Regression Fit of the Data to Eqs. (2) and (3)

Microspheres	B (%)	K_2 (day^{-1})	T_{50} (day)
PLGA 7525 (MS 1)	77.67±3.95	0.38±0.07[a]	79.33±0.84[a]
PLGA 7525/Resomer RG 502H: :3:1[b] (MS 2)	95.94±1.34[a]	0.26±0.03	24.64±2.23[a]
PLGA 7525/Resomer RG 502H: :1:1 (MS 3)	86.46±2.3	1.63±0.25	9.82±0.83
PLGA 7525/Resomer RG 502H: :1:3 (MS 4)	72.42±1.88	1.14±0.21	9.06±0.14
Resomer RG 502H (MS 5)	78.25±1.28	1.46±0.08[a]	5.09±0.05[a]

Values are represented as mean ± SEM (n = 3–4/batch).
[a] Represents significant difference at $p < 0.05$.
[b] Modified sigmoid equation fits the data, whereas others are fit to dual-release equation.

1 to 3.75% for MS 2 and 10.12% for MS 3. No significant difference between MS 1(23.93%) and MS 4 (28.77%) was observed relative to A. The overall rates of drug release diminished for blend microspheres (MS 2, MS 3, and MS 4) relative to the parent microspheres (MS 1 and MS 5). The highest release rate was observed for MS 5 (43.68%/day), and the lowest was observed for MS 2 in which no significant phase I was observed.

T_{50} is an index of duration of phase II. MS 1 exhibits the longest T_{50} values (79.73 days), whereas the shortest value was observed for MS 5 (5.09 days). The blend microspheres generated intermediate T_{50} values approaching the lower molecular weight polymer as its content was raised in the microsphere matrix (Table IV; MS 2, 24.64 days; MS 3, 9.84 days; and MS 4, 9.06 days). Such descending trends of T_{50} values indicate faster onset of phase III in case of polymer blends in relation to the PLGA 7525 microspheres (MS 1).

Phase III parameters B (percentage of drug released during phase III) and K_2 (drug-release rate constant as a result of matrix degradation cum drug diffusion) are given in Table IV. It is evident from this table that more than 70% of the entrapped drug is released during phase III that begins immediately after phase II. Thus, a shorter phase II is absolutely necessary for the maintenance of drug levels above MIC at target site. Polymer blend microspheres (MS 2) released 95.94% of entrapped drug during phase III, indicating a negligible contribution from phase I. Other microsphere batches also released about 70–90% of entrapped drug during phase III and only 20–30% during phase I. Drug-release rate constants during phase III (K_2) are significantly lower for MS 1 (0.38 ± 0.07 day^{-1}) and MS 2 (0.26 ± 0.03 day^{-1}) as compared with MS 3 (1.63 ± 0.25 day^{-1}), MS 4 (1.14 ± 0.21 day^{-1}), and MS 5 (1.46 ± 0.08 day^{-1}). As the amount of Resomer RG 502H was raised in the microsphere blend, the drug-release rate constants (K_2) became closer to Resomer RG 502H than PLGA 7525.

Differential scanning calorimetry (DSC) thermograms were processed, and T_g's were calculated for both the polymers and the microspheres. PLGA 7525 polymer has a T_g of 46.85°C, whereas Resomer RG 502H exhibits a T_g of 41.4°C. However, GCV-loaded microspheres prepared from individual polymers exhibited considerably lower T_g's (PLGA 7525 microspheres, 28.9°C; Resomer RG 502H microspheres, 24.9°C), and the blend microspheres exhibit single T_g, suggesting miscible nature of the component polymers (MS 2, 29.2°C; MS 3, 25.5°C; MS 4, 24.7°C).

DISCUSSION

Controlled drug delivery systems have been developed for ganciclovir. These systems include microspheres, nanoparticles, liposomes, and nonbiodegradable implants (11,20–22). Among these delivery vehicles, microspheres seem to be the most promising therapeutic candidates, as the particles are easy to prepare and administer. PLGA polymers can be extensively employed in the preparation of GCV microspheres because of their biodegradable nature. GCV release from PLGA microspheres occurs in three phases because of its hydrophilic nature. As discussed previously, the duration of phase II plays an important role in determining the overall success of the therapy. A limited phase II warrants more frequent administration of microspheres, and a very long phase II might result in failure to maintain GCV levels above MIC in the retina. Release-modifying agents have been incorporated to alter the duration of phase II and also to enhance the amount of drug released during phase I. However, incorporation of additional substances in the microspheres may cause local irritation and toxicity. Polymer blending has been known to be an ideal strategy to alter the duration of phase II without the addition of any other agents. Release profiles were fitted to a set of empirically developed equations to mathematically characterize the effect of polymer blending on all three phases of GCV release.

Drug entrapment studies show that the amounts of GCV entrapped in microspheres increase significantly with polymer blends in relation to individual parent polymers (Table II). Similar increase in entrapment efficiency of pentamidine because of PLGA blending has been reported by Graves *et al.* (10). Such increase in entrapment efficiencies could be a result of the efficient packing of the microsphere matrix during microsphere-hardening stage, which inhibits GCV leakage into the aqueous dispersion medium. As the molecular weight difference between the polymers is large (126,000 and 8000 Da), intermolecular spaces between the large PLGA molecules are efficiently filled by the smaller PLGA molecule. Such a packing could lead to increased drug entrapments as compared to individual parent PLGA polymers. Particle size of the microspheres remained constant

among batches. SEM studies reveal that microspheres possess good spherical nature and uniform surface patterns. Production of microspheres with no surface pores because of the small ratio value of dispersed/continuous phases (1:300) is in accordance to the predictions made by Li *et al.* (23).

Drug-release parameters estimated by nonlinear regression fit of the data to Eqs. (2) and (3) are summarized in Tables III and IV. Percentage of drug released during phase I is similar for microspheres prepared from individual PLGA polymers (MS 1, 23.13 ± 3.81% and MS 5, 21.31 ± 1.31%). However, the release rate constant (K_1) for PLGA 7525 microspheres (MS 1, 1.42 ± 0.17 day^{-1}) is smaller relative to Resomer RG 502H microspheres (MS 5, 2.05 ± 0.28 day^{-1}). Such increase could be a result of greater diffusion as a result of increased hydrophilicity of the smaller PLGA molecule in MS 5 than PLGA 7525 in MS 1. Moreover, duration of phase I among polymer blend microspheres became longer with higher contents of Resomer RG 502H probably because of increased matrix hydrophilicity. Percentage of drug released and release rate constants during phase I diminished for polymer blend microspheres MS 2, MS 3, and MS 4 possibly because of more efficient packing of the matrix. Such reduction in drug-release rates during phase I for blended microspheres was previously observed for pentamidine release (10). Lower initial drug-release rates from blend microspheres along with greater entrapment values could be attributed to efficient matrix packing between the polymer molecules. With elevated amounts of Resomer RG 502H, the hydrophilicity of the matrix is raised resulting in longer phase I (MS 4 > MS 3 > MS 2). Based on the current studies, it can be hypothesized that GCV release during phase I from PLGA 7525 microspheres is primarily a result of diffusion through spaces within the matrix because of improper packing of polymer molecules. Upon replacement of 25% of PLGA 7525 with Resomer RG 502H, the matrix spaces are filled resulting in the absence of major drug release during phase I (MS 2, Fig. 4). Further, elevation in Resomer RG 502H results in enhanced matrix hydrophilicity, which, in turn, results in faster drug diffusion through the matrix (MS 3 and 4; Figs. 5 and 6). In the same line, Resomer RG 502H microspheres could be expected to exhibit longer phase I. However, because of polymer degradation, phase III is initialized even before the completion of phase I for these microspheres, resulting in merging of phases I and III with barely noticeable phase II (Fig. 7). Further studies are still required to exactly determine the molecular weights of polymers to be blended to observe the best matrix packing efficiency to yield high drug entrapments and near-zero-order drug release.

Glass transition temperatures (T_g) for GCV-loaded microspheres were considerably lower relative to pure polymers. A decrease in T_g could be a result of the plasticizing effect of the entrapped drug, traces of water, and residual methylene chloride present in the microsphere matrix or presence of smaller PLGA that might be generated during the sonication process (24). PLGA 7525 microspheres exhibited a higher T_g (28.9°C) relative to Resomer RG 502H microspheres (24.9°C). Increase in T_g with polymer molecular weight has been reported previously (25). The blend microspheres exhibited a single inflection in DSC thermograms, revealing the miscible nature of the polymers. However, T_g of the blend microspheres MS 2 was 29.2°C, which is similar to T_g of MS 1 (28.9°C). Therefore, MS 2 blend requires temperatures similar to MS 1 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 (MS 2) suggests stronger binding between PLGA 7525 and Resomer RG 502H molecules in the microsphere matrices. Such observation therefore reinforces the hypothesis that the blend (MS 2) possesses better polymer packing because of stronger binding among its constituent polymers. Other blends exhibited T_g's similar to MS 1 than MS 5 (MS 3, 25.5°C; MS 4, 24.7°C). This suggests that MS 3 and MS 4 behave similar to MS 5, as the major portion of their matrix is constituted by Resomer RG 502H.

T_{50} is an index of duration of phase II. Bodmer *et al.* (3) reported that phase III during release of BSA from PLGA matrices was initiated once the polymer molecular weight was reduced to 10,000 Da. Therefore, T_{50} can be considered to be the time necessary for the reduction of constituent polymers to reduce to a critical value by ester bond hydrolysis. Beyond T_{50}, microspheres lose the capacity to withhold the drug. The exact value of this polymer molecular weight limit can depend on both drug and polymer. Ideally, a moderately long T_{50} (20–25 days) with high drug-release rate during phase I (AK_1) would be perfect for reaching and maintaining therapeutic levels in the retina for 1–1.5 months following single administration. However, our results indicate that PLGA 7525 microspheres have a very large T_{50} value of 79.33 days (Table IV). Moreover, lack of appreciable drug release during the phase II precludes their use in drug delivery. With the replacement of PLGA 7525 with lower molecular weight and more hydrophilic Resomer RG 502H, T_{50} values dropped significantly from 79.73 days for MS 1 to 24.64 days for MS 2 to 9.86 days for MS 3 and 9.08 days for MS 4. Resomer RG 502H microspheres (MS 5) exhibit the least T_{50} value of 5.09 days, possibly because of the greater degradation rate of the polymer as compared with other polymers and blended mixtures. These data also suggest that as the hydrophilicity of the microsphere matrix increases, duration of phase II diminishes, i.e., microspheres degrade more rapidly as matrix hydrophilicity increases.

Phase III follows phase II during which GCV release from the microspheres is controlled by matrix degradation cum drug diffusion. It is evident from the B values (Table IV) that the amounts of GCV released during phase III are three to four times greater than those released during phase I. Greatest amounts are released for MS 2 (95.94 ± 1.34%). It indicates no significant drug release during phase I possibly due to the best packing among all the microsphere batches because of greater binding energy between the constituent polymers. The release rate constants of GCV during phase III (K_2) are similar for MS 1 and MS 2 and are significantly smaller than MS 3, MS 4, and MS 5. *In vitro* polymer degradation studies indicate that polymers with greater lactide content degrade at slower rates in aqueous buffers (12). We observed that GCV release rate constant from Resomer RG 502H microsphere (MS 5) is four to five times higher than the release rate constant from PLGA 7525 microspheres (MS 1). Moreover, no significant difference between MS 1 and MS 2 GCV release rate constants (0.38 ± 0.07 and 0.26 ± 0.03 day^{-1}, respectively) indicates that drug release from MS 2

(PLGA 7525/Resomer RG 502H: :3:1) during phase III is determined by PLGA 7525 component and not by Resomer RG 502H. However, as the amount of Resomer RG 502H in the matrix increases (MS 3 and MS 4), GCV release rates during phase III are guided by Resomer RG 502H. This is evident because of the lack of significant difference between the K_2 values for MS 3, MS 4, and MS 5 (Table IV).

None of the *in vitro* release profiles reported here meet the qualifications for a long-acting GCV release formulation either because of poor GCV release during phase I and long phase II (MS 1 and MS 2) or rapid overall rates of GCV release (MS 3, MS 4, and MS 5). However, an ideal controlled release formulation can be developed by physical blending of the prepared microspheres. Such a blend, with different populations of microspheres, would release GCV at different times resulting in a constant release.

In conclusion, effect polymer blending on GCV release from PLGA microspheres has been thoroughly investigated. Equations have been developed, and their utility in characterizing drug release from PLGA microspheres has been established. These studies reiterate the utility of polymer blending to effectively hasten the process of drug release without the use of any additives. Our observations also indicate the utility of small molecular weight PLGA molecules as drug-release-enhancing agents from large molecular weight PLGA microspheres. Moreover, polymer blending can also result in entrapment of greater amounts of drug as compared to individual polymers. Overall, polymer blending can be utilized to custom-prepare drug delivery systems meeting specific patient needs.

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