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Design of Controlled Release PLGA Microspheres for Hydrophobic Fenretinide

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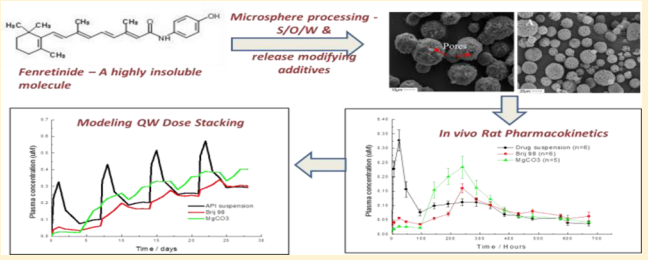
Supporting Information

ABSTRACT: Fenretinide, a chemotherapeutic agent for cancer, is water-insoluble and has a very low oral bioavailability. Hence, the objective was to deliver it as an injectable depot and improve the drug solubility and release behavior from poly(lactide-*co*-glycolide) (PLGA) microspheres by incorporating nonionic surfactants with fenretinide. Enhancement of drug solubilization was observed with Brij 35 or 98, Tween 20, and Pluronic F127, but not Pluronic F68. Co-incorporation of Brij 98 with fenretinide significantly changed the microsphere morphology and improved the fenretinide release profile. The most optimal microsphere formulation, with 20% Brij 98 as excipient, showed an initial in vitro burst around 20% and a sustained release over 28 days in a solubilizing release medium at 37 °C. The effect of addition of $MgCO_3$, drug loading, and polymer blending on the release of fenretinide from PLGA microspheres was also investigated and observed to enhance the drug release. Two sustained release formulations, one incorporating 20% Brij 98 and the other incorporating 3% $MgCO_3$ in the oil phase, were selected for dosing in Sprague–Dawley rats and compared to a single injection of an equivalent dose of fenretinide drug suspension. These two formulations were chosen due to their high encapsulation efficiency, high cumulative release, and desirable in vitro release profile. The drug suspension resulted in a higher initial release in rats compared to the polymeric formulations, however, sustained release was also observed beyond 2 weeks, which may be attributed to the physiological disposition of the drug in vivo. The two PLGA based test formulations provided the desired low initial burst of fenretinide followed by 4 weeks of in vivo sustained release.



KEYWORDS: *PLGA, microspheres, bioavailability, excipient, sustained release*

INTRODUCTION

Fenretinide (*N*-(4-hydroxyphenyl)retinamide, 4-HPR) is a synthetic retinoid with high antitumor activity, and a promising candidate for cancer chemoprevention and chemotherapy due to its high tolerability and low incidence of long-term side effects.[1] To date, fenretinide has been administered orally as an oil based formulation in soft gelatin capsules in clinical trials for the treatment of various cancers. Unfortunately, fenretinide in oral formulations has poor bioavailability and requires excessive or multiple administrations to achieve therapeutic blood concentrations of fenretinide.[2,3] In addition, preclinical studies have demonstrated that intravenously injected fenretinide is rapidly eliminated from the body[4,5] and, therefore, cannot maintain a high enough antitumor activity.[6] Clinical results indicate that therapeutic action appears to be limited to the intervention period and appears to cease after discontinuation of drug administration.[6−8] These studies suggested that further clinical development of fenretinide would require novel delivery systems that are able to maintain a steady effective level over a prolonged period of time.

Biomaterials prepared from copolymers of lactic and glycolic acids (PLGA) are currently among the most commonly used in medicine.[9,10] PLGA particulate systems, such as microparticles/nanoparticles and implants,[11] have been shown to be capable of carrying and delivering a variety of drug classes such as vaccines,[12] peptides,[13] and proteins[14,15] as well as hydrophobic drugs.[16] Some advantages of using PLGA injectable delivery systems include a reduction of injection frequency, sustained therapeutic drug levels, site-specific drug delivery, and formulations that can be tailored for any number of desired release profiles.[17] There is some research about polymer–fenretinide complex and polymeric micelles to improve drug

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Table 1. Formulation Conditions and Loading Data for Fenretinide-Loaded Microspheres with Surfactants/$MgCO_3$ as Additives

formulation no.	PBS in W_1	surfactant (w/w %)	L_T,[a] w/w %	$MgCO_3$, w/w %	PLGA concn, w/w %	PLGA type	L_A, w/w %
1			20		25	503	19.9 ± 0.4
2	1×		20		25	503	21.3 ± 0.7
3		Brij 98 (20)	20		25	503	21.0 ± 0.2
4		Brij 98 (10)	20		25	503	21.5 ± 1.1
5		Brij 98 (5)	20		25	503	19.9 ± 0.4
6		Brij 35 (10)	20		25	503	23.0 ± 1.0
7		F127 (10)	20		25	503	21.2 ± 1.2
8	1×	Brij 98 (20)	20		25	503	24.5 ± 2.1
9			20	3	25	503	21.6 ± 0.7
10			20	5	25	503	20.0 ± 1.1
11		Brij 98 (20)	20	3	25	503	23.1 ± 0.4
12	1×		20	3	25	503	18.3 ± 0.2
13	5×		20		25	503	22.2 ± 2.2
14	5×		30		25	503	32.6 ± 1.6
15	5×		40		25	503	41.6 ± 2.3
16	5×		20		50	502 H	21.5 ± 1.3
17	5×		20		35	502 H/503 1:1	22.3 ± 3.0
18		Brij 98 (20)	20		50	502 H	13.8 ± 2.4
19		Brij 98 (20)	20		35	502 H/503 1:1	19.0 ± 2.8

[a] L_T and L_A are the theoretical loading and actual loading of fenretinide, respectively.

solubility, permeability, bioavailability, and retention of drug within the tumor.[18−23]

Efforts have been made to deliver fenretinide utilizing PLGA nanoparticles, microparticles, and in situ implant delivery system by some researchers including our lab.[24−26] Thus, the objective and novelty of this study was to expand on previous findings and explore the potential of utilizing additives, nonionic surfactants, and salt ($MgCO_3$) incorporated into the PLGA matrix to (a) increase the fenretinide continuous release from PLGA, and (b) improve drug transport in vivo from the releasing polymer into the systemic circulation.

MATERIAL AND METHODS

Materials. Fenretinide was provided by Merck & Co. Methylene chloride, acetonitrile, phosphoric acids, and $MgCO_3$ were purchased from Aldrich Chemical. Poly(lactide-*co*-glycolide) (PLGA) (Resomer RG 502 H and RG 503) were obtained from Boehringer Ingelheim. Poly(vinyl alcohol) (PVA, 88% hydrolyzed, M_w 25,000) was supplied by Polysciences, Inc. Brij 98 and Brij 35 were purchased from Sigma, and Pluronic F127 was purchased from BASF. Fenretinide was micronized in a mortar using a cryogenic procedure. $MgCO_3$ was ground and sieved to smaller than 20 μm, as observed by SEM (data not shown).

Solubility of Fenretinide in Aqueous Media. For determining the solubility of fenretinide in aqueous media, excess amounts of fenretinide were placed in 15 mL of media (phosphate buffer saline (PBS) with surfactants at different concentrations) and incubated at 37 °C on a horizontal shaker for 3 days. After centrifugation and syringe filtration, the samples were assayed by high performance liquid chromatography (HPLC). Samples below the HPLC detection were lyophilized and reconstituted into a small amount of THF.

Preparation of Microspheres. The standard oil−water (O/W) microencapsulation technique with methylene chloride as solvent could not be used to make microspheres since fenretinide is insoluble in methylene chloride. Microspheres were therefore prepared using a solid−oil−water (S/O/W) method in this study.[27,28] Briefly, micronized fenretinide was mixed with a solution of PLGA (Resomer RG 503 or RG 502 H or a 50:50 mixture of RG 502 H and RG 503) in methylene chloride. The mixture was homogenized at 10,000 rpm (Tempest IQ^2 Homogenizer, VirTis Company, Gardiner, NY) for 1 min. Then 4−25 mL of 5% PVA solution was added and the mixture was vortexed at maximum speed (approximately 3000 rpm) for 1 min. It should be noted that 4 mL of PVA solution was sufficient to form microspheres during the emulsion step, but for formulations with nonionic surfactants as excipients, 8−25 mL of PVA solution was used because microspheres did not form at the lower PVA solution volumes due to the higher viscosity of the surfactant-containing polymer/drug/solvent suspension. The formed S/O/W emulsion was immediately transferred to 75−100 mL of 0.5% PVA aqueous solution under stirring at a constant rate. 150 μL of PBS aqueous solution (1× or 5×) was added into the inner water phase to make porous microspheres for some formulations. In the case of incorporating nonionic surfactants as additives, surfactants were codissolved in methylene chloride with PLGA. For incorporating $MgCO_3$, the solid salt particles were suspended in the PLGA methylene chloride solution. After evaporation of methylene chloride for 3 h at ambient temperature, the microspheres were collected by sieve (20−90 μm) and washed thoroughly with water. Finally, the microspheres were lyophilized for 24 h and stored in the refrigerator before further investigation. A total of 19 formulations were made in this study, which are listed in Table 1.

Morphology of Microspheres. The morphology of microspheres after freeze-drying was analyzed by scanning electron microscopy (SEM). Microsphere samples were sputter-coated with gold for analysis by SEM (Hitachi S3200 Variable Pressure SEM).

Loading of Microspheres. Microspheres were dissolved in 0.5 mL of THF, followed by 10 mL of ethanol to precipitate the PLGA. After centrifugation and filtration, the drug loading was determined by HPLC. Each formulation was analyzed in triplicate. Reversed phase high performance liquid chromatographic (RP-HPLC, Waters Alliance) analysis of fenretinide was performed by a Nova Pak C-18 column (3.9 × 150 mm,

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Waters). A mixture of acetonitrile and 0.1% phosphoric acid (63:37 v/v) or mixture of methanol and 0.1% phosphoric acid (80:20 v/v) was used as mobile phase. Running time was 30 min with a flow rate of 1.0 mL/min. UV absorbance was measured at 365 nm, and actual loadings of fenretinide in microspheres are given in Table 1.

In Vitro Release Procedures. Due to the interaction of drug with the dialysis bag, release of drug from PLGA microspheres was carried out in mesh bags (nylon materials, Midwest Filter Corporation, Lake Forest, IL) with 1 μm pore size. The release medium was PBST (phosphate buffer saline with 0.1% Tween 20). Before investigating the release behavior of microspheres using mesh bags, we conducted the release experiment of pure fenretinide particles from mesh bag to ensure that mesh bag (a) had no affinity to the drug and (b) did not pose any rate limit. Fenretinide release behavior from mesh bag is consistent with its dissolution kinetics, as shown in Figure S1.

Microspheres were placed in the mesh bags and sealed with an impulse heat sealer (model: AIE-210C, American International Electric Inc., Whittier, CA). The mesh bags were placed in 3.6 L of PBST. The samples were continuously agitated at constant rate and maintained at 37 °C. Release medium was changed periodically as needed to maintain the sink condition. At preselected time points (days 1, 3, 7, 14, 21, 28), the mesh bags were taken out, freeze-dried, and analyzed for drug remaining in the microspheres by HPLC.

In Vivo Release. Four groups each containing six female Sprague–Dawley rats (~190 g) from Harlan Laboratories (Madison, WI) were used for this study. Each rat received a single 0.2 mL intramuscular injection of the microsphere or drug suspension into the left thigh muscle. The injection site was marked and observed during the course of the study for signs of irritation, redness, or swelling. Blood was drawn from the jugular vein into K_3EDTA vials at predose, 6, 24, 48, 96, 144, 192, 240, 288, 336, 384, 432, 480, 528, 600, and 672 h postdose. Plasma was separated by centrifugation (5 min at 5000 rpm), and plasma samples were analyzed using LC/MS/MS. All animals had access to food and water ad libitum during the course of the study. The animals were observed twice daily during the course of the study for any signs of general health related issues, and any unusual clinical signs were noted. These studies were conducted under a protocol approved by Merck IACUC.

RESULTS AND DISUSSION

Solubility of Fenretinide in Surfactant Aqueous Solutions. Fenretinide is practically insoluble in water with a solubility less than 0.1 ng/mL. Very slow dissolution rates of crystalline drug and often very slow release rates from PLGA microsphere were obtained in standard release media.[24] Nonionic surfactants, such as Tween 20 or Tween 80, are often added in aqueous media to alter the release kinetics of hydrophobic drugs because the surfactants can improve the wetting of microparticles and enhance drug solubility.[29] Therefore, we developed the concept of incorporating nonionic surfactants as additives into PLGA microspheres to improve the release behavior of hydrophobic drugs in the following ways: (a) the formation of micelles in the aqueous pores of microspheres was expected to increase the solubility of fenretinide and diffusion of drug through aqueous pores, (b) lowering of interfacial tension of the pore water was also expected to facilitate the transport of drug from PLGA matrix to aqueous pores, and (c) the polyethylene chain of surfactants might partition into the PLGA matrix and act as a plasticizer for the polymer and thus accelerate the release of drug.

The effect of surfactant type and their concentration on solubility of fenretinide is given in Figure 1. Significant increase

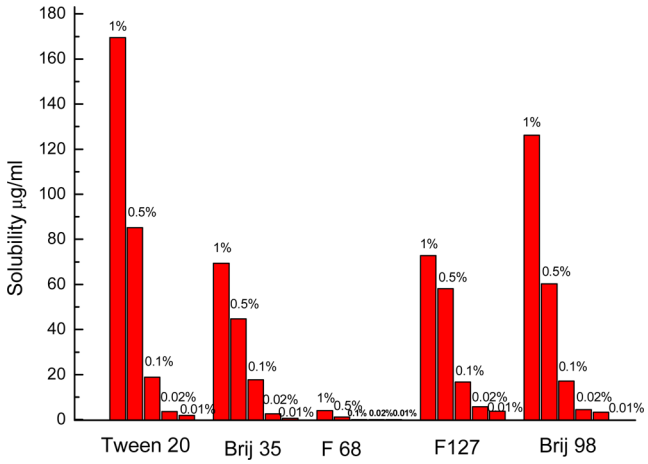

Figure 1. Solubility of fenretinide in surfactant aqueous solutions. Surfactant levels are given as % w/w.

in the apparent level of fenretinide solubility due to the presence of surfactant micelles is observed for 4 out of 5 of the surfactants and is consistent with the previously reported results.[30] The solubility is also very sensitive to surfactant concentration. Critical micelle concentration (CMC) of surfactants influenced the solubilization effect (CMC of surfactants: 0.025 mM for Brij 98, 0.09 mM for Brij 35, 0.059 mM for Tween 20, 0.019 mM for F127, and 8.33 mM for F68[31,32]). Brij 98, Brij 35, and poloxamer 127 (F127) were selected for PLGA formulation as microsphere additives based on the solubility data and the following surfactant attributes: (a) optimal hydrophilic–lipophilic balance (HLBs) suitable for solubilization (surfactants with a HLB between 13 and 18 are considered good for solubilization), (b) good biocompatibility,[33] (c) established use in biomaterial and pharmaceutical applications to improve the surface antifouling and for delivery of low molecular weight drugs and peptides,[34,35] and (d) availability in solid form.

Effects of Surfactants on Microsphere Morphology and Fenretinide Release from Microspheres. For successfully encapsulating solid fenretinide into microspheres using the S/O/W method, mechanical grinding was required to ensure that the crystalline drug was less than 5 μm (data not shown). SEM pictures of several microsphere formulations are shown in Figure 2. From Figure 2A, it is clear that a significant amount of crystalline drug is present on the surfaces of microparticles prepared without nonionic surfactants. The appearance of these drug crystals on the surface of microparticles is probably the result of relatively low viscosities of the organic phase in this formulation. The low viscosity allows the micronized fenretinide crystals to migrate to the surface of the organic droplet. Due to low fenretinide solubility in water phase, the drug crystals remain at the O/W interface until the particles harden.[28] By adding salt to the inner water phase (PBS in W_1), porous microspheres were obtained because of the increased osmotic gradient and the flux of water from the outer water phase into the W_1/O phase.

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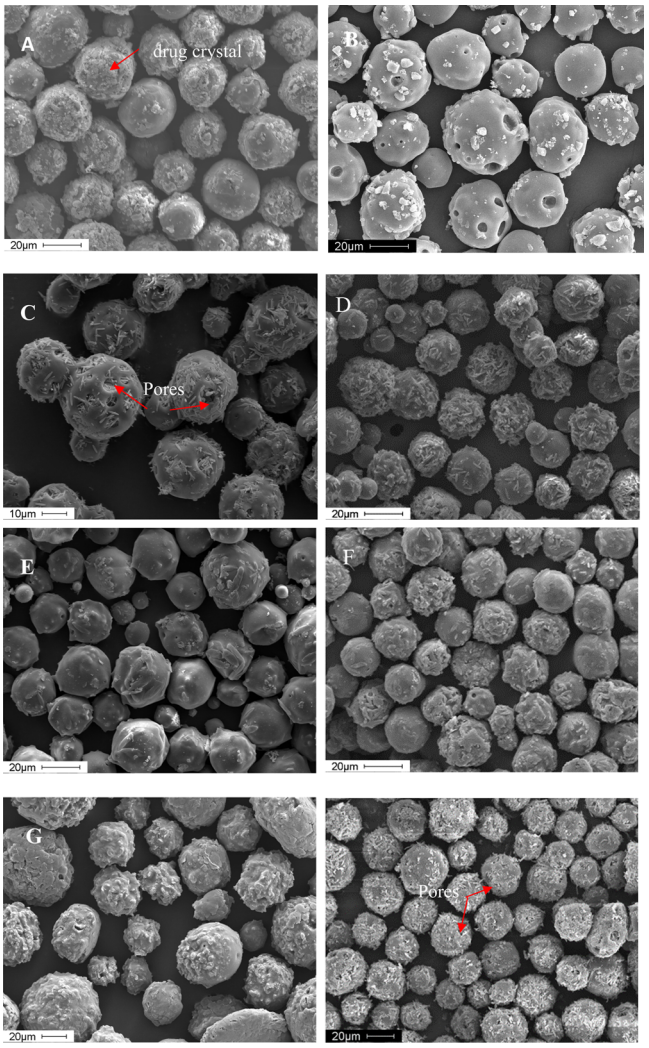

Figure 2. Scanning electron micrographs of microspheres prepared without any excipients (A, formulation 1) ; with 150 μL of PBS (1×) in W_1 (B, formulation 2), 20% Brij 98 in oil phase (C, formulation 3); 10% Brij 98 in oil phase (D, formulation 4); 5% Brij 98 in oil phase (E, formulation 5); 10% Brij 35 in oil phase (F, formulation 6); 10% F127 in oil phase (G, formulation 7); 20% Brij 98 in oil phase + 150 μL of PBS (1×) in W_1 (H, formulation 8) using RG 503.

The microspheres coencapsulating fenretinide with surfactants, Brij 98, Brij 35, and poloxamer 127 (formulations 3–7, Figures 2C–2G), which were codissolved in methylene chloride with PLGA as additives, were spherical as observed by SEM, but had a rough surface without pores with the exception of formulation 3 (Figure 2C). The morphology of microspheres prepared with Brij 98 was directly related to the concentration of surfactant. In the case of microparticles prepared using 20% Brij 98, the microspheres were characterized by the presence of some pores (formulation 3). Microspheres containing 5% and 10% surfactant did not present obvious pores (formulations 4 and 5, Figures 2D and 2E). Solid particles, which were likely solid surfactant based on morphology, appeared on the surface of microspheres in the surfactant-containing formulations. It seems that increased levels of needle-like projections on the microsphere surface were observed when Brij 98 was increased from 5% to 20% (Figures 2C, 2D, and 2E). The presence of surfactant on the microsphere surface could reasonably be attributed to the phase separation of surfactant and PLGA during solvent evaporation. Since surfactants were codissolved in methylene chloride with PLGA, upon contact of methylene chloride droplets with the aqueous phase, phase separation into a PLGA-rich phase and a surfactant-rich phase could occur since hydrophilic surfactants (high HLB) have a strong tendency to migrate toward the O/W interface. When 20% Brij 98 was incorporated into the organic phase, microspheres presented some pores on their surfaces, which can be attributed to the high molecular weight as well as its high solubility in both methylene chloride and water. Such a molecular feature has been reported to be favorable for generating pore structures when leaching out from the matrix.[36]

Effects of various surfactants on the in vitro release are shown in Figure 3. The large burst release (36% and 50% in the first 24 h) exhibited by control batches of microspheres (formulations 1 and 2, respectively) is attributed in part to the presence of drug crystals on the surface of the microparticles. Formulation 2 displayed faster release than the other microsphere formulations. It is obvious that a high porosity will allow the release medium to penetrate the particles more easily and favor the drug to be released faster by pore diffusion.

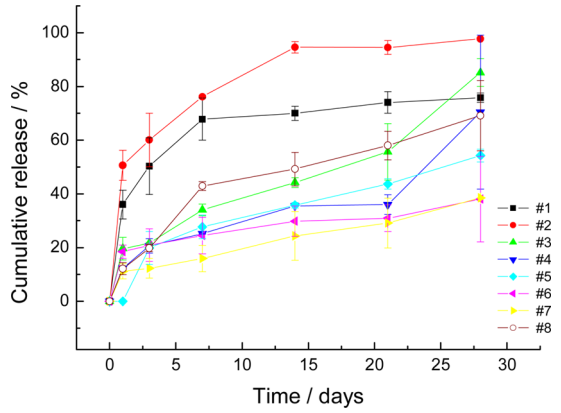

Figure 3. Effects of surfactants on the in vitro release of fenretinide from microspheres formulations 1–8: formulation 1, no excipient; formulation 2, 100 μL of PBS in W_1; formulation 3, 20% Brij 98 in O; formulation 4, 10% Brij 98 in O; formulation 5, 5% Brij 98 in O; formulation 6, 10% Brij 35; formulation 7, 10% Pluronic F127; formulation 8, 20% Brij 98 in O and 100 μL of PBS in W_1.

Incorporation of additives significantly affected the fenretinide release pattern. Microspheres containing surfactants reduced burst release and provided a more constant rate of release than the control batches of microspheres (formulations 1 and 2). Drug release was biphasic, showing a small initial burst followed by a continuous release. The burst release was slower than the control batch microspheres likely due to improved encapsulation and/or increased density of the polymer at the surface. The rate of drug release during the second phase from microspheres containing surfactants was faster than that of the nonporous microspheres (formulation 1), which exhibited a lag phase after 7 days. It is postulated that leaching of water-soluble surfactants into the release medium with time and degradation of PLGA leads to the pores being filled with water. This effect and the release of monomers and oligomers from degradation of PLGA will reduce the barrier to diffusion of fenretinide resulting in the consistent post 7 day release. Remaining surfactant in the microspheres also

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increased drug solubility and potentially plasticized the polymer matrix, both of which may have attributed to the acceleration in the release of fenretinide. Figure S2 gives the SEM of microspheres incorporating 20% Brij 98 (formulation 3) that were incubated in PBST over time. A significant amount of Brij still remained in the pores of microspheres after 14 days, but began to noticeably decline by 21 days.

In addition, the release profile was dependent on the concentration of Brij 98 with very substantial increase in release rate, particularly when the surfactant level was increased from 10% to 20% w/w. These critical loading phenomena have also been observed previously when blending of PEG 10,000 was increased from 10 to 20% in PLA microspheres,[37] and when bovine serum albumin (BSA) loading was increased from 10% to 20% in PLGA millicylinders.[38]

To further improve the release of fenretinide via increased microsphere porosity, both surfactants and PBS were incorporated into microspheres (formulation 8, Figure 2H). An improved release rate was observed with formulation 8 (incorporating 20% Brij 98 in the organic phase and PBS in the inner water phase) as compared to formulation 3 (incorporating with 20% Brij 98 in the organic phase and no PBS) at the early stage, but slowed down at 4 weeks, as shown in Figure 3. Microsphere formulations with Brij 35 and poloxamer 127 as excipients (formulations 6 and 7) displayed very slow release kinetics. This was probably due to the relatively low solubilization of fenretinide by these surfactants and the accumulation of these surfactants on the surface of microspheres which may prevent the diffusion of fenretinide.

Effects of $MgCO_3$ on the Morphology and Release from Microspheres. In a previous study from our group, PLGA-COOH millicylinders with pore-forming agent $MgCO_3$ were found to exhibit a more desirable release of hydrophobic drug, 2-methoxyestradiol.[39] Hence, $MgCO_3$ was used as an excipient in this study to improve the release of fenretinide from RG 503 PLGA microspheres. Inclusion of $MgCO_3$ produced a more porous microsphere structure (Figures 4A and 4B) when compared to the control microspheres (Figure 2A), which is due to the increased osmotic gradient and influx of water from the outer water phase. Improved release kinetics was observed with microspheres loaded with 3 and 5 wt % w/w of $MgCO_3$, as seen in Figure 5. Adding PBS salt to the microspheres in addition to $MgCO_3$ did not significantly change the release behavior (formulation 12). Although Brij and $MgCO_3$ improve release kinetics when used alone in microsphere formulations, as noted above, microspheres prepared by including both $MgCO_3$ and Brij 98 in the organic phase demonstrated the slowest release despite a porous microsphere structure (Figures 4C vs 2C). This observation can be explained by (a) improvement in encapsulation with Brij 98 and an increase in polymer density at the surface and (b) reduction in the rate of polymer degradation in the presence of Mg base.[39]

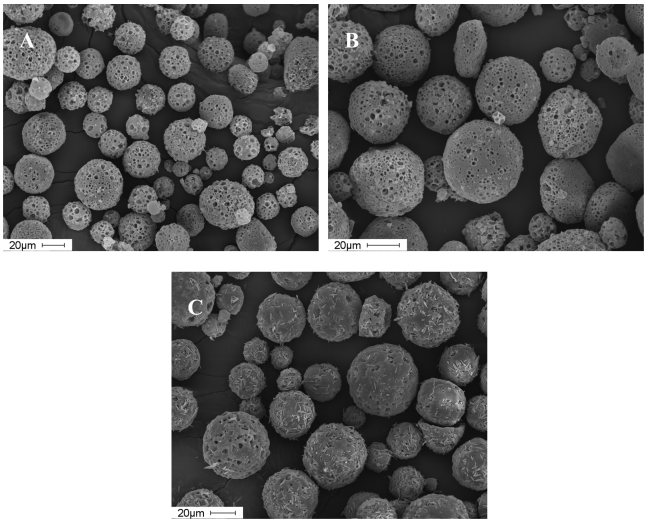

Figure 4. SEM of microspheres with 3% $MgCO_3$ in oil phase (A, formulation 9); 5% $MgCO_3$ in oil phase (B, formulation 10); 3% $MgCO_3$ and 20% Brij 98 in oil phase (C, formulation 11) using RG 503.

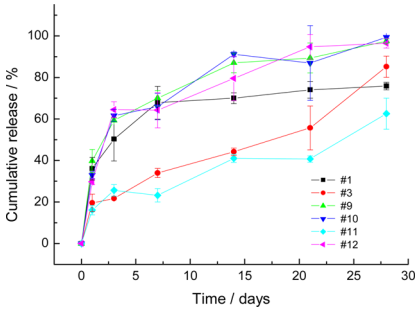

Figure 5. Effects of $MgCO_3$ on the in vitro release of fenretinide from microspheres: formulation 1, no excipient; formulation 3, 20% Brij in O; formulation 9, 3% $MgCO_3$ in O; formulation 10, 5% $MgCO_3$ in O; formulation 11, 20% Brij + 3% $MgCO_3$ in O; formulation 12, 3% $MgCO_3$ in O + PBS in W_1.

Effect of Drug Loading and Polymer Type on the Morphology and Release of Microspheres. For microspheres with 20% fenretinide loading (formulation 13, Figure 6A), there were drug particles distributed on the surfaces. However, microspheres with 30% and 40% fenretinide loading (formulations 14 and 15, Figures 6B and 6C, respectively) had clusters of drug particles covering almost the entire microsphere surfaces.

The influence of fenretinide loading on release kinetics is shown in Figure 7. It can be observed that the initial burst effect was affected by the fenretinide loading in the PLGA microspheres. As fenretinide loading increased, the 1 day burst of fenretinide from PLGA microspheres decreased. Lowest initial burst was observed with microspheres loaded with 40% drug. However, the cumulative release from microspheres with different loadings was similar after 3 days. The initial burst appeared to be controlled by the drug present on the surface of microspheres, and dissolution and diffusion of fenretinide from the microsphere surfaces. The crystals of fenretinide in the 20% drug loading formulation were finely dispersed on the PLGA microsphere surface (Figure 6A). In contrast, at higher initial drug loading, drug crystals appeared in clusters on the PLGA surface (Figures 6B and 6C), which may have decreased dissolution rate.

Similar to the RG 503 PLGA microspheres containing Brij 98 in organic phase, needle-shaped Brij 98 was observed on the surface of microparticles (formulations 18 and 19) when encapsulating fenretinide with RG 502 H PLGA or with the 1:1 mixture of RG 502 H and RG 503 and Brij 98, as shown in Figure 6. The microspheres had a rough surface with pores,

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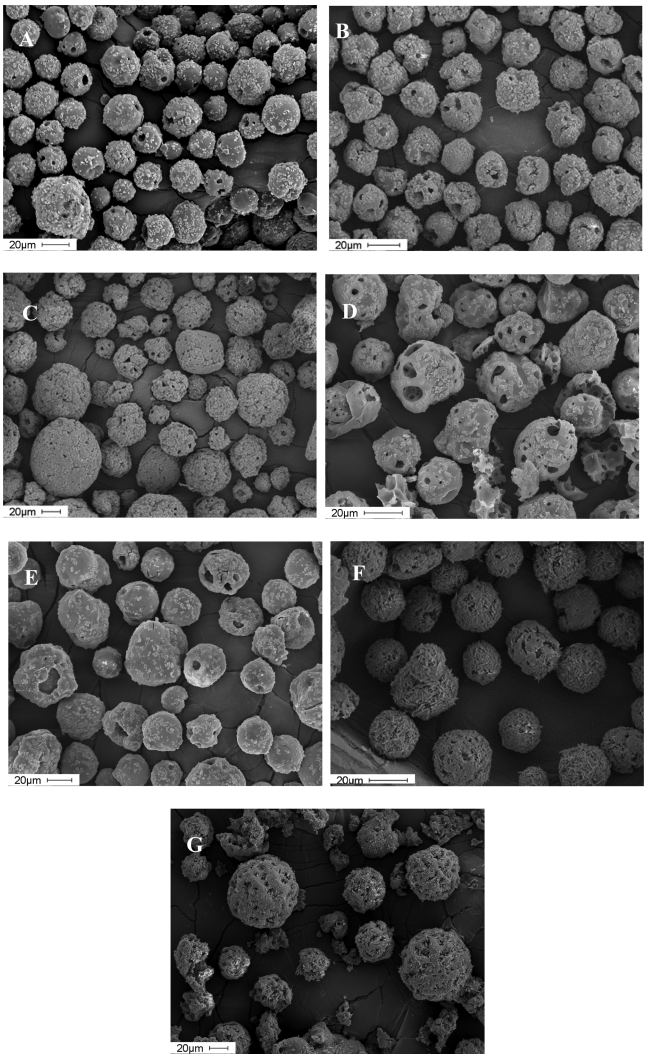

Figure 6. SEMs of fenretinide loaded microspheres: (A) loading 20%, 503 (formulation 13); (B) loading 30%, 503 (formulation 14); (C) loading 40%, 503 (formulation 15); (D) loading 20%, 502 H (formulation 16); (E) loading 20% 502 H:503 1:1 (formulation 17); (F) loading 20%, 502 H, Brij 98 20% in oil phase (formulation 18); (G) loading 20%, 502 H:503 1:1, Brij 98 20% in oil phase (formulation 19).

which might be attributed to the leaching of Brij 98 into aqueous phase and the low viscosity of the polymer solution.

Free-acid end-group PLGA (RG 502 H) and a 1:1 mixture of RG 502 H and RG 503 were used to speed up the degradation of the polymer and release of fenretinide in comparison to ester end-capped PLGA (RG 503). The influence of PLGA on drug release is given in Figure 8. Microspheres made with RG 502 H PLGA and PBS salt as a porosity inducer displayed the fastest release behavior among all the formulations. It is obvious that a higher porosity and faster degradation accelerated by autocatalysis favor faster drug release. Physical blending of two polymers is well-known to affect the release profiles of microspheres.[40] As expected, blending hydrophobic RG 503 with relatively hydrophilic RG 502 H accelerated release of fenretinide compared with the microspheres prepared by hydrophobic 503.

The addition of Brij 98 as excipient to various PLGAs improved the release profile of fenretinide to some extent. Microspheres with Brij 98 provided a more constant rate of

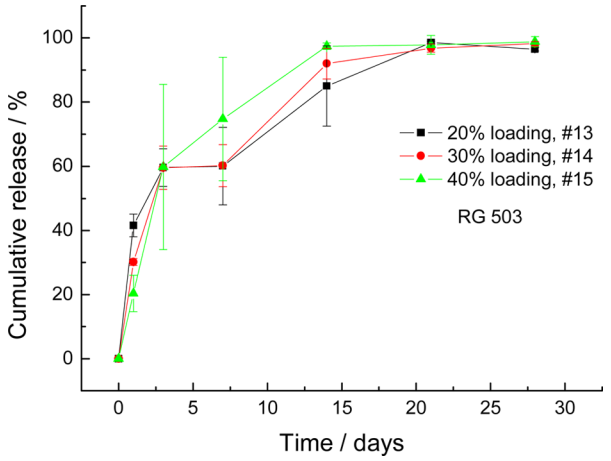

Figure 7. Effect of drug loading on the fenretinide in vitro release from microspheres: formulation 13, 20% drug loading; formulation 14, 30% drug loading; formulation 15, 40% drug loading.

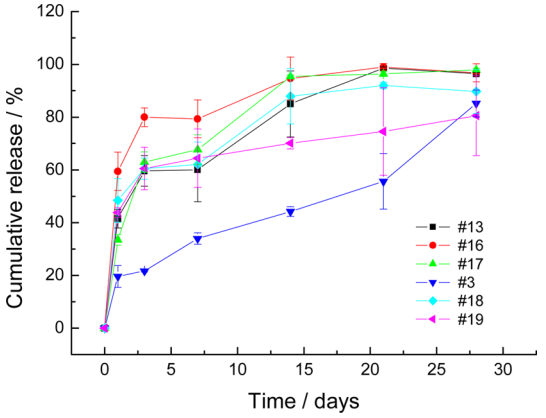

Figure 8. Effect of PLGA type on the in vitro drug release from microspheres: formulation 3, PLGA 503, 20% Brij 98; formulation 13, PLGA 503, 5× PBS; formulation 16, PLGA 502 H, 5× PBS; formulation 17, PLGA 502 H/503 1:1, 5× PBS; formulation 18, PLGA 502 H, 20% Brij 98; formulation 19, PLGA 502 H/503 1:1.

release than porous microparticles by using PBS in the inner water phase (Figure 8).

In Vivo Release. Formulation 3, incorporating 20% Brij 98, and formulation 9, incorporating 3% $MgCO_3$ as excipient, were chosen for in vivo release evaluation in rats due to their high encapsulation efficiency (as shown in Table 1), high cumulative release, and desirable release profile, as shown in Figure 9A. Fenretinide suspension was dosed as a control. The mean plasma concentration versus time profiles of fenretinide after intramuscular administration of formulations 3 and 9 and the drug suspension are shown in Figure 9B.

Intramuscular administration of the fenretinide drug suspension, containing 4.2 mg of fenretinide (median particle size distribution ca. 5−10 μm), produced a high initial plasma drug concentration (C_{max} = 0.33 ± 0.04 μM) at day 1. By comparison, formulations 3 and 9 with Brij 98 and $MgCO_3$ as excipients containing 4.2 mg of fenretinide per dose produced only low plasma level during the first days post administration. The plasma concentration of fenretinide reached a C_{max} of 0.16 ± 0.02 μM and C_{max} of 0.24 ± 0.04 μM at 10 days for formulations 3 and 9 respectively, which were lower than the C_{max} obtained with the drug suspension. Since the release of

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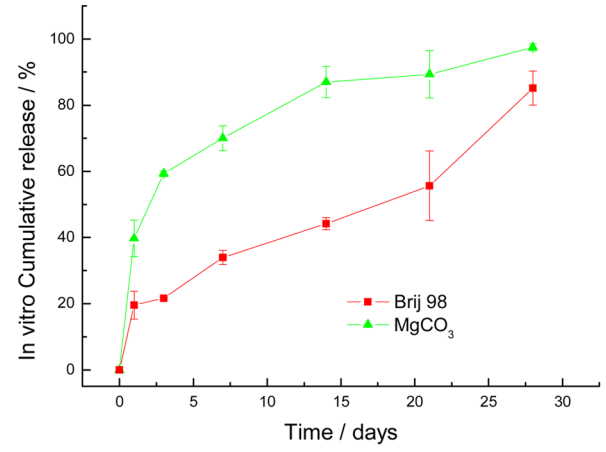
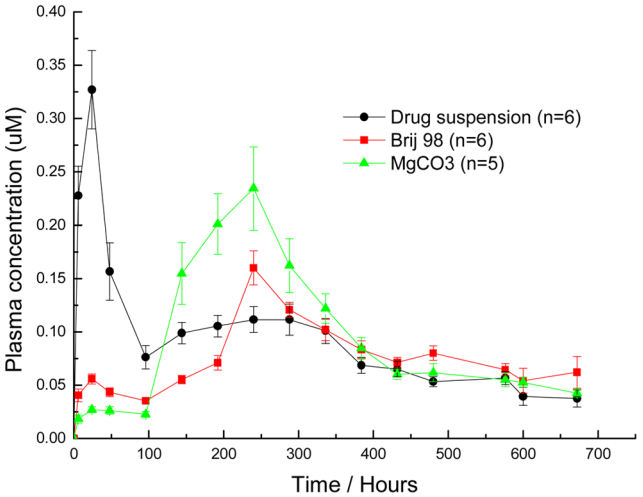
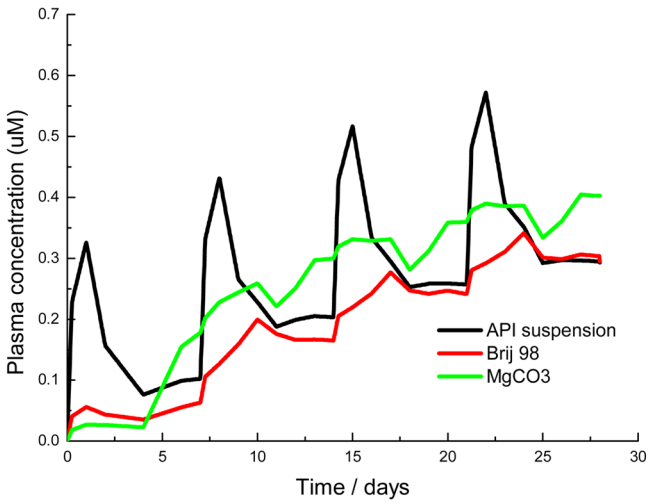


(A)



(B)



(C)

Figure 9. In vitro cumulative release curve (A), in vivo pharmacokinetics of fenretinide from drug suspension and microsphere formulations in rats (B), and modeling dose stacking based on once weekly administration (C) (formulation 3 with Brij 98 and formulation 9 with $MgCO_3$).

fenretinide from the drug suspension is a function of drug dissolution in the intramuscular space in comparison to the release from the polymeric microspheres, where polymer degradation and diffusion through the matrix are added resistances to release, there was a significant burst effect observed with the drug suspension formulation. The longer T_{max} for the microsphere formulations as compared to the drug suspension (240 h vs 24 h) further corroborates the postulated release mechanism. However, the plasma concentrations resulting from suspension formulation were maintained 5 days after injection, which might be attributable to low solubility of the suspension and potential foreign body response to injected drug suspension leading to particle trafficking to the lymphatics[41] and or aggregation with formation of a fibrous capsule.[42,43] It may also be attributable to the slow release of accumulated drug from fat tissue. Fenretinide is known to have long half-life and accumulates in tissues resulting in sustained plasma levels long after administration has ceased.[44] Of the two microsphere formulations, microspheres containing $MgCO_3$ (high surface porosity) showed higher fenretinide area under the curve (AUC) and C_{max} as compared to microspheres with Brij 98. This is consistent with the in vitro release profile (Figures 3 and 5), where 100% and 80% fenretinide release was observed after 30 days for the $MgCO_3$ and Brij 98 containing formulations, respectively.

In order to estimate the amount of drug released from the tested formulations in vivo, the relative bioavailability was calculated from the AUC of intramuscularly injected dose. The relative bioavailabilities were 81.8% and 101% for formulations 3 and 9 respectively, assuming complete in vivo release from drug suspension, which is listed in Table 2. The results indicate that all encapsulated drug was completely released from formulation 9, with less than 20% of drug still available in formulation 3 microspheres at the end of study at 4 weeks. These results are consistent with in vitro data. In order to assess the in vivo release from the microsphere formulations, the plasma PK profiles were deconvoluted against the intravenous data. The deconvolutions were conducted using previously published iv data in rats.[4] These data demonstrated that the microsphere containing $MgCO_3$ showed higher fenretinide release than Brij 98 microspheres, suggesting that the high surface porosity for the $MgCO_3$ microspheres facilitated better drug release (data not shown).

There were no deaths or any significant adverse events during the course of the study, which is a preliminary indication that these microsphere formulations might have adequate biocompatibility.

Further, to compare the steady state plasma levels after multiple administration of the prototype formulations, multiple dose PK simulations after once weekly intramuscular injections of 4.2 mg of fenretinide (suspension, Brij 98, and $MgCO_3$ formulations; Figure 9C) were conducted over 4 weeks based on the single dose PK data. These simulations were conducted using nonparametric superposition in Phoenix 6.3 (Certara, Princeton, NJ), using linear computation model. The terminal elimination rate constant was estimated in the model using regression of the terminal portions of the plasma concentration time profiles for each microsphere formulation. These values were 0.065, 0.043, and 0.066 h^{-1} for suspension, Brij 98, and $MgCO_3$ formulations, respectively. Subsequently, the PK profiles after multiple doses were simulated using an accumulation ratio computed from the terminal slopes of the single dose PK data. Based on these simulations, it is evident that the $MgCO_3$ containing microsphere formulation provides

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Table 2. Pharmacokinetic Parameters Following Single Intramuscular Administration of Microspheres and Fenretinide Suspension in Rats

formulation	dose, mg	C_{max}, μM	T_{max}, h	AUC_{0-672h}, μM·h	rel bioavailability, %
fenretinide suspension	4.2	0.33 ± 0.04	24	62.6 ± 3.10	
microspheres containing 20% Brij 98, no. 3	4.2	0.16 ± 0.02	240	51.3 ± 2.49	82
microspheres containing 3% $MgCO_3$, no. 9	4.2	0.24 ± 0.04	240	63.5 ± 7.47	101

superior trough levels at steady state compared to the suspension and Brij 98 containing formulation.

In vitro release and in vivo pharmacokinetic data were used to develop a level A in vitro−in vivo correlation (IVIVC) for the microspheres. The IVIVC demonstrated poor correlation with large predicted error particularly for AUC, which was significantly underpredicted using the current in vitro release data. This indicates that the current in vitro model is not adequate to accurately predict the in vivo performance of these fenretinide microsphere formulations. This could be due to the instability of drug (fenretinide is sensitive to light and oxygen), the release medium used to conduct the release study, differences in degradation of the polymer microspheres in vitro and in vivo, and the accumulation of burst release of fenretinide in fat tissue and gradually depleting from drug-bound tissues. This may suggest that current in vitro model may need to be adapted with changes to the formulation to accurately predict the in vivo performance. Further research to gain a more mechanistic understanding of in vitro release from polymeric microspheres to enable design of biorelevant in vitro assays is ongoing.

CONCLUSION

A high drug loading PLGA based microsphere depot for controlled release of hydrophobic fenretinide was developed by incorporating the nonionic surfactant, Brij 98, and/or the salt, $MgCO_3$, into the PLGA matrix. By introducing these excipients, the in vitro and in vivo release was slow and continuous up to 1 month after a small burst release. The injected microspheres were well tolerated by the rats with no evidence of local adverse events. The results show that PLGA microsphere delivery systems for fenretinide could be promising for further development.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.molpharmaceut.5b00961.

Fenretinide release behavior and dissolution kinetics and SEMs of microspheres (PDF)

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Notes

The authors declare no competing financial interest.

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