A Short-term (Accelerated Release) Approach to Evaluate Peptide Release from PLGA Depot Formulations

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ABSTRACT An accelerated method to evaluate peptide release from poly(dl-lactide-co-glycolide) (PLGA) depot formulations in short time is described. Peptide-loaded microspheres were made from hydrophilic 50:50 PLGA by a dispersion-solvent extraction technique, and peptide release was studied at 37°C and at higher temperatures in various media. For all accelerated conditions, release was faster at temperatures above the glass transition, Tg, of the host polymer. Complete release of peptide from 8600 MW PLGA was achieved in 35 hours at 50°C in buffered and nonbuffered media containing 0.5% polyvinyl alcohol (PVA). Type of release media and concentration of PVA influenced the release profiles. A PVA concentration of 0.1 to 0.5% was found to prevent aggregation of microspheres at higher temperatures, with an increase in release at the higher PVA concentration. Peptide release was associated with a reduction of pH of the releasing media and increased mass loss. Complete peptide release at pH 4 from 8.6 kd and 28 kd PLGA at 50 and 60°C occurred within 30-40 hours and correlated well with the real-time release at 37°C and pH 7.0. At the higher molecular weight, a slightly longer accelerated release time and higher temperature were required to correlate with the real-time release. The data suggest that by optimization of release conditions such as temperature, surfactant concentration, buffer component, and pH, an accelerated study could be employed to evaluate depot formulations for a given polymer type.

KEY WORDS: peptide loaded microspheres, depot formulation, accelerated release, elevated temperature, PLGA

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INTRODUCTION

In recent years, a large number of recombinant proteins and synthetic peptides have been developed as potential therapeutic agents (1). Poor absorption of these agents by the oral route has necessitated delivery by alternative routes, principally the parenteral route. Several peptides have been successfully incorporated into a poly(dl-lactide-co-glycolide) (PLGA) matrix as a depot formulation for parenteral use (2,3). This is currently an important area of investigation not only for peptides or proteins, but also for vaccine delivery. Depot formulations made from biodegradable polymers release the incorporated drugs over several days, weeks, or months depending on the polymer type and concentration, drug loading, and porosity of the matrix (4,5). Accelerated stability testing at high temperatures is often employed to predict shelf life of drugs. Previously, high temperatures were applied to shorten degradation time for polylactides (6) and to reveal the drug-release mechanism from polylactides and other polymer matrices (7,8). Drug release at these conditions is accelerated due to polymer softening and enhanced hydration and degradation. While the real-time (long-term) *in vitro* release study at physiological conditions is essential to evaluate these systems, an accelerated (short-term) *in vitro* release method would be helpful for rapid assessment of formulation and processing variables. The purpose for utilizing an accelerated method is twofold:

1. differentiate formulations prepared from a similar polymer varying in molecular weight, drug loading, particle size, and morphology and
2. correlate short-term release with real-time release in order to predict real-time release.

The USP describes several ways to establish an *in vitro-in vivo* correlation of drug release from controlled release products. The most acceptable

method is the 1:1 correlation or superimposibility of the release profiles obtained from *in vitro* and *in vivo* studies. The same concept was applied to make a correlation between the real-time release at 37°C in days vs short-term release in hours.

In this study, peptide-loaded microspheres were prepared from two 50:50 PLGA polymers of different molecular weight (8.6 kd, 16 kd, and 28 kd) and their long-term release determined at 37°C. Two of the microsphere batches prepared from the 8.6 kd and 28 kd polymers were selected for study at elevated temperatures employing different buffer conditions and surfactant concentrations in order to mimic real-time release.

MATERIALS

Leuprolide (1,205 MW) as the acetate was purchased from Bachem Inc., Torrance, CA. The PLGA polymers were obtained from Boehringer Ingelheim Inc., Germany (50:50, 8,600 MW, and 28,000 MW) and Mitsui Chemical Co., Tokyo, Japan (50:50, 16,300 MW) in the hydrophilic forms (carboxylic acid end group). All other chemicals used were of analytical reagent grade.

METHODS

Preparation of Microspheres

The microspheres were prepared by an aqueous solvent extraction technique using a temperature gradient for solvent removal described previously (9). The method involved dissolving the peptide and polymer in a CH_2Cl_2/CH_3OH cosolvent system (CH_2Cl_2/CH_3OH ratio = ~2.62 wt/wt). The polymer concentration varied between 20 and 28% wt/wt. The organic phase was dispersed into the aqueous phase containing 0.35% polyvinyl alcohol (PVA) and allowed to mix initially for 15 minutes before the temperature was gradually raised to 40°C. The extraction was carried out at 40°C for 1 h. The microspheres obtained from the cool-down solution by filtration were washed with distilled water, dried under vacuum at room temperature, and stored in a desiccator at -20°C. The typical yield of microspheres in this process was ~90%. The encapsulation efficiency calculated from the actual loading (10-12%) and target loading (12.5%) was about 80-96%. The microspheres obtained by this process were porous in the size range 25-125 µm.

Long-Term In Vitro Release

Peptide-loaded microspheres (10 mg) were incubated in 0.033 M phosphate buffer (PB, 10 ml), pH 7.0 at 37°C. The samples were occasionally shaken (twice/week) for 1 minute to simulate the static *in vivo* condition, and the peptide release was determined from the residual microspheres by high-performance liquid chromatography (HPLC).

Accelerated Short-Term Release

Peptide-loaded microspheres (10 mg) were incubated in 10 ml media under continuous shaking for specified times at elevated temperatures with different buffer components and surfactant concentrations. Surfactant was used to prevent agglomeration of microspheres at temperatures above the Tg of polymer. Agglomerated microspheres had negligible peptide release at higher temperatures. Peptide release was assayed from both the supernatant and the residual microspheres. There was a good mass balance (~100%) between the peptide release measured from the supernatant and residual microspheres at all conditions. A 0.1 to 0.5% PVA in 0.1% PB was used to optimize effect of PVA concentration on release. Unbuffered media (distilled water) and PB at 0.1 M and 0.01 M were used to observe ionic effect on release in 0.5% PVA. On the other hand, 0.1 M PB and acetate buffers at pH 7.0 and 4.0, respectively, were used to optimize short-term release at elevated temperatures.

HPLC Assay

The peptide was assayed by a reversed phase HPLC technique (10). The system, obtained from Dionex (Sunnyvale, CA), comprised a gradient pump, an auto injector, and a variable wave length detector. The assay conditions were as follows: column: Bondapak C-18 (5 µm; 3.9 x 300 mm, i.d., Waters, Milford, MA); mobile phase: acetonitrile:water:TFA (32:68:0.1,vol/vol/vol); flow rate: 1.1 ml/min; injection volume: 30 µl; detection: 220 nm.

Mass Loss Study

One hundred milligrams of blank microspheres were carefully weighed and incubated in 100 ml distilled water and 0.1 M acetate buffer containing 0.5% PVA at various temperatures. At appropriate

time intervals, the samples were filtered through a 0.8-µ'b5m filter, rinsed with distilled water, and dried for 24 hours in a vacuum oven to a constant weight. The percentage mass remaining was calculated as shown below:

% mass remaining = m_d/m_i X 100%

m_i = initial mass of microspheres

m_d = mass loss after degradation.

The pH of the dissolution media was monitored during the mass loss study.

RESULTS AND DISCUSSION

Long-Term Release

Figure 1 shows the long-term (real-time) *in vitro* release of the peptide from three 50:50 PLGA with different molecular weight. Complete peptide release occurred at 28 days from 8.6 kd, 35 days from 16.3 kd, and 42 days from 28 kd molecular weight polymer. The initial release was higher from the low molecular weight polymer. The particles made from these polymers had similar drug loading, particle size, and surface morphology; hence, polymer molecular weight predominantly controlled the release. In order to predict and differentiate real-time release from particles made from these polymers, short-term investigations were undertaken at accelerated temperatures.

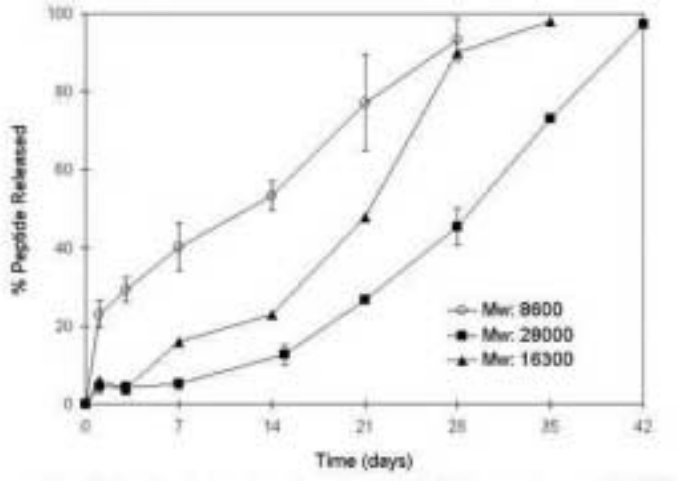

Figure 1. Long-term *in vitro* release of peptide from 50:50 PLGA microspheres in 0.033 M PB, pH 7.0 at 37°C .

Optimization of Short-Term Release Conditions

Polymer Molecular Weight Effect

Initially microspheres prepared from two molecular weights of 50:50 PLGA were evaluated at 50°C and the amount of release in 30 hours measured. Table 1 shows that while 50°C appeared to be a suitable temperature for the 8.6 kd polymer to generate maximum release in short time, the release from the 28 kd polymer, with Tg being close to 50°C, was minimal. These studies revealed that it was necessary to elevate the temperature sufficiently above the Tg to complete release from both these polymers (data not shown).

Generally Tg of polymer increases corresponding to increase in molecular weight (11), and as the molecular weight of host polymer increases, longer extended release is usually observed at a given temperature. Complete release of peptide from microspheres of these polymers occurred within a 25- to 40-day range at 37°C. To correlate with long-term release, higher temperatures were therefore required to develop short-term methods for various polymers. Microspheres prepared from 8.6 kd PLGA were further used to investigate conditions and parameters that might affect accelerated release.

Table 1. Effect of molecular weight of 50:50 PLGA on peptide release at 50°C in 0.1M PB.

Polymer (MW)	Tg (°C)*	% Released in 30 hours
8,600	40.04	86
28,000	46.16	10

* From reference 11.

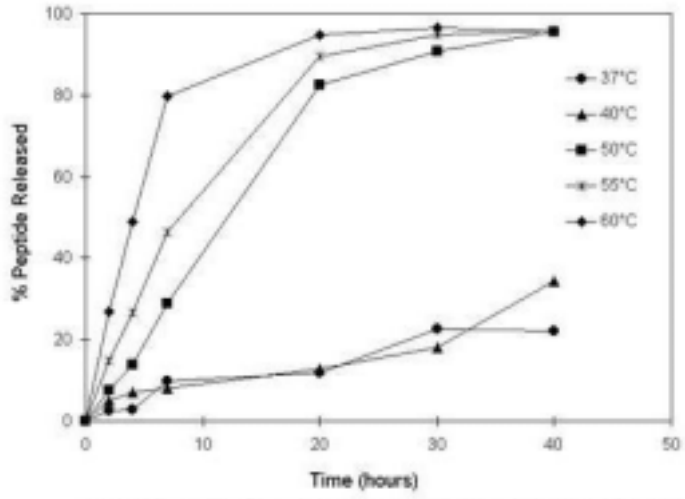

Figure 2. Effect of temperature on the short-term release of peptide from 50:50 PLGA (8.6 kd) microspheres in 0.5% PVA (no buffer).

Temperature Effect

Figure 2 shows release from particles in 0.5% PVA without buffer at different temperatures. Peptide release was faster at temperatures above the Tg of the host polymer. The release enhancement was more pronounced in going from 40 to 50°C than from 50 to 60°C due to the Tg of the polymer being between 40 and 50°C. The temperature effect also occurred with acetate and phosphate buffers containing 0.5% PVA (data not shown). Complete release of peptide was achieved in 30-35 hours at 50-60°C in all media. At temperatures above the Tg, polymer softening led to enhanced diffusional release. The data show that a 24-30 hour test at higher temperatures could be used to monitor short-term release from the 8.6 kd polymer

Surfactant Effect

Surfactant was found to be crucial in optimizing the *in vitro* release. Mircospheres incubated in water or buffer above 40°C without surfactant aggregated and adhered to the glass container, resulting in poor release. A PVA concentration of 0.1 to 0.5% was found to prevent aggregation of microspheres at higher temperatures. Figure 3 shows an increase in release at the higher PVA concentrations at 50°C in 0.1 M PB at the 1- and 20-hour time points. PVA ensured a condition where the particles remained suspended at elevated temperatures similar to that at 37°C for the long-term (real-time) method.

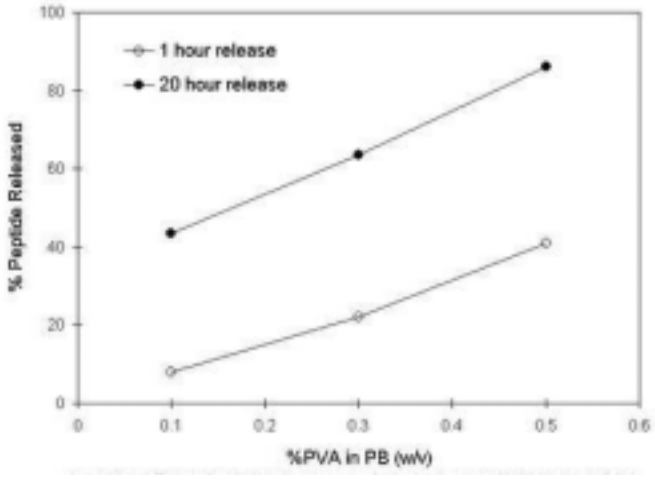

Figure 3. Effect of PVA concentration on peptide release from 50:50 PLGA (8.6 kd) microspheres in 0.1 M phosphate buffer, pH 7.0 at 50°C.

Buffer Concentration Effect

The buffer strength of the media influenced the release profiles. Figure 4 indicates that an increase in the phosphate buffer concentration at pH 7.0 and 50°C can change the release profile significantly. At 0.1 M buffer concentration, a much faster release occurred, compared to both the 0.01 M buffer and the nonbuffered systems (0.5% PVA in water), the latter being slightly acidic (pH = 5.5). The basic amino acids in leuprolide have been found to interact with the acid end groups of PLGA (12); therefore, increasing the ionic strength of the release media will reduce the interaction between peptide and polymer, resulting in faster peptide release.

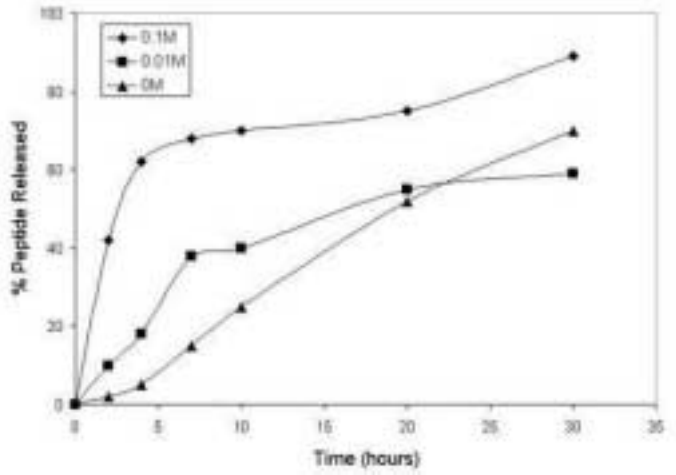

Figure 4. Effect of buffer concentration on peptide release from 50:50 PLGA (8.6 kd) microspheres in 0.5% PVA at 50°C.

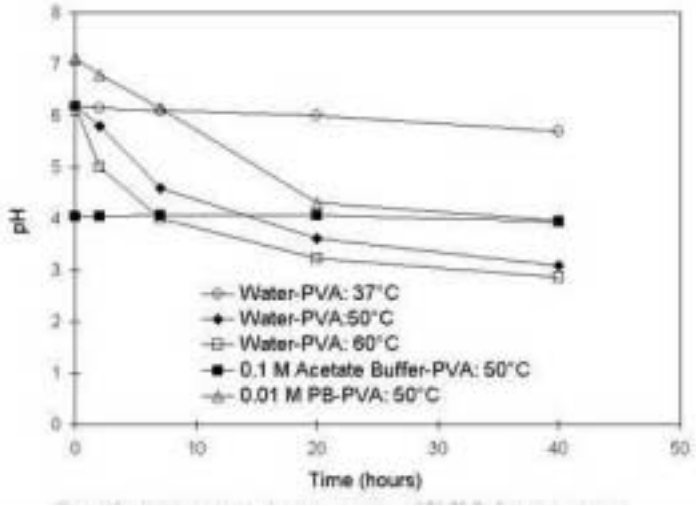

Figure 5. pH change in the dissolution medium of 50:50 PLGA microspheres (8.6 kd) at different temperatures.

In Vitro Release at Higher Temperature

At temperatures higher than Tg of polymer, the molecular mobility of drug will be increased and the polymer will be in a less glassy state, leading to an accelerated drug release by diffusion. However, at temperatures above Tg of the polymer, the pores in microspheres were somewhat obscured due to polymer softening. An accelerated release of the hydrophilic peptide across the polymer matrix is therefore expected, via a combination of diffusion and mass loss mechanisms. Accelerated peptide release at higher temperatures was associated with the reduction of pH of the media as shown in Figure 5.

The formation of acidic components via polymer cleavage creates an acidic microenvironment inside the microspheres. The behavior has been reported previously in real-time release studies from microspheres prepared from fast degrading 50:50 PLGA (12). The pH reduction of the dissolution media during peptide release corresponded to increasing temperature in the different media (Figure 5). The 0.1 M acetate buffer had sufficient buffer capacity to resist change of pH. The pH of this buffer was close to that of an acidic microenvironment that forms within the PLGA matrix (13). Figure 6 shows the mass loss profiles from the 50:50 hydrophilic PLGA matrix. The loss was about 20% in water and acetate buffer in 40 hours at 50 and 60°C, while about 40% in PB in 40 hours at 50°C.

These results demonstrate that peptide release under accelerated temperatures occurs via a combination of the following processes:

1. release above Tg of polymer;
2. and time dependent mass loss;
3. effect causing faster dissolution of drug and polymer.

Short-Term Versus Long-Term (Real-Time) Correlation

Figure 7 shows short-term *in vitro* peptide release (solid lines) from 8.6 kd and 28 kd polymers using the optimized conditions in 0.1 M acetate buffer at pH 4.0. The long-term release is shown as dotted lines. The correlation between the short- and long-term release was established by plotting different levels of release in days vs hours as shown in Figure 8.

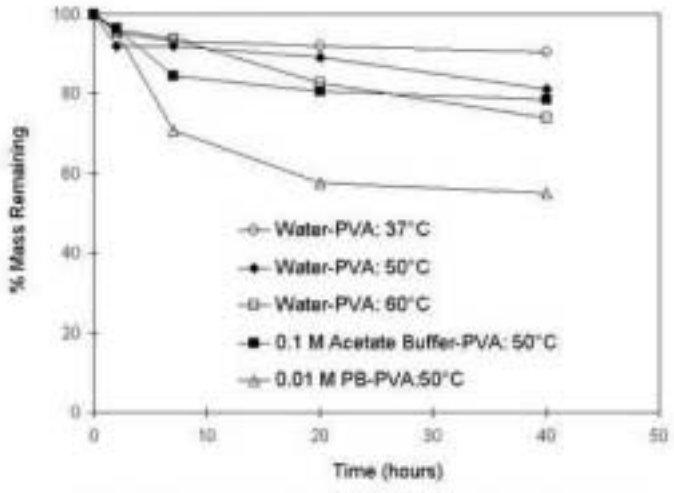

Figure 6. Mass loss in the dissolution medium of 50:50 PLGA microspheres at different temperatures.

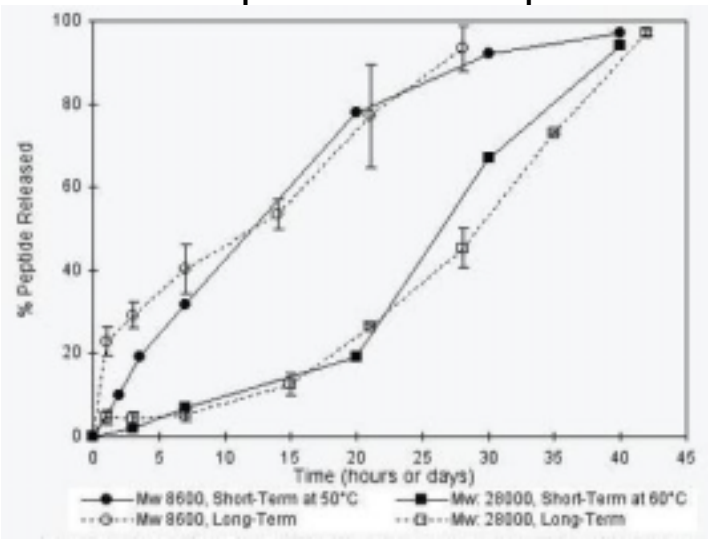

Figure 7. Short-term *in vitro* release of peptide from 50:50 PLGA microspheres in 0.1 M acetate buffer, pH 4.0 at elevated temperatures.

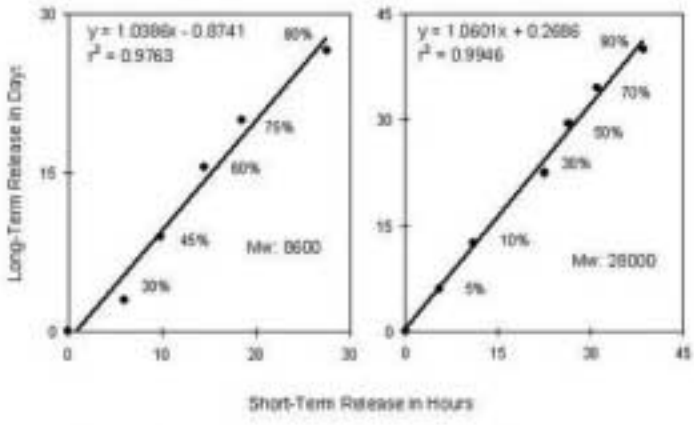

Figure 8. Short-term vs long-term correlation of peptide release from 50:50 PLGA microspheres.

The correlation coefficients (r^2) obtained were 0.976 for 8.6 kd polymer and 0.996 for 28 kd polymer at 50 and 60°C, respectively. Acidic buffer appeared to provide better correlation than neutral buffer or water system through microenvironmental effects, which play a significant role in real-time release from such polymer systems. Microspheres prepared from other polymers of different molecular weights would be necessary to further establish this approach of evaluating release in a predictable manner.

In summary, short-term accelerated release at elevated temperatures can differentiate the release of peptide from different polymers and correlate with the real-time release at 37°C. Through optimization of the experimental variables (eg, temperature, surfactant concentration, buffer component, pH) the current approach may be applied to evaluate depot formulations for a given polymer type. The method can be easily adapted to the official USP dissolution apparatus and employed as a rapid quality control test during microsphere development or commercial manufacturing. Studies are underway to use this approach to correlate short-term release with real time release of 90-, 120-, and 180-day forms.

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