A Model-dependent Approach to Correlate Accelerated With Real-Time Release From Biodegradable Microspheres

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ABSTRACT

The purpose of this study was to determine the feasibility of applying accelerated in vitro release testing to correlate or predict long-term in vitro release of leuprolide poly(lactide-co-glycolide) microspheres. Peptide release was studied using a dialysis technique at 37°C and at elevated temperatures (50°C-60°C) in 0.1M phosphate buffered saline (PBS) pH 7.4 and 0.1M acetate buffer pH 4.0. The data were analyzed using a modification of the Weibull equation. Peptide release was temperature dependent and complete within 30 days at 37°C and 3 to 5 days at the elevated temperatures. In vitro release profiles at the elevated temperatures correlated well with release at 37°C. The shapes of the release profiles at all temperatures were similar. Using the modified Weibull equation, an increase in temperature was characterized by an increase in the model parameter, α, a scaling factor for the apparent rate constant. Complete release at 37°C was shortened from ~30 days to 5 days at 50°C, 3.5 days at 55°C, 2.25 days at 60°C in PBS pH 7.4, and 3 days at 50°C in acetate buffer pH 4.0. Values for the model parameter β indicated that the shape of the release profiles at 55°C in PBS pH 7.4 (2.740) and 50°C in 0.1M acetate buffer pH 4.0 (2.711) were similar to that at 37°C (2.677). The E_a for hydration and erosion were determined to be 42.3 and 19.4 kcal/mol, respectively. Polymer degradation was also temperature dependent and had an E_a of 31.6 kcal/mol. Short-term in vitro release studies offer the possibility of correlation with long-term release, thereby reducing the time and expense associated with long-term studies. Accelerated release methodology could be useful in the prediction of long-term release from extended release microsphere dosage forms and may serve as a quality control tool for the release of clinical or commercial batches.

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INTRODUCTION

Parenteral microspheres formulated using biodegradable polymers such as polylactide (PLA) and poly(lactide-co-glycolide) (PLGA) have been studied and successfully used for the treatment of a variety of disease states. Table 1 lists currently marketed formulations, indications for use, and duration of action. These depot formulations can deliver a sustained dose in vivo for varying periods of time, from weeks to months,[1-6] primarily owing to low degradation rates of the bulk eroding PLA and PLGA polymers.

With the increased use of parenteral microspheres having extended duration of action in vivo, drug release assessment becomes an important aspect in the development of clinically safe and effective formulations. However, evaluation of drug release under real-time conditions, in vivo and in vitro, would encompass the duration of action of the microspheres, which would be both time consuming and expensive, especially in the preliminary stages of formulation development and for quality control release of a marketed drug product. A quick and reliable method for assessing and predicting drug release in a short period would be cost-effective. In addition, this method should be simple, reproducible under the conditions of study, and applicable to biodegradable microsphere formulations that release drug for varying periods of time. This was highlighted in the guidelines, which resulted from an American Association of Pharmaceutical Scientists/International Pharmaceutical Federation (AAPS/FIP) sponsored conference on dissolution/in vitro release testing of novel/special dosage forms.[7]

Recognizing that drug release is governed by the slowly degrading polymer, research in the past decade has focused on accelerating polymer degradation using elevated temperatures,[8] buffer type, and pH[9-13] and comparing the results to physiologically degraded material. Buchholz[8] examined the effect of 2 temperatures, 37°C and 80°C, on the in vitro degradation of $_{D,L}$-PLA. In another study by Bergsma et al,[10] degradation behavior of $_{L}$- and $_{D,L}$-PLA was studied at 90°C. Degradation at temperatures between 25°C and 50°C of polyglycolic acid (PGA) sutures[11] and

Table 1. Examples of Currently Marketed Microsphere Formulations of Extended Duration*

Product	Drug/Polymer	Indication for Use	Duration of Action
Lupron Depot	Leuprolide/PLA or PLGA	Prostate cancer, endometriosis	1, 3, 4 months
Trelstar Depot	Triptorelin/PLGA	Prostate cancer	1 month
Suprecur MP	Buserelin/PLGA	Endometriosis	1 month
Nutropin	Human growth hormone/PLGA	Growth deficiencies	2 weeks, 1 month
Sandostatin LAR	Octreotide/PLGA-glucose	Acromegaly	1 month
Somatuline LA	Lanreotide/PLGA	Acromegaly	1 month
Arestin	Minocycline/PLGA	Periodontitis	2 weeks
Risperdal Consta	Risperidone/PLGA	Schizophrenia	2 weeks

*PLA indicates polylactide; PLGA, poly(lactide-co-glycolide).

50:50 PLGA devices[12] has also been reported. Makino et al[9] reported the degradation of $_{L}$- and $_{D,L}$-PLA microcapsules at varying temperatures, ionic strength, and pH of medium. All these studies showed that polymer degradation rate was accelerated at elevated temperatures, thereby reducing the time required for in vitro experiments. All other parameters kept constant, no change in the mechanism of polymer degradation was reported at higher temperatures. However, changes in the type of buffer or pH (acidic or alkaline) have been reported to accelerate polymer degradation, owing to the difference in the mechanism of PLA and PLGA ester hydrolysis.[9]

An application of these findings was demonstrated by Shameem et al[14] who investigated peptide release from PLGA microspheres by using elevated temperature, buffer, and surfactant to accelerate release kinetics and reduce the time required for in vitro release studies. Release was assessed using the "sample and separate" method with residual peptide analysis from the microspheres at predetermined time points. Peptide release was shown to be higher at the elevated temperature and correlated with real-time release at 37°C.

In a previous study,[15] a novel dialysis technique using a commercially available dialyzer composed of a regenerated cellulose membrane was used to assess peptide diffusion at varying temperatures and release from PLGA microspheres at 37°C. Results from the diffusion and in vitro release studies on peptide-loaded microspheres revealed the advantages of this technique over the commonly used "sample and separate" method including ease of sampling, total buffer replacement, and simulation of in vivo conditions.

Therefore, the feasibility of using the novel dialysis technique to assess short-term release at elevated temperatures along with correlation to long-term release at 37°C was explored in this study. A PLGA microsphere formulation of the peptide, leuprolide (luteinizing hormone-releasing hormone [LHRH] agonist), intended for 1-month duration of action, was selected for in vitro studies.

Materials and Methods

Materials

Leuprolide acetate purchased from Bachem Inc (Torrance, CA). PLGA polymer 50:50 (molecular weight [MW] 32 kDa) was obtained from Boehringer Ingelheim Inc (Ingelheim, Germany). Float-a-Lyzer (25 kDa molecular weight cutoff [MWCO] mL capacity, regenerated cellulose membrane) was purchased from Spectrum Labs (Rancho Dominguez, CA). All other chemicals used were of analytical reagent grade.

Methods

Preparation and characterization of microspheres

PLGA microspheres were prepared by a dispersion method followed by solvent extraction/evaporation. Briefly, a solution of leuprolide in methanol was added to a 22% (wt/wt) solution of polymer in methylene chloride to form a homogeneous dispersed phase. The dispersed phase was added to an aqueous solution containing 0.35% polyvinyl alcohol (continuous phase) under stirring with a Silverson L4R mixer (Silverson Machines, East Longmeadow, MA) at a predetermined speed. The solvents were removed by stirring for 2 hours at 40°C. The resulting microspheres were recovered by filtration, washed to remove traces of polyvinyl alcohol and residual solvent, and dried under vacuum at room temperature for 3 days to ensure low moisture, and then stored in a dessicator. The microspheres were characterized for particle size by laser diffractometry, surface morphology by scanning electron micrography (SEM), and peptide content.

Peptide content of microspheres

Ten milligrams of the peptide-loaded microspheres were dissolved in 2 mL dimethyl sulfoxide. The peptide was extracted from organic solvent by adding 4 mL of 0.1M acetate buffer pH 4.0 followed by agitation for 1 hour and then assayed using a gradient high-performance liquid chromatography (HPLC) method using C-18 column (3.9 × 300 mm,

μBondapak, Waters, Milford, MA) at a flow rate of 1 mL/min, and UV detection wavelength of 215 nm. Mobile phase A contained 30% vol/vol acetonitrile and 0.1% trifluoroacetic acid in water, while mobile phase B comprised a 0.1% solution of trifluoroacetic acetic acid in water.

Long-term peptide release

Peptide release was assessed using a dialysis method.[15] Briefly, a predetermined amount of microspheres were transferred to the Float-a-Lyzer and suspended in 5 mL of 0.1 M phosphate buffered saline (PBS) pH 7.4. The dialyzer was then introduced into a graduated glass cylinder containing 55 mL buffer at 37°C. The contents of the outer media were continuously stirred to prevent the formation of the unstirred water layer. At predetermined intervals 1 mL of supernatant was withdrawn followed by buffer replacement. Analysis of peptide in the supernatant was assessed by an isocratic reverse phase HPLC method using a C-18 column (3.9 × 300 mm, μBondapak, Waters) using an acetonitrile-water mixture containing 0.1% trifluoroacetic acid at a flow rate of 1.1 mL/min and UV detection wavelength of 220 nm.

Accelerated (short-term) drug release

Prior to assessing short-term release at elevated temperatures, peptide stability was assessed at 60°C. Release experiments were performed between 50°C and 60°C in 0.1 M PBS pH 7.4 using the Float-a-Lyzer. Peptide release assessment and subsequent HPLC analysis at accelerated conditions was similar to that described for the long-term release study.

Molecular weight determination

Polymer degradation at different temperatures was assessed by gel permeation chromatography (GPC). The GPC system consisted of 2 Ultrastyragel columns connected in series (7.8 × 300 mm each, one with 10^4 Å pores and one with 10^3 Å pores), a delivery device (Shimadzu LC-6A, Tokyo, Japan), UV detector set at λ = 210 nm (Shimadzu), and software to compute molecular weight distribution (Maxima 820, Waters). Sample solutions in tetrahydrofuran (THF) at a concentration of 5 mg/mL were filtered through a 0.45-μm filter (Millipore, Billerica, MA) before injection into the gel permeation chromatography (GPC) system and were eluted with THF at 0.4 mL/min. The weight-average MW of each sample was calculated using monodisperse polystyrene standards, MW 1000 to 50 000 Da.

Data analysis

The Weibull function was used to model in vitro release data at real-time (37°C) and elevated temperatures using GraphPad Prism software (GraphPad Software Inc, San Diego, CA). Goodness of fit of the model, standard error, and a 95% confidence interval for the model parameters were computed. The energy of activation (Ea) was calculated using the Arrhenius equation.

Results and Discussion

Peptide loading in PLGA microspheres was found to be 14%. Figure 1 shows the in vitro release of leuprolide at 37°C. Following an initial burst of ~5% within 6 hours, peptide release from the microspheres was diffusional for 4 to 7 days, which could be attributed to polymer hydration. Once hydrated, release rate increased due to polymer erosion and drug diffusion, and complete release was achieved by 30 days.

Figure 2 shows the release of leuprolide at elevated temperatures (50°C-60°C). The release profiles were similar to those at 37°C in that they exhibited triphasic behavior—an initial burst, followed by slow release due to hydration, and finally a more rapid drug release due to polymer erosion and drug diffusion. Peptide release was clearly dependent on temperature, an increase in temperature manifesting in faster release from the microspheres. The onset of faster

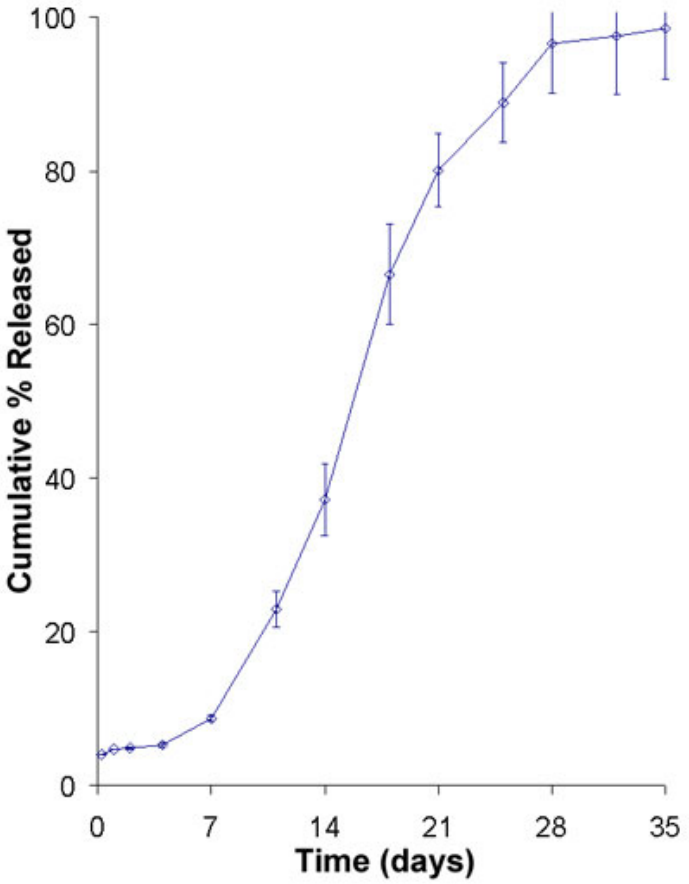

Figure 1. Long-term release of leuprolide from microspheres in 0.1M PBS pH 7.4.

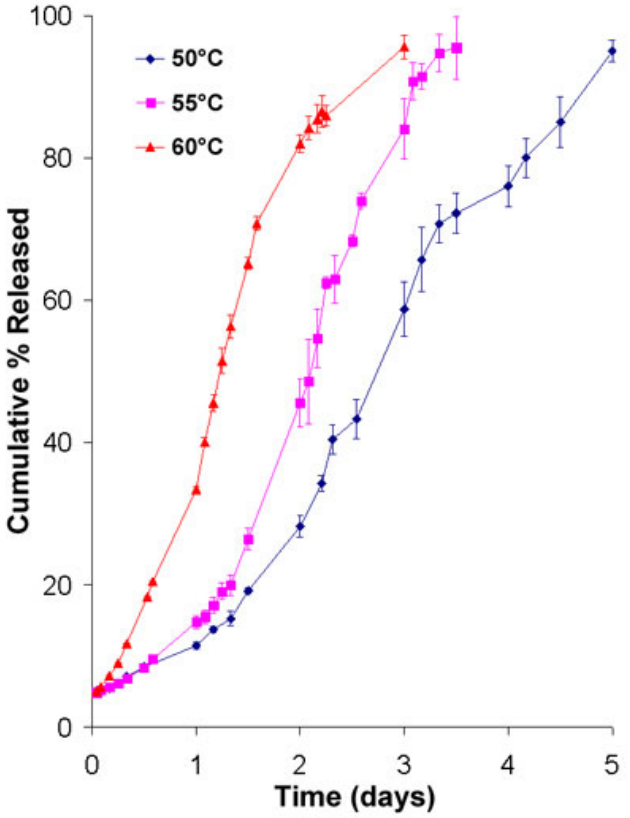

Figure 2. Release of leuprolide from microspheres at 50°C, 55°C, and 60°C in 0.1M PBS pH 7.4.

release phase is believed to be due to the more rapid hydration at the higher temperatures.

Comparison of peptide release profiles at 37°C with 50°C, 55°C, and 60°C (Figure 3) suggested that release at the elevated temperature could be correlated with that at 37°C and used to predict long-term drug release owing to similarity in release profiles over the temperatures studied. Depending on the temperature of the short-term study, real-time drug release for leuprolide PLGA microspheres could be obtained within 3 to 5 days: 5 days at 50°C, 3.5 days at 55°C, and 2.25 days at 60°C. This finding represents a 6- to 8-fold reduction in the time required for a release study. The short-term release study was found to be reproducible ($P > .05$) when performed in triplicate at 55°C (data not shown).

Qualitatively, release profiles at the elevated temperature studies appeared predictive of long-term release. However, a measure of the correlation between short-term release at 50°C, 55°C, and 60°C with long-term release would be useful to interpret results, if the data or the release profiles could be described in a quantitative manner using a mathematical and/or statistical approach. In other words, data analysis should highlight any possible differences that could exist between in vitro release at 50°C, 55°C, and 60°C when compared with long-term release from leuprolide PLGA microspheres.

A comparison of dissolution profiles between 2 drug products may be accomplished by using a "model-independent"[16] or a "model-dependent" (curve fitting) approach.[17] In the model-independent approach, the data are described by sample times (eg, $t_{50\%}$, $t_{90\%}$, mean dissolution time [MDT], similarity factor [f_2], or a dissimilarity factor [f_1]).[18] The model-dependent approach is recommended for a "data rich" scenario in which a mathematical function may be used to describe in vitro release data.[17] Once the mathematical function is selected, the in vitro release profiles are evaluated

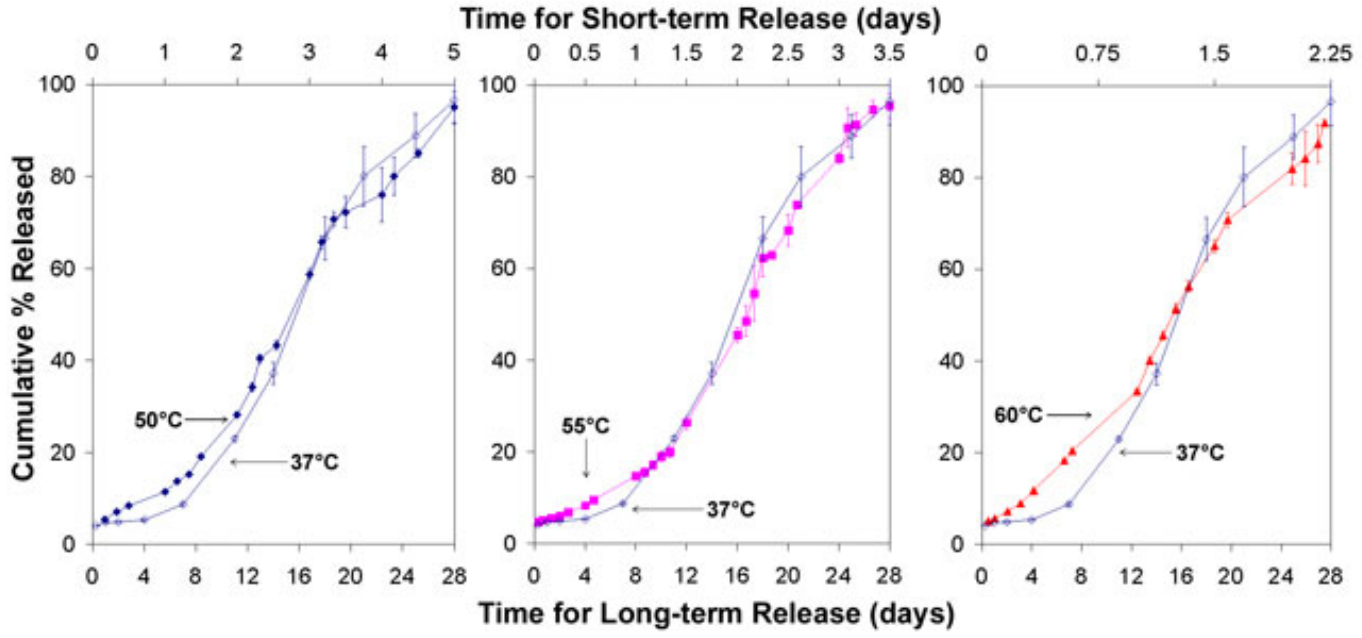

Figure 3. Comparison of long-term with short-term release at 50°C, 55°C, and 60°C in 0.1M PBS pH 7.4.

in terms of the model parameters that describe the release profile. The model-independent approach requires measurement of point estimate measures, which may not account for variances and covariances within/between lots[16] or identical sampling schemes. The model-independent approach is not desirable for routine comparison studies from a quality control standpoint and would not be suitable for this study, given that sampling occurs at varying time points. Hence, the model-dependent approach was selected.

The model-dependent or curve fitting approach has been successfully used to compare in vitro dissolution profiles of solid dosage forms.[19-22] Triphasic or sigmoid release curves were found to be best described by the Weibull function.[23,24] The usefulness of the Weibull function in modeling drug release from extended release dosage forms such as biodegradable microspheres was recognized at the AAPS/FIP conference.[7] The attendees recommended this function as a curve fitting approach to model in vitro release. The Weibull equation is used to model release with delivery systems that show

1. zero to minimal initial burst release,
2. zero to minimal diffusion-mediated release rate, or
3. erosion-dominated process coupled with minimal diffusive release rates.

Therefore, the Weibull function (Equation 1) was used to fit the in vitro release data from leuprolide PLGA microspheres at long- and short-term conditions.

$$X/X_{inf} = 1 - \exp\left[-\alpha\ (t^{\beta})\right] \qquad (1)$$

where X = percentage drug released at time t and is complete when X_{inf} = 100%, α = scale factor corresponding to the apparent rate constant, and β = shape factor.

In Equation 1, α defines the time scale of the process, and β characterizes the shape of the curve as exponential ($\beta = 1$), sigmoid or S-shaped with upward curvature followed by a turning point ($\beta > 1$) or parabolic, with a higher initial slope and after that consistent with exponential ($\beta < 1$).[17]

Curve fitting of the in vitro release data at 37°C and 50°C to 60°C in 0.1M PBS pH 7.4 was accomplished by nonlinear regression (GraphPad Prism software), and the results are shown in Table 2. Values for the model parameter α, which is indicative of the rate constant, increased from 4.339×10^{-4} day^{-1} at 37°C to 0.5068 day^{-1} at 60°C in 0.1M PBS pH 7.4, demonstrating a temperature dependence, which was expected. In addition to α, the Weibull function describes a parameter β, which is indicative of the shape of the curve. The differences in the release profiles between 50°C and 60°C can be seen from the β values at the respective temperatures. Although the release profiles at all the elevated temperatures studied were triphasic and sigmoidal, β values were greater than 2 for in vitro release between 37°C and 55°C and dropped to 1.696 at 60°C. The low value at 60°C implied a change in the shape of the curve due to an increase in release rate at the elevated temperature.

The 95% confidence interval (CI) for β at 37°C was broad (2.327-3.040) and relatively narrow at the elevated temperatures. The mean value of β at 55°C (2.558) lay between the 95% CI at 37°C (2.327- 3.040), suggesting that the shape of the release profile at 55°C was similar to that at 37°C. Values for β at 50°C (2.048) and 60°C (1.696) did not fall within the 95% CI at 37°C implying that the in vitro release profile at these elevated temperatures was statistically different from that at 37°C. The goodness of fit (R^2) for the analyses was >0.98 for the release data at all the temperatures. The slightly low values of R^2 were thought to be a consequence of a poor model fit (solid line) during the initial phase of release (Figures 4A and 5A).

In order to obtain a better fit, the data were normalized to accommodate the assumptions for the Weibull function (ie, zero to minimal burst release [burst release occurs due to surface-associated and/or unincorporated drug and therefore is not involved in drug release from the polymeric matrix, which is addressed by the Weibull function]). The initial burst was excluded from the percentage cumulative in vitro release data, prior to analysis using the Weibull expression. For parenteral controlled delivery systems such

Table 2. Results of Weibull Analysis for In Vitro Peptide Release (Including Burst Release)*

Parameter	37°C, 0.1M PBS pH 7.4	50°C, 0.1M PBS pH 7.4	55°C, 0.1M PBS pH 7.4	60°C, 0.1M PBS pH 7.4	50°C, 0.1M Acetate Buffer pH 4.0
α (day^{-1})	4.339×10^{-4}	9.2410^{-2}	0.1151	0.5068	0.1662
SE (α)	2.044×10^{-5}	1.020×10^{-2}	1.212×10^{-2}	2.095×10^{-2}	2.475×10^{-2}
95% CI (α)	-1.145×10^{-5} - 8.792×10^{-4}	7.099×10^{-2} -0.1138	9.005×10^{-2} - 0.1401	0.4629 - 0.5507	0.1127 - 0.2196
β	2.684	2.048	2.558	1.696	2.671
SE (β)	0.1636	0.0966	0.1225	0.0794	0.2099
95% CI (β)	2.327 - 3.040	1.845 - 2.251	2.305 - 2.811	1.530 - 1.862	2.218 - 3.125
R^2	0.9953	0.9887	0.9883	0.9909	0.9846

*PBS indicates phosphate buffered saline; and CI, confidence interval.

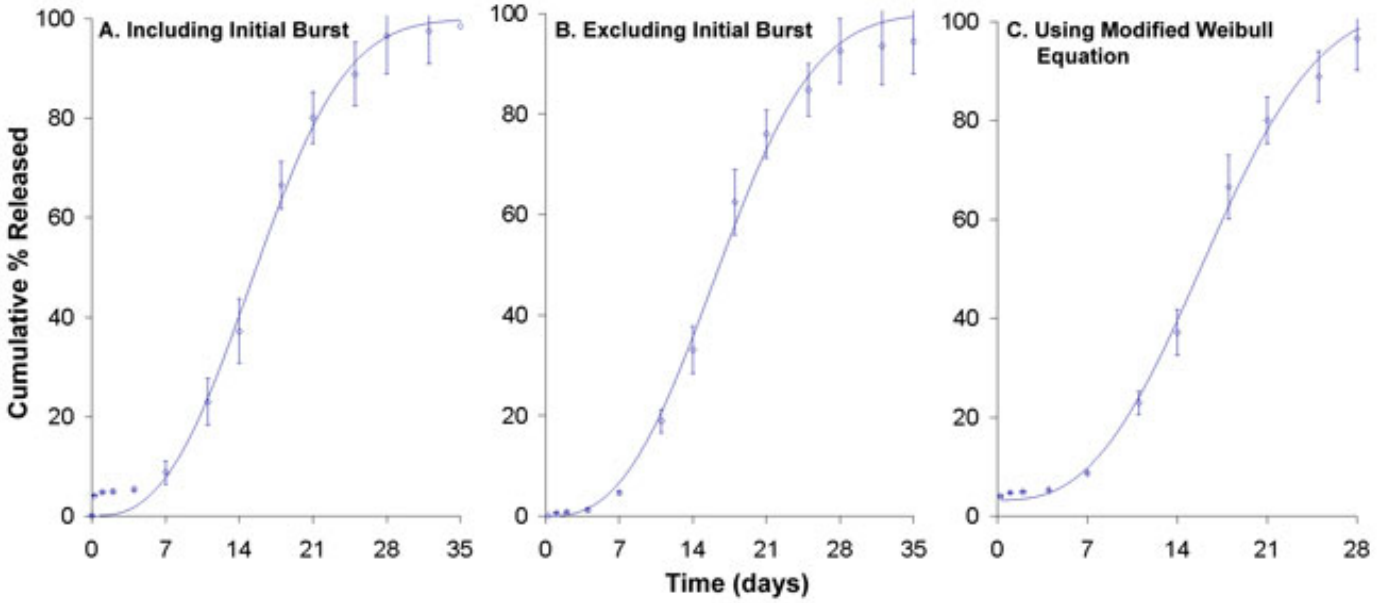

Figure 4. Results of the Weibull fit for long-term release of leuprolide from PLGA microspheres at 37°C in 0.1M PBS pH 7.4. Model fit shown as a solid line.

as polymeric microspheres, a high initial burst is desired in some cases to provide a loading dose of drug. This may be achieved by reducing the particle size (increasing surface area) or by addition of drug to the formulation, postmanufacturing. After this initial bolus, drug release is controlled by hydration and erosion of the polymer matrix, which continues for extended periods of time. Elimination of the initial burst during analysis would allow modeling of drug release from the polymer matrix based on the encapsulated drug (ie, release due to diffusion and erosion). Since the predominant processes governing release from the microspheres are polymer hydration and erosion, the release may be normalized to exclude the burst. Results of the model fit for 37°C and 50°C to 60°C in 0.1M PBS, pH 7.4, are shown in Figures 4B and 5B, respectively. The solid line represents the fit using nonlinear regression and the actual data points along with standard deviation represented by the markers. By observation alone, the goodness of fit (R^2) is apparent and is further evident in Table 3. Values for the model parameter α, which is indicative of the rate constant, increased from 3.787×10^{-4} at 37°C to 0.4115 day^{-1} at 60°C and demonstrated a temperature dependence. The values for α, a scaling factor for the rate constant (Table 3), were numerically lower for all the temperatures when the initial

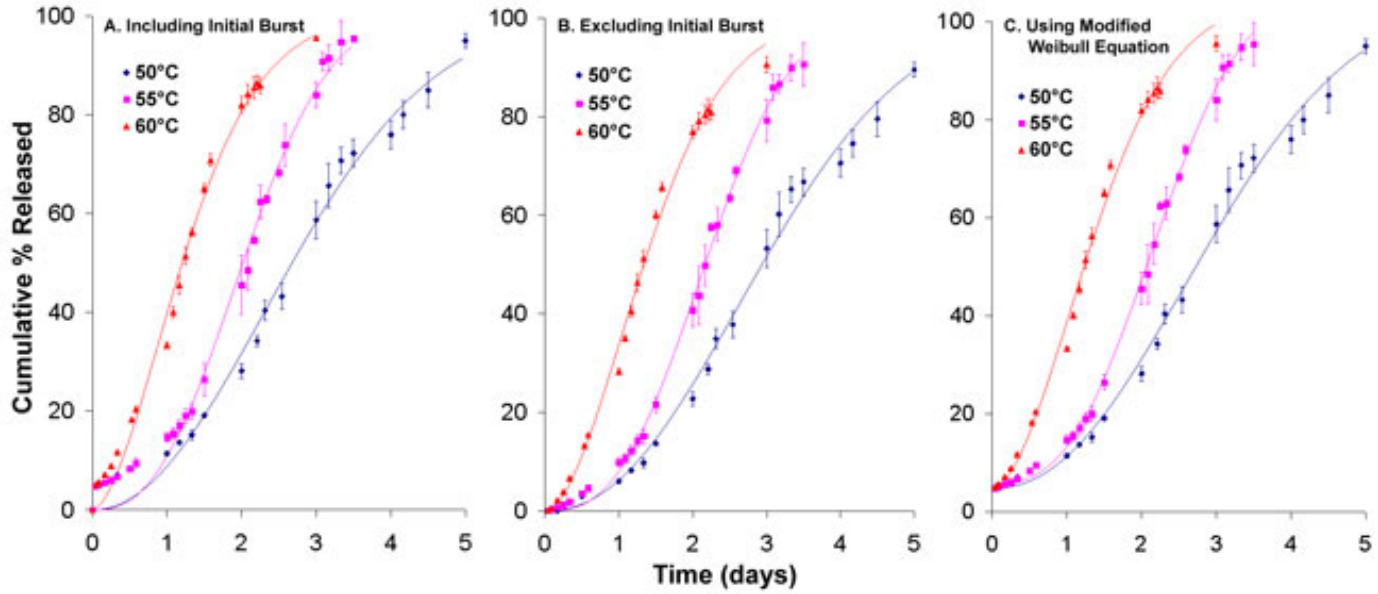

Figure 5. Results of the Weibull fit for short-term release of leuprolide from PLGA microspheres at elevated temperatures in 0.1M PBS pH 7.4. Model fit shown as a solid line.

Table 3. Results of Weibull Analysis for In Vitro Peptide Release (Eliminating Burst Release)*

Parameter	37°C, 0.1M PBS pH 7.4	50°C, 0.1M PBS pH 7.4	55°C, 0.1M PBS pH 7.4	60°C, 0.1M PBS pH 7.4	50°C, 0.1M Acetate Buffer pH 4.0
α (day^{-1})	3.787×10^{-4}	6.468×10^{-2}	8.353×10^{-2}	0.4115	0.1197
SE (α)	1.808×10^{-5}	6.440×10^{-3}	4.648×10^{-3}	1.387×10^{-2}	0.0194
95% CI (α)	-1.932×10^{-5} - 7.767×10^{-4}	5.109×10^{-2} - 7.827×10^{-2}	7.3910^{-2} - 9.315×10^{-2}	0.3824 - 0.4407	7.827×10^{-2} - 0.1612
β	2.677	2.201	2.740	1.795	2.711
SE (β)	0.1623	0.08344	0.06183	0.06074	0.1978
95% CI (β)	2.320 - 3.034	2.025 - 2.377	2.612 - 2.868	1.667 - 1.923	2.280 - 3.142
R^2	0.9950	0.9928	0.9975	0.9950	0.9857

*PBS indicates phosphate buffered saline; and CI, confidence interval.

burst was not considered. This result could be because when the initial burst was considered, cumulative percentage release of peptide was higher, leading to a slight increase in α values.

However, when model fit was attempted by eliminating the initial burst, in vitro release obtained experimentally was less than 100% when plotted (Figures 4B and 5B) and is undesirable. Therefore, the Weibull equation was modified to include the initial burst:

$$X/X_{inf} = X_{burst}/X_{inf} + \{1 - \exp[-\alpha(t^{\beta})]\} \qquad (2)$$

where X_{burst} = percentage burst release of peptide. Figures 4C and 5C and Table 4 show the result of the model fit to the experimental data using the modified Weibull equation.

Results for the shape parameter β at 37°C were similar (2.684, 2.677 vs 2.757) when the initial burst was included, eliminated, or with the modified equation (Tables 2-4). In comparison, slightly higher values for β were obtained at all the elevated temperatures (Tables 3-4) when the initial burst was eliminated from the in vitro release data or with the modified Weibull equation. A comparison of β values at 50°C (2.182), 55°C (2.776), and 60°C (1.788) with the 95% CI at 37°C (2.425-3.089) suggested once again that the shape of the short-term release curve at 55°C was similar to that at 37°C. The β value at 60°C (1.788) was lower than at 50°C (2.182) and 55°C (2.776) indicating the rapid increase in release rate (0.07816 day^{-1} at 55°C vs 0.418 day^{-1} at 60°C) caused the change in the shape of the profile. Therefore, data analysis using the modified Weibull function showed that short-term in vitro release at 50°C and 55°C in 0.1M PBS, pH 7.4, were mathematically and statistically similar with long-term release at 37°C in the same media. Figure 6 shows a correlation of time required for release at 37°C and at 55°C in 0.1M PBS, pH 7.4. There is extremely good correlation between 15% and 85% peptide release. This plot serves to show that there is a good correlation between the 2 temperatures, the goodness of fit (R^2 = 0.98) confirming results obtained after nonlinear regression analysis of the data. The slope of the curve reveals that release achieved within 1 day at 55°C is equivalent to 7.5 days at 37°C.

Release of peptide was triphasic at 37°C and the elevated temperatures, showing an initial burst followed by predominantly diffusional release due to polymer hydration, which

Table 4. Results of Model Fit for In Vitro Release (Modified Weibull Equation)*

Parameter	37°C, 0.1M PBS pH 7.4	50°C, 0.1M PBS pH 7.4	55°C, 0.1M PBS pH 7.4	60°C, 0.1M PBS pH 7.4	50°C, 0.1M Acetate Buffer pH 4.0
α (day^{-1})	3.1210^{-4}	6.764×10^{-2}	7.816×10^{-2}	0.4180	0.1298
SE (α)	1.337×10^{-5}	9.352×10^{-3}	5.209×10^{-3}	2.389×10^{-2}	2.572×10^{-2}
95% CI (α)	3.743×10^{-6} - 6.024×10^{-4}	4.782×10^{-2} - 8.747×10^{-2}	6.736×10^{-2} - 8.896×10^{-2}	0.3675 - 0.4684	7.252 - 0.1871
β	2.757	2.182	2.776	1.788	2.746
SE (β)	0.1438	9.455×10^{-2}	6.379×10^{-2}	6.564×10^{-2}	0.2112
95% CI (β)	2.425 - 3.089	1.981 - 2.382	2.644 - 2.908	1.650 - 1.927	2.276 - 3.127
X_{burst}	3.278	4.792	5.840	4.675	5.335
SE (X_{burst})	1.121	1.368	0.5713	1.098	2.294
95% CI (X_{burst})	0.6932 - 5.864	1.892 - 7.692	4.656 - 7.025	2.358 - 6.992	0.2225 - 10.45
R^2	0.9965	0.9929	0.9978	0.9978	0.9867

*PBS indicates phosphate buffered saline; and CI, confidence interval.

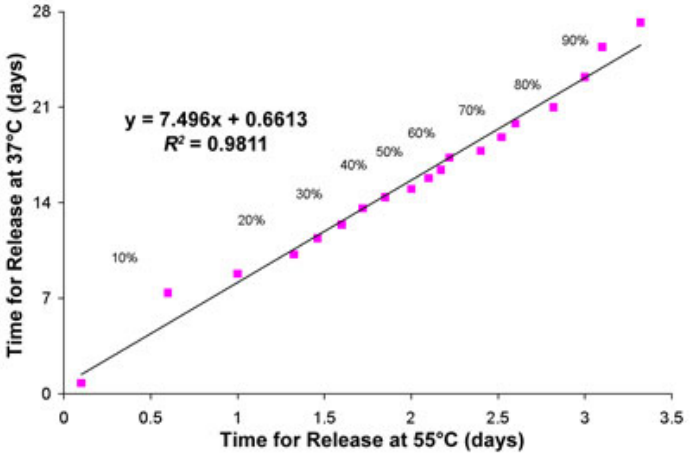

Figure 6. Short-term (55°C) versus long-term correlation of leuprolide release from PLGA microspheres in 0.1M PBS pH 7.4.

caused bulk hydrolysis of the polymer leading to the erosional phase of release. Figure 7 shows the influence of temperature on the diffusional phase of peptide release (<10%-15% peptide release). Temperature did not change the initial burst of peptide from the microspheres. However, it increased the rate of subsequent diffusional and erosional release as indicated by the slopes at the respective temperatures. The energetics of diffusional release was calculated using Arrhenius treatment, which expresses the temperature dependency of a reaction by a relationship between the activation energy (Ea), absolute temperature (T), and the specific rate constant (k) at that temperature.

$$k = Ae^{-Ea/RT}, \tag{3}$$

where A is a constant and R is the universal gas constant (1.987 cal/mol).

The Ea for diffusional release (Figure 8) was calculated to be 42.3 kcal/mol. An analysis of the erosional phase of peptide release also revealed a temperature dependence (Figure 9), the slopes from 50°C to 60°C being indicative of faster release rates at higher temperatures. Of interest, once hydrated, peptide release profiles during the erosion phase for all the temperatures appeared to be similar (Figure 10), with faster rates at short-term when compared with long-term release. The center graph shows that the release at 37°C and 55°C are nearly superimposable. Using the Arrhenius equation, the Ea for the erosional phase of release (Figure 11) was calculated to be 19.4 kcal/mol, which is less than half of that obtained for the diffusional phase of peptide release. From the Ea values, it could be inferred that polymer hydration was the rate limiting step in peptide release. This was expected as PLGA polymers are known to hydrate slowly prior to bulk hydrolysis of the

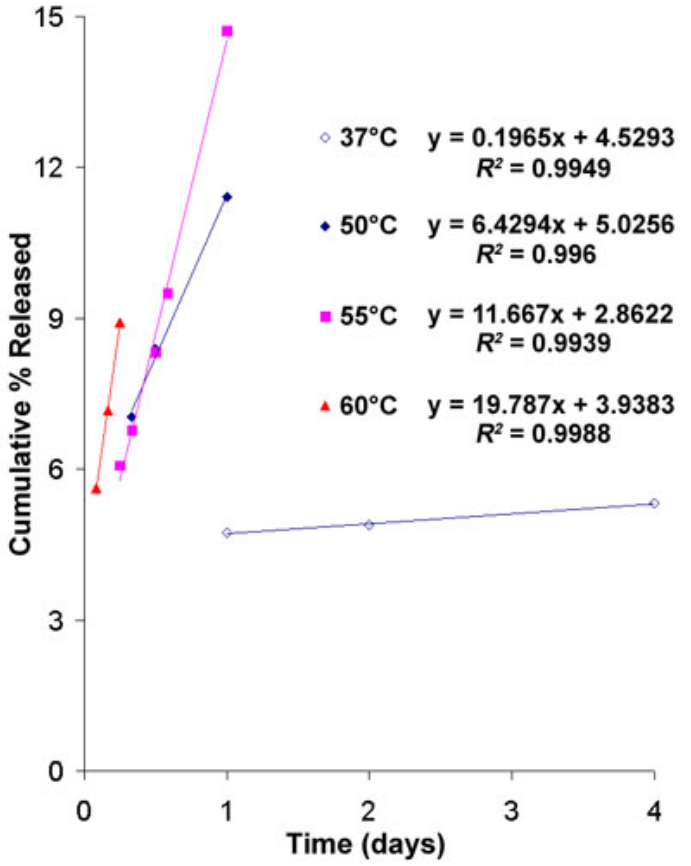

Figure 7. Effect of temperature on the diffusional phase of peptide release in 0.1M PBS pH 7.4.

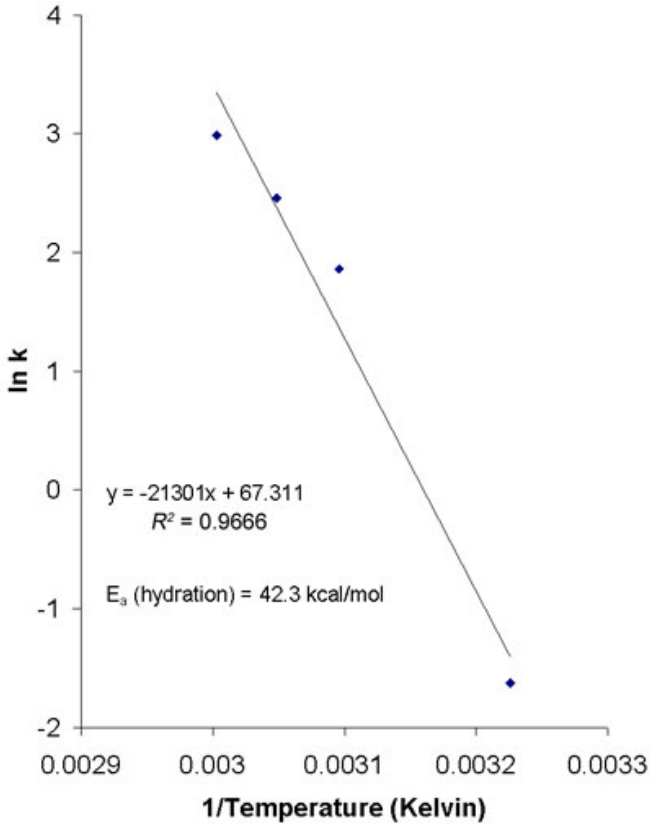

Figure 8. Arrhenius plot for the diffusional phase of peptide release in 0.1M PBS pH 7.4.

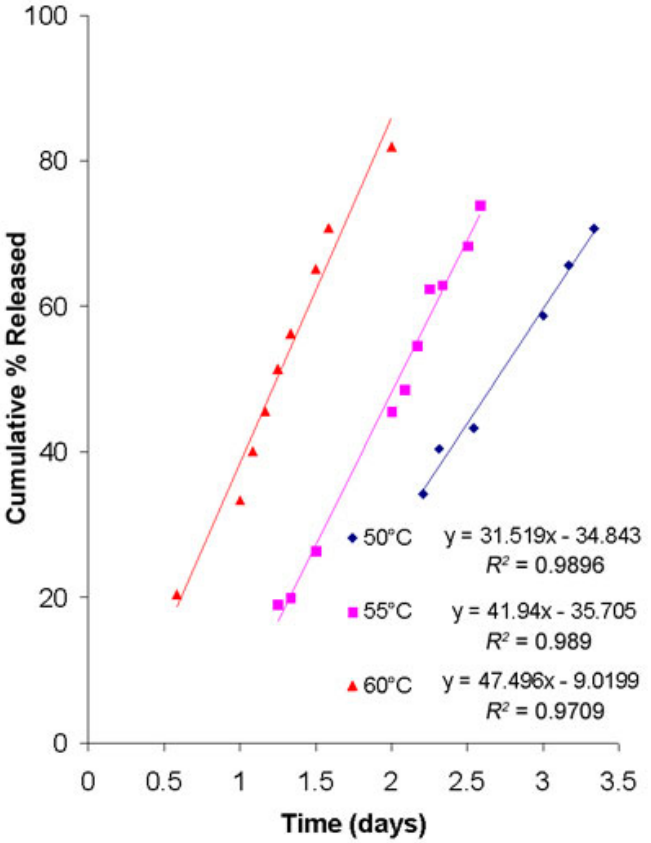

Figure 9. Effect of temperature on the erosional phase of peptide release at elevated temperatures in 0.1M PBS pH 7.4.

polymer, which accelerates due to cleavage of the ester bonds of the polymer backbone forming acid end groups.

The effect of pH on accelerated release is illustrated in Figure 12. Since previous studies showed that peptide release from microspheres was faster at acidic pH,[14] a comparison of the peptide release at 50°C in 0.1M PBS at pH 7.4 and 0.1M acetate buffer pH 4.0 is shown in Figure 12A. Complete release at acidic pH occurred within 3 days as compared with 5 days at nearly neutral pH, in which case polymer degradation would be slower. This result may be explained using the theory of general acid-base catalysis, where cleavage of the ester bonds in the polymer is induced by an excess of H^+ or OH- ions in the media, with faster hydrolysis obtained under alkaline conditions. This was reported by Makino et al[9] who observed a significantly faster decrease of weight-average MW of poly(D,L-PLA) microcapsules in highly alkaline media (pH 9.6) than in highly acidic (pH 1.6) or nearly neutral conditions (pH 7.4).

In this study, although the mechanism of catalysis is different with H^+ ions than with nearly neutral pH, where water molecules act as a catalyst, both moieties cause polymer degradation due to hydrolysis of the ester bond, the reaction being faster at acidic pH. Accelerated release in acetate buffer was able to simulate long-term release at 37°C in 0.1M PBS at pH 7.4 (Figure 12B), suggesting that parameters such as pH and buffer type could be used along with elevated temperatures to shorten the time required for an in vitro release study.

Using the modified Weibull function to analyze short-term release at 50°C in 0.1M acetate buffer pH 4.0, the model parameters α and β were generated (Figure 12C) and compared with long-term in vitro release data (Table 4). Nonlinear regression of the data showed a goodness of fit (R^2) of 0.9867. As expected, the value of the α, a scaling factor for the apparent rate constant at 50°C in 0.1M acetate buffer

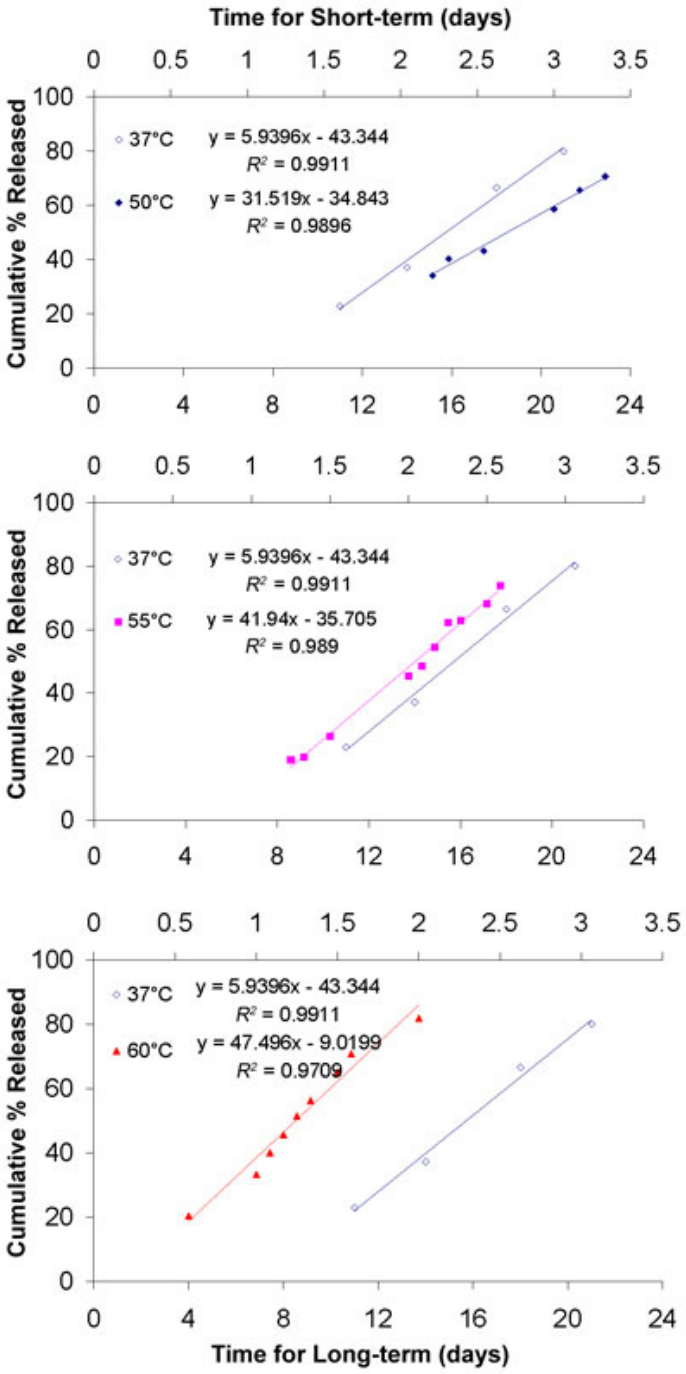

Figure 10. Comparison of release profiles at short- and long-term during erosional phase of peptide release in 0.1M PBS pH 7.4.

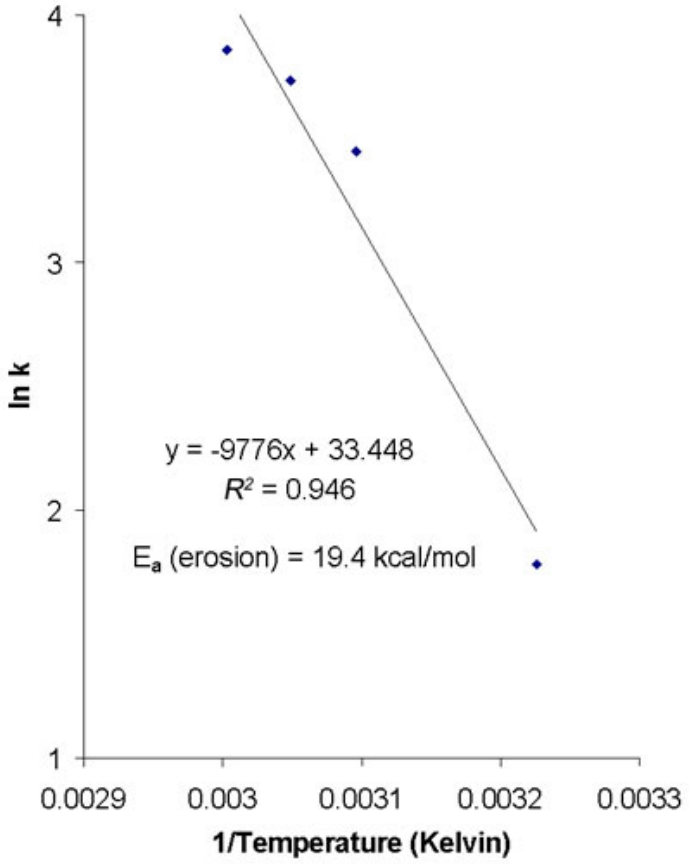

Figure 11. Arrhenius plot for the erosional phase of peptide release in 0.1M PBS pH 7.4.

pH 4.0 was higher (0.1298 day^{-1}) when compared with 3.1210^{-4} and 0.07816 day^{-1} at 37°C and 55°C in 0.1M PBS pH 7.4, respectively. Values for the shape factor β (Table 4), were similar to that obtained at 37°C (2.757 vs 2.746, respectively) and at 55°C in 0.1M PBS pH 7.4 (2.776). The mean value for β at 50°C in 0.1M acetate buffer pH 4.0 lay in the 95% CI of β for long-term release (2.425-3.089), suggesting that short-term release at 50°C in 0.1M acetate buffer pH 4.0 was similar to long-term release at 37°C in 0.1M PBS pH 7.4.

The molecular weight change of polymer at 37°C, 50°C, and 55°C in 0.1M PBS pH 7.4 is shown in Figure 13. As expected, polymer degraded faster at higher temperatures. Table 5 lists the values of the first order polymer degradation rate constant k at the elevated temperatures. The Ea for polymer hydrolysis (Figure 14) calculated using the Arrhenius equation was 31.6 kcal/mol.

From this study, it can be seen that short-term studies on extended release dosage forms can provide useful information on the behavior of the formulation under long-term (37°C) conditions. In addition to predicting real-time in vitro release from biodegradable microspheres, short-term accelerated studies could also be used to

1. characterize batch-to-batch variation in clinical and commercial batches (quality control),
2. predict long-term in vitro behavior and possibly in vivo release of a formulation,
3. aid design and development of microspheres containing therapeutic agents, and
4. serve as a product release specification, once conditions for a short-term study are optimized.

Since the results obtained from short-term studies can be extrapolated to provide crucial information regarding real-time behavior of a formulation in a matter of hours to a few

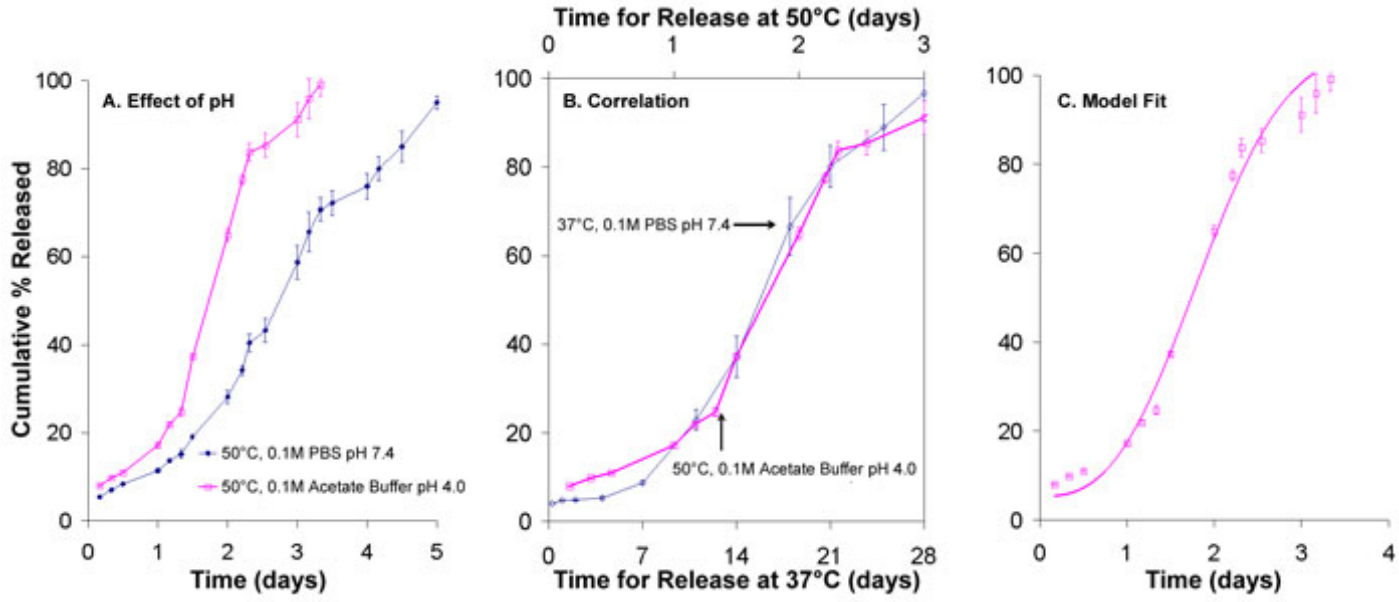

Figure 12. (A) Effect of pH on peptide release at 50°C; (B) correlation with release at 37°C in PBS pH 7.4; and (C) model fit for data at 50°C in 0.1M acetate buffer using modified Weibull function.

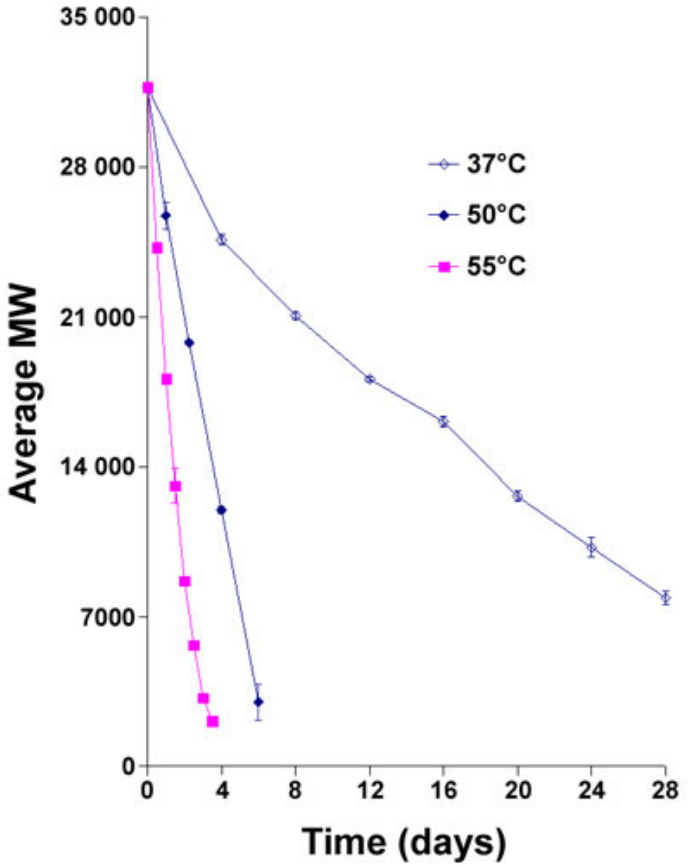

Figure 13. Effect of temperature on polymer degradation in leuprolide PLGA microspheres in 0.1M PBS pH 7.4.

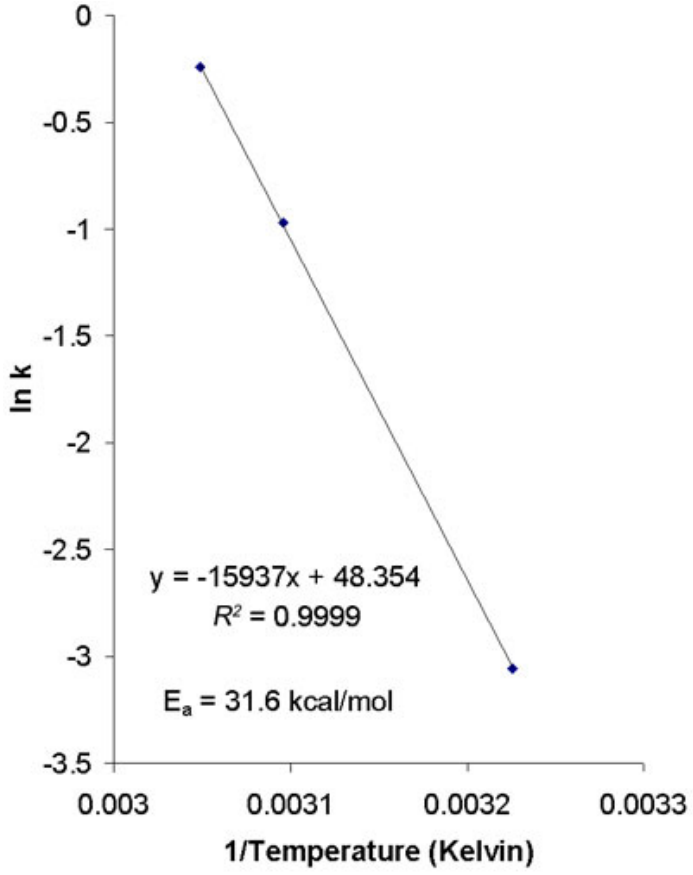

Figure 14. Arrhenius plot for polymer degradation in 0.1M PBS pH 7.4.

days, the effect of parameters that influence drug release need to be well characterized and optimized for a drug candidate-polymer delivery system. These include temperature, buffer type and concentration, pH, surfactant, and agitation, all of which affect release kinetics. These parameters are important in the design of in vitro release studies, short- or long-term, and influence the rate at which the polymer hydrates initially followed by subsequent degradation (mechanisms governing drug release). Parameters used in the selection of a short-term study should increase drug release rate without changing the release profile (ie, hasten the rate of polymer hydration and degradation). The effect of the selected parameters on the release rate and profile can be assessed using a model dependent approach such as the Weibull function. Data analysis can identify parameters that can optimize short-term conditions to correlate with real-time release. Once the parameters have been selected, robustness of the short-term or accelerated methodology should be assessed by testing for batch reproducibility (within a lot and between lots) and lots that do not meet manufacturing specifications, followed by linear or nonlinear regression (as shown in this study) to characterize and quantify differences in release. This research will enable use of accelerated studies as a quality control tool to test clinical and commercial batches.

Table 5. First Order Rate Constants for Polymer Degradation in 0.1M PBS pH 7.4

Temperature (°C)	First Order Rate Constant (k, day^{-1})	R^2
37	0.0470	0.9890
50	0.2419	0.9918
55	0.7846	0.9860

CONCLUSIONS

An accelerated, short-term in vitro drug release method for microspheres should provide for (1) rapid assessment of formulations, especially in the development phase, and (2) correlation with, and prediction of, release at 37°C, thereby permitting its use as a batch-release specification.

In addition, accelerated studies would be a useful quality control technique to assess batch-to-batch variation.

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