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A Retrospective Mathematical Analysis of Controlled Release Design and Experimentation

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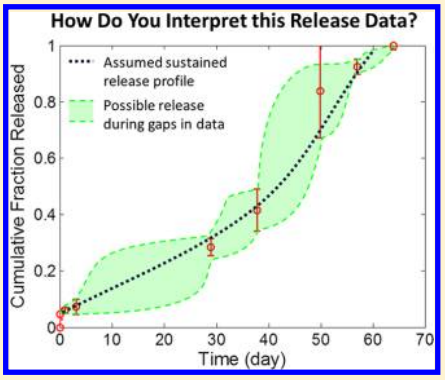
Supporting Information

ABSTRACT: The development and performance evaluation of new biodegradable polymer controlled release formulations relies on successful interpretation and evaluation of *in vitro* release data. However, depending upon the extent of empirical characterization, release data may be open to more than one qualitative interpretation. In this work, a predictive model for release from degradable polymer matrices was applied to a number of published release data in order to extend the characterization of release behavior. Where possible, the model was also used to interpolate and extrapolate upon collected released data to clarify the overall duration of release and also kinetics of release between widely spaced data points. In each case examined, mathematical predictions of release coincide well with experimental results, offering a more definitive description of each formulation's performance than was previously available. This information may prove particularly helpful in the design of future studies, such as when calculating proper dosing levels or determining experimental end points in order to more comprehensively evaluate a controlled release system's performance.



KEYWORDS: *in vitro release, mathematical modeling, PLGA, microspheres*

INTRODUCTION

Controlled release technology holds tremendous potential to advance medical treatment as well as facilitate scientific inquiry. For instance, controlled release formulations made from biodegradable polymer matrices (BPMs) improve drug safety and patient adherence, barriers that are responsible for 10% of hospitalizations and over $100 billion in medical expenses annually.[1] Controlled release formulations have also served as the tool that has enabled scientific studies on a broad range of topics including animal behavior (via antisense DNA delivery),[2] vasculo- or angiogenesis (via growth factor delivery),[3] and neonatal vaccination (via antigen−adjuvant delivery).[4] Yet, for successful use in medicine or research, BPMs must be carefully designed or tuned to deliver drug within a specific dosing range and time frame (or release profile). Currently, this development process is nontrivial since each of a number of factors, including polymer composition and size, drug size and charge, matrix geometry and porosity, fabrication procedure, and drug loading (as just a few examples), affects the rate and duration of drug release.[5] Because of this, design tends to progress heuristically through the iterative adjustment of formulation properties and assaying of formulation performance.[5,6] This dramatically increases both the time and cost associated with development and evaluation of new controlled release systems over other formulation technologies.

To provide a first-pass estimation of formulation performance, simple *in vitro* release assays are utilized where released drug is sampled from the surrounding supernatant or exudate over time. These assays are useful because they provide a cost-effective means of determining how a formulation should be redesigned to achieve a target behavior or alternatively the dosing required in the context of subsequent *in vitro* or *in vivo* tests of effectiveness.[7] However, designing *in vitro* release studies to effectively capture the full range or duration of a formulation's release kinetics can be nontrivial for two key reasons: (1) It is often unclear how long to carry out an assay, as BPM formulations can release drug for days, weeks, or months, depending on their composition.[5] (2) A formulation's rate of drug release can fluctuate dramatically over the course of an assay, making it difficult to time data collection without foreknowledge of when changes in the rate of release occur. In fact, as many as three phases of release (initial burst, lag phase, and secondary burst) have been documented from biodegradable polymer microparticles depending on their composition.[8] Because of these issues, release assays often yield data that leaves researchers uncertain of a formulation's peak and trough drug concentrations or the duration of drug delivery, complicating design of subsequent cell and animal studies.

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The challenges faced in interpreting the sometimes incomplete data generated by *in vitro* release assays are similar to those encountered in analysis of pharmacokinetic (PK) data. Like *in vitro* release assays, the data collected in PK studies provides evidence of drug concentration only at discrete points in time. However, in PK studies today, it is now standard to employ proven mathematical models that allow researchers to accurately interpolate and extrapolate upon collected data.[9] This type of analysis is essential for the accurate computation of the important PK processes adsorption, distribution, metabolism, and excretion.[10] If comparable model-driven analysis were regularly applied to *in vitro* release data as well, then key performance parameters such as rate and duration of release could be determined for incomplete or sparse data sets. Further, model predictions could permit sample points to be optimally timed in subsequent *in vitro* or *in vivo* assays if these parameters need to be validated more fully. Such analysis would be particularly valuable when designing formulations of the extremely popular polyester materials, like poly(lactic-*co*-glycolic) acid (PLGA), that often release drug in a pattern of bursts that are often difficult to anticipate.[11]

Accordingly, the current study retrospectively evaluates existing sets of release data using mathematical predictions of controlled release in order to generate additional insight into the formulations' release behaviors. Specifically, data sets for evaluation were selected to illustrate common issues, like lack of closure or widely spaced sampling times, that make their results difficult to definitively interpret. The mathematical model used for this evaluation has been previously validated for its predictive capabilities on active agents ranging from small molecules to large proteins, much like those considered herein.[8a,12] However, when appropriate, additional *in vitro* release assays were carried out to validate sets of interpolative and extrapolative model predictions. Overall, these mathematical evaluations of *in vitro* release data have the potential to yield useful information about formulation performance and insight that can guide the design of future studies.

MATERIALS AND METHODS

Materials. Poly(lactic-*co*-glycolic) acid (PLGA) RG502 ($Mw_o \approx 9$ kDa) was purchased from Boehringer Ingelheim. Enfuvirtide (T-20) was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH, from Roche. Nitro oleic acid (NO_2-OA) was composed by an equal mixture of 10-NO_2-octadec-9-enoic acid and 9-NO_2-octadec-10-enoic acid and synthesized as previously described.[13] Solvents, assay reagents, and other chemicals were purchased from Thermo Fisher Scientific.

Microparticle Fabrication. Microparticles were prepared using the emulsion processing technique as described.[14] Briefly, 200 mg of RG502 PLGA was dissolved in 4 mL of dichloromethane (oil phase). One hundred microliters of an agent stock solution (either 2 mg/mL enfuvitide in 2.4 mg/mL sodium carbonate solution or 1 mM NO_2-OA in methanol) was added to the oil phase, which was then mixed for 10 s using a probe sonicator (Sonics and Materials Inc.). This mixture was then homogenized at 2,300 rpm for 1 min in 60 mL of 2% PVA (MW ~ 25,000, 98% hydrolyzed) solution, using a homogenizer (Silverson L4RT-A). This emulsion was immediately poured into 80 mL of 1% PVA solution, and dichloromethane was allowed to evaporate. After 3 h, the particles were centrifuged (1500*g*, 10 min, 4 °C) and washed 4× in deionized water. Microparticles were then resuspended in 5 mL of deionized water, frozen on dry ice, and lyophilized (Virtis Benchtop K freeze-dryer; operating at 60 mTorr).

Microparticle Characterization. Microparticles were sized using the volume impedance method with a minimum of 10,000 measurements on a Beckman Coulter Counter (Multisizer 3). Microparticle surface morphology and shape were examined using a scanning electron microscope (JEOL JSM-6330F). The loading of the enfuvirtide microparticles was measured by dissolving 5 mg of microparticles in 250 μL of dimethyl sulfoxide (DMSO). Peptide was then extracted with the addition of 1 mL of 0.05 M NaOH/5% sodium dodecyl sulfate (SDS) and measured using the microBCA assay. The loading of NO_2-OA could not be measured by this method as the presence of SDS interfered with the detection of this agent.

***In Vitro* Release Assay.** Release data was measured accumulatively, as done previously.[14] A known amount of microparticles was suspended in 500 μL of phosphate buffered saline (DPBS, pH 7.4, GIBCO, Invitrogen) and placed on an end-to-end rotator at 37 °C. At regular intervals the microparticle suspensions were centrifuged, allowing the supernatant to be collected and particles to be resuspended in an equal volume of DPBS.

Enfuvirtide concentration in the supernatant was detected either by microBCA or by high pressure liquid chromatography (HPLC). The microBCA assay was carried out using a Spectramax M5 microplate spectrophotometer (Molecular Devices) as detailed in the manufacturer's protocol (Pierce, Thermo Fischer). For HPLC, detection was carried out as done previously.[15] A Dionex Ultimate 3000 HPLC system (Thermo Fisher) was used with a XTerra RP18 5 μm 3.0 × 15 mm column (Waters Corporation), and enfuvirtide was detected on a Dionex RF2000 fluorescence detector (ex = 280 nm, em = 350 nm). Solvents and gradients were kept consistent with previous work, yielding peaks for enfuvirtide at 4.86 min and insulin (internal standard) at 2.6 min.

NO_2-OA concentration was quantified using high-performance liquid chromatography−electrospray ionization mass spectrometry (HPLC−ESI MS/MS) as previously reported.[13,16] Briefly, the released NO_2-OA was chromatographically resolved using a 20 × 2 mm cartridge column (Mercury MS Gemini 3 μm C18, 110 Å, Phenomenex) with a flow rate of 0.75 mL/min using a water (A)/acetonitrile (B) solvent system containing 0.1% acetic acid. A linear gradient of B from 11% to 100% was developed in 3.5 min and used to separate the ions, followed by their detection on a triple quadrupole mass spectrometer (API 5000, Applied Biosystems/MDS Sciex) using the specific 326.3/46 and 344.3/46 transitions for NO_2-OA and $^{13}C_{18}$-NO_2-OA respectively. For quantification, peak areas of NO_2-OA and $^{13}C_{18}$-NO_2-OA were calculated using Analyst 1.4.2 quantification software (Applied Biosystems/MDS Sciex), and the ratio of analyte to internal standard was calculated to determine the concentration.

Collection of Published Data. Published data was collected from the figures of manuscripts using Plot Digitizer software (v. 2.4.1), as done previously.[8a,12] Release profiles in these figures were enlarged to a size of 600 by 400 pixels allowing accurate measurement of the scales on their axes. Data points were then manually targeted, yielding numerical coordinates for each point. All sets of published release data collected were assayed accumulatively, by measuring the concentration of drug or protein released into the medium.

Model Predictions. All mathematical predictions of controlled release were made using the model developed by

Rothstein et al., as done previously.[8a] A summary of the model, its equations, and a glossary of variables appears in the Supporting Information (pages 1−2). These equations were coded in MATLAB(v7.12) and solved using the finite element method with COMSOL(v3.5a). To initialize solutions, values for the drug's molecular weight (Mw_A), the polymer's initial molecular weight (Mw_o) and the microparticle radius (R_p) were assigned based on the published materials and methods of each system considered. The value for occlusion size (R_{occ}) was calculated from SEM images of the microparticle internal morphology, when available, and otherwise was back-calculated from the average magnitude of the given system's initial burst as derived previously.[8a] As R_{occ} only contributes to the prediction of initial burst magnitude, this back-calculation does not interfere with the model's predictions regarding the timing or rate of the secondary burst, which are of primary focus in this work. The polymer degradation rate (kC_w) was held constant for each copolymer ratio as follows: 50:50 PLGA, kC_w = 0.08636 day^{-1}; 75:25 PLGA, kC_w = 0.0342 day^{-1}; and PLA, kC_w = 0.0169 day^{-1}. With these input parameters, the model could then predict the given system's release profile. The results were then plotted against the respective set of experimental data for statistical analysis.

As the mathematical model used currently does not account for kinetics of the initial burst (only the magnitude[8a]), data points within the first 72 h of release were omitted from this statistical analysis. The accuracy of mathematical predictions was quantified by the normalized residual squared error (nRSS = residual sum of squares divided by the number of data points compared). For the Results section on widely spaced data points, equivalent calculations were made to quantify the prediction's deviation from linearity with a specified gap between two data points (nRSSi). Both of these metrics, unlike the r^2, do not unduly weight values near the mean.

RESULTS

Evaluation of published data reveals two major areas of opportunity where *in silico* predictions may be able to better inform the design and validation of degradable controlled release formulations. First, if data is collected infrequently enough to produce a jump in cumulative release, the duration and kinetics of release can be difficult to resolve and an *in silico* prediction can help interpolate between widely spaced data points. Second, release studies may also (for any number of reasons) be terminated prior to the completion of release, leaving subsequent kinetics undocumented and perhaps even leaving the impression that the partially documented profile is complete. In this case, *in silico* predictions can be used to extrapolate upon the existing release data. Both of these areas of opportunity are illustrated in more detail with the following examples:

Wide Spacing of Data Sampling Points. A number of data sets include intervals in sampling that result in low resolution of the release profile. This can occur for a number of reasons, including an expectation that release will follow a different pattern, unavailability of the researcher for empirical sampling, or concern that more closely spaced intervals would not allow for enough drug release to permit detection. However, when data sampling is too infrequent, important changes in the rate of drug delivery may be difficult to identify. This was the case when enfuvirtide release was measured from PLGA microparticles at 10 day intervals (Figure 1A). The low sampling rate was selected to ensure detection of enfuvirtide, a therapeutic peptide, by the micro BCA (μBCA) protein assay, which had a detection limit of 980 ng/mL. The resulting gaps in data accounting for 33% and 44% of drug release leave open many interpretations (broken lines, Figure 1A), which can be clarified by the model's prediction (solid lines, Figure 1A). Subsequently, release from these microparticles was measured using high pressure liquid chromatography (HPLC) which has a detection limit below 39.1 ng/mL for enfuvirtide, despite being less cost and time efficient. This detection method allowed enfuvirtide release to be measured every 1 to 2 days, filling in the gaps left by the prior study (Figure 1B) in order to validate any conclusions made through model predictions. The resulting HPLC-detected release profile closely follows the profile predicted by the earlier mathematical analysis, providing strong evidence that the interpolation based on model predictions accurately describes the actual enfuvirtide release behavior.

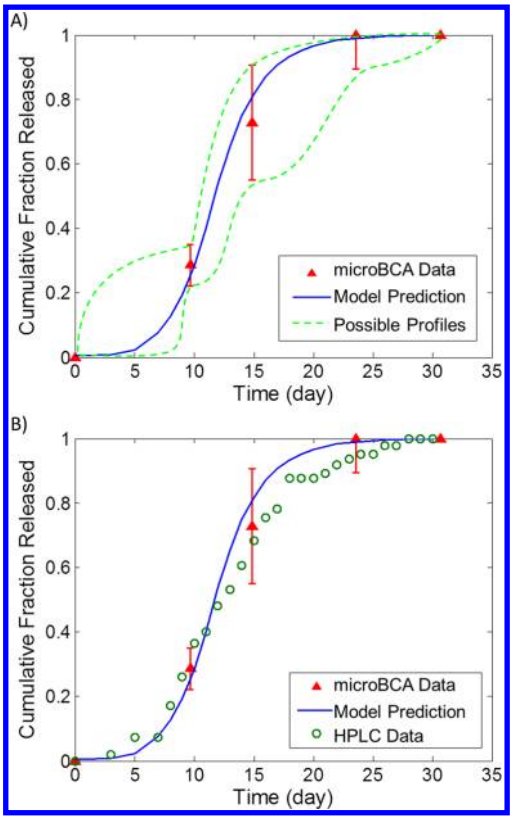

Figure 1. Testing model predictions for the interpolation of *in vitro* release data from enfuvirtide loaded 9 kDa 50:50 PLGA, 20 μm particles. (A) Initial release measurements using the μBCA assay with widely spaced intervals (triangles). Broken lines are meant to illustrate the range of qualitative interpretations that could be made using the reported data and standard deviations as bounds. An interpolative prediction was made using the mathematical model which would suggest that actual release profile (solid line). (B) Subsequent release measurements made by highly sensitive HPLC experiments at short intervals confirm the accuracy of the interpolative prediction (circles). (nRSS = 0.0030.)

As with our data, the literature includes a number of release data with significant gaps between sample points. For instance, in one assay of lysozyme release, over 40% of the drug is delivered during a 28 day window where no data was collected (Figure 2A).[17] As a result, the timing of the transition from first burst to second burst is unclear and could follow any number of patterns within the range marked by green curves. In this

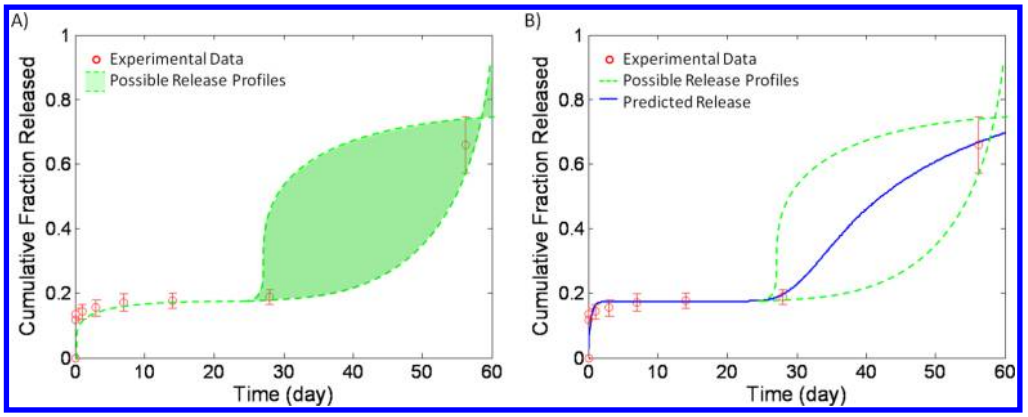

Figure 2. Evaluations of *in vitro* data for lysozyme encapsulated in 12 kDa 50:50 PLGA.[17] (A) *In vitro* release data points (circles) jump from 18% at day 28 to 66% by day 56, leaving a range of potential interpretations of the measured release data based on the reported standard deviations (broken lines, shaded area). (B) Mathematical results indicate a definitive release profile for this formulation (solid line).

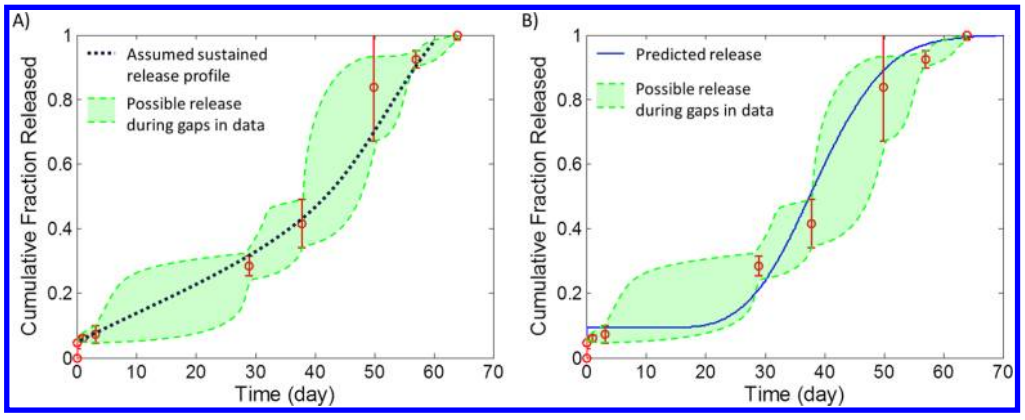

Figure 3. Evaluations of *in vitro* data for superoxide dismutase (SOD) encapsulated in 40−70 kDa 50:50 PLGA microparticles (14 μm).[18] (A) Gaps in this data (circles) suggest that SOD release could proceed in a sustained, linear fashion (dotted line) following the initial burst. (B) Mathematical results indicate a nonlinear release profile for this formulation (line).

Table 1. Interpolative Predictions

						gap size		error		
encapsulated agent	R_p (μm)	R_{occ} (μm)	Mw_o (kDa)	kC_w (day^{-1})	Mw_A (Da)	(day)	(%)	overall (nRSS)	linear (nRSSi)	ref
carbonic anhydrase	0.533	0.205	12	0.08636	68900	28	45	0.0020	0.0246	17
leuprolide	20	1.8	10	0.08636	1210	7	43	0.0014	0.0021	19a
octreotide	10.5	0.525	28	0.08636	1020	7	45	0.0025	0.0029	19b
dexamethasone	5.5	2.86	70	0.08636	392	13	37	0.0061	0.0061	20
gentamicin	276	69	36.2	0.08636	477	8	45	0.0012	0.0030	21a
butorphanol	200	40	29.7	0.3278	327	1	44	0.0062	0.0035	21b

specific case, mathematical analysis of this data suggests that release is predicted to continue in a sustained manner between data points without a more prominent burst of drug with possible upper and lower bounds at 30 or, alternatively, 50 days (Figure 2B). Importantly, the variation between this prediction and ideal linearity (a point-to-point interpolation in the gap; nRSSi = 3.0×10^{-4}) is similar to what is calculated as the overall model deviation from all other experimental data points (nRSS = 3.8×10^{-4}). In another study, superoxide dismutase release was measured at 6 points over 60 days (Figure 3A).[18] Because these sample points were spaced unevenly, significant gaps occur from days 3 to 29 and days 38 to 50, accounting for 15% and 40% of release, respectively. This leaves researchers a broad range of qualitative interpretations (the bounds of which are illustrated with shading). Potential interpretations include the possibility that release proceeds linearly over the 60 days (Figure 3A, broken line). However, in this specific case, the model prediction suggests that release is not sustained for the duration of 60 days. In contrast, a lag-burst pattern is predicted through the first gap where little release occurs between days 3 and 20, followed by rapid release from day 20 onward (Figure 3B). During this window (days 3 to 29), the prediction's deviation from point-by-point linear interpolation ($nRSSi_1$ = 1.27×10^{-2}) far exceeds the error inherent in the model's prediction (nRSS = 4.7×10^{-3}; $nRSSi_1$ > nRSS), suggesting that the predicted deviation from linearity in the gap is indeed statistically much greater than the collective deviation from other data points. In the second gap (days 38 to 50), the prediction's deviation from linearity ($nRSSi_2$ = 4.9×10^{-3}) is actually comparable to the error inherent in the model's prediction ($nRSSi_2 \approx$ nRSS), suggesting that no significant change in release rate is expected to occur during this gap.

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Significant gaps also appear in release data from PLGA microparticles loaded with model enzymes,[17] hormone antagonists,[19] and anti-inflammatory agents,[20] among others[21] (a summary of statistics IS included in Table 1, and corresponding release profiles appear on page 3 of the Supporting Information). In some cases, retrospective analysis predicts that release will not deviate from a sustained or linear manner during the gap between data points[19b,20,21b] (Table 1, nRSSi < nRSS or nRSSi ≈ nRSS). However, in other cases significant changes in release rate occur during gaps (Table 1, nRSSi > nRSS).[17,19a,21a]

Assay Termination Prior to Complete Release. Many sets of release data document only one burst when additional release would be expected.[8,11] This is not surprising given that the additional release, often in the form of a secondary burst, may occur only after weeks, months, or years, while most of the functional assays or animal models using these formulations require just days or weeks to complete. Indeed, our studies on the novel anti-inflammatory agent, NO_2-OA, began with a single batch of particles and 1 week release assay to confirm that this novel agent could be encapsulated and delivered intact from microparticles (Figure 4A). Specifications for this formulation, including size, polymer molecular weight, and internal matrix morphology, were measured and used to initialize a model prediction that extrapolates beyond the range of the preliminary data (Figure 4B). In order to test the accuracy of this prediction, the preliminary formulation was reproduced and assayed in triplicate for the full predicted duration of its release profile, with a sampling frequency selected to capture key changes in the rate of release. This second assay confirmed that the formulation's release profile closely follows the model prediction (Figure 4C). Notably, our initial release curve only captured less than 25% of the total release behavior, which was captured by the model prediction.

Like this prior example, the literature includes a number of examples where release assays are, for whatever reason, terminated prior to closure. Indeed, one set of PLA microparticles loaded with the protein superoxide dismutase (SOD) was assayed for 50 days without showing additional release beyond the initial burst. However, predictions indicate that SOD release will reach completion only in an additional 200 days (Figure 5).[18] Importantly, this will result in release of 40% of the encapsulated drug following day 150 if this formulation were placed *in vivo* and not removed before this time. In another study, recombinant human growth hormone (rhGH) release was measured for 28 days, recording delivery of 78% of the anticipated payload.[22] Mathematical predictions show that release, which appears to asymptote, actually began a secondary burst on day 20 (Figure 6). This burst continues until day 40, whereas no additional information is provided past day 30 by the assay. Predictions were also evaluated for data sets from PLA and PLGA formulations loaded with many other proteins, peptides, and small molecules[3a,23] (Table 2; Supporting Information, pages 4–5). For a number of these systems, the release is predicted to continue for more than 2 months beyond the end of *in vitro* data collection.[23b,c,f]

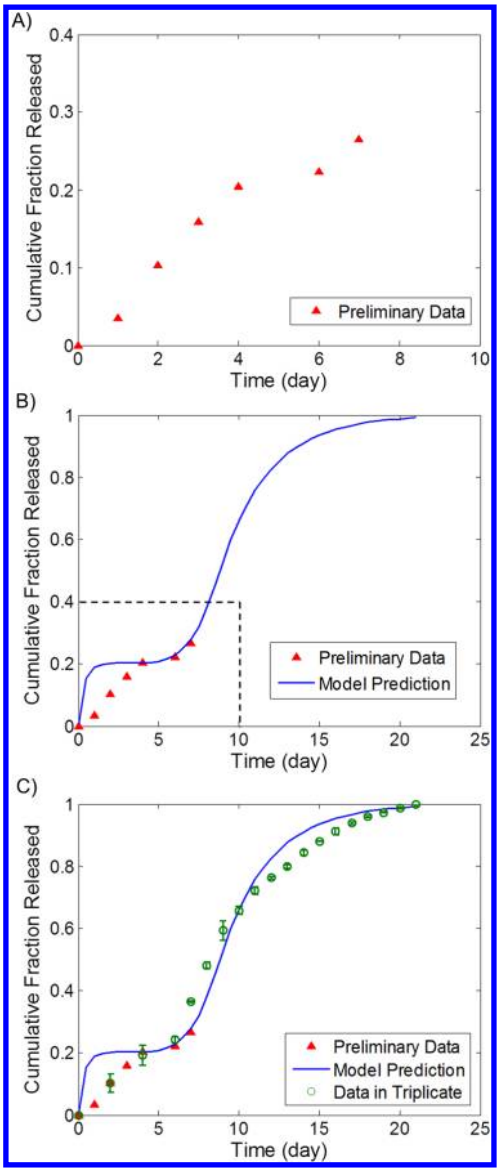

Figure 4. Testing an extrapolative model predicted for the *in vitro* release of NO_2-OA from 9 kDa 50:50 PLGA 20 μm particles. (A) Data from preliminary *in vitro* release assay. (B) Predicted release behavior for the same formulation. (Note, the model used does not include equations for capturing kinetics of the initial burst.) (C) Release from formulations produced in triplicate closely followed the model prediction. (nSSR = 0.0020.)

DISCUSSION

Retrospective analysis of *in vitro* release is the use of modern technology and understanding of release phenomena toward the interpolation and exploration of prior studies to gain further information on a formulation's performance in the absence of additional experimental data. For instance, published release assays can terminate before drug release is complete or contain widely spaced intervals of time between data points, complicating interpretation of a formulation's drug delivery kinetics. While such data may be suitable for planning initial functional assays that evaluate a formulation's activity, a more detailed understanding of a formulation's release kinetics would be useful when refining a formulation design or planning animal studies, like those for long-term dosing or evaluating disease outcome. Further, when translating formulations to human use, a full understanding of how a biodegradable polymer matrix releases an encapsulated agent is extremely important. To most efficiently plan such studies or possibly even make the best decision as to whether or not the studies would be fruitful, prior information is needed on (a) how the expected rate of release varies between data points, and/or (b) if or when additional

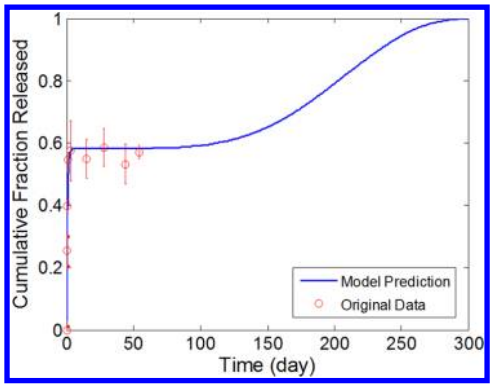

Figure 5. Release of superoxide dismutase SOD from 106 kDa poly(lactide) microparticles. *In vitro* data (circles) documents only 60% of release.[18] Prediction indicates that the remaining 40% of drug will be released between days 150 and 250.

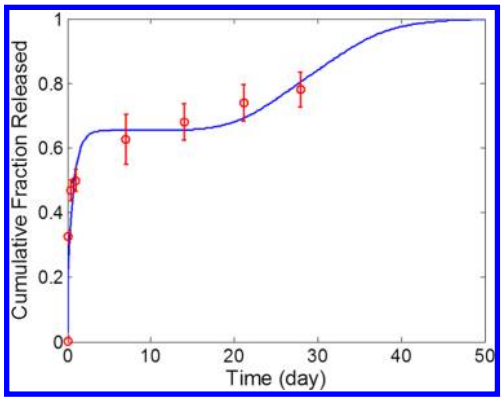

Figure 6. Release of rhGH from 45 kDa 50:50 PLGA microparticles. *In vitro* data (circles) was collected for only 28 days.[22] *In silico* predictions (line) show release continuing until day 45.

release can be expected beyond the release assay's termination. One possibility to obtain this information would be to iteratively conduct a series of long-term release assays. However, mathematical analysis of existing release data may offer a tremendous time and cost savings in obtaining this useful information.

In order to be useful for retrospective analysis, a mathematical model must effectively describe or predict a formulation's release kinetics, particularly in the regions where data are sparse or nonexistent. The model used herein has been previously validated on a broad range of bulk and surface eroding systems of various shapes and sizes using a wide variety of encapsulated drugs.[8a] Importantly for this study, the model has demonstrated consistent and accurate predictions of both the timing and rate of secondary burst release for small molecule-, peptide-, and protein-loaded microparticulate systems. This makes the model a strong fit for the broad range of agents considered herein. Its predictive capabilities are essential for handling analysis of data sets that lack information about the second burst and therefore require extrapolation. The predictive power of the current model sets it apart from others in the fields, which either lack the extensive validation needed to support predictions or focus on systems beyond the scope of the present work.[8b,24] This latter class of models should enable retrospective analysis of the kinetics of the initial burst, poorly soluble agents, or matrix implants, hydrogels, and novel controlled release systems.[8b,24]

The model-aided analysis used herein can help determine if a formulation actually provides an acceptable rate of drug delivery when widely spaced data points leave uncertainty as to its release kinetics. One outcome of this analysis is the ability to use more cost-effective, rapid detection techniques, which would otherwise be eliminated from consideration due to their poor sensitivity. For instance, in the evaluation of enfuvirtide release, mathematical predictions can confirm the utility of a microBCA assay in the detection of release over a more expensive and lengthier HPLC protocol (Figure 1). Without supporting model predictions, the 10 day sampling interval necessitated by the microBCA assay's poor detection limit could result in missing key changes in release behavior, potentially leading researchers to misinterpret the formulation's variable release rate as "sustained" or "linear". This misinterpretation could be extremely costly especially since true, sustained or linear release is the most critical desirable aspect of many modern release formulations.

Indeed, the "linear release" design criterion applies to any formulation intending to produce constant serum concentration, encompassing well over 50% of the top 200 best-selling drugs on the market today.[25] For example, Figures 2 and 3 contain data sets with gaps of at least 20 days and more than 40% release that could be assumed to span intervals of sustained release. Indeed in the case of lysozyme release, mathematical predictions suggest that release is actually sustained from day 30 onward using the described formulation (Figure 2). This predicted profile is consistent with those documented in other sets of release data from lysozyme-loaded PLGA microparticles of comparable chemistry and molecular weight.[26] In evaluating release of superoxide dismutase, however, predictions suggest that SOD release follows a burst−lag−burst pattern, instead of a constant, sustained one, which would have been desirable for an antioxidant (Figure 3). Similar behaviors are reported for a number of other protein loaded PLGA microparticles.[8b] Having the ability to evaluate

Table 2. Extrapolative Predictions

						end of assay (day, %)				
encapsulated agent	R_p (μm)	R_{occ} (μm)	Mw_o (kDa)	kC_w (day^{-1})	Mw_A (Da)			end of release (day)	error (nRSS)	ref
VEGF	2.55	1.84	34	0.0864	21000	28	74	49	2.30×10^{-4}	3a
BSA	22.5	5.85	18.9	0.0864	66500	34	26	74	2.20×10^{-4}	23a
GM-CSF	25	1.23	40.4	0.0864	22000	7	5	91	2.66×10^{-5}	23b
VEGF	2.2	1.05	110	0.0634	21000	30	51	113	9.64×10^{-5}	23c
BSA	17.5	5.25	130	0.0864	66500	25	33	66	3.06×10^{-4}	23d
levofloxacin	1180	802.4	80	0.0169	361	46	69	91	7.39×10^{-5}	23e
paclitaxel	25	6.75	100	0.0169	854	30	27	340	1.70×10^{-4}	23f

protein release data as sustained or pulsatile, without frequent sampling, should become increasingly valuable as work on the controlled release of costly cytokines, chemokines, growth factors, and other biomolecules continues to expand.[27] In the absence of plots, statistical metrics comparing a linear interpolation to the model prediction, such as the normalized residual sum of squares (nRSSi), can indicate if changes in the rate of release are indeed occurring between data points. When assumed point-to-point interpolation differs significantly from a model prediction, but the data points do not, changes in the rate of release are occurring between sampling points.

Mathematical analysis can also aid in the understanding of release kinetics when an experiment does not reach completion because it is (for whatever reason) terminated after just the initial burst phase of release. Recording only this initial release data is often useful when exploring formulations for an entirely new drug candidate or when planning preliminary studies with cell functional assays which last for just days or weeks. However, the presence or absence of delayed release of drug could be extremely important when refining a formulation design or planning extended animal studies. A case study is provided by a controlled release formulation of nitro-fatty acid, a novel anti-inflammatory lipid whose stability in the acidic microclimate of degrading PLGA microparticles remains unknown. Accordingly, a short, 2 week release assay revealed both NO_2-OA's stability and its initial rate of release. However, this assay documented less than 30% of drug delivery. Clues to the duration of release can come from data on erosion, which are responsible for governing release in many hydrolyzable polymer systems.[8b] Similar polymer microparticles erode most significantly between days 8 and 30,[28] defining a window when pore formation and erosion-mediated release may occur. Model predictions during this time period have the potential to provide further detail into the actual release behavior. For instance, model predictions suggest that this formulation exhibits release continuing in a sustained manner through day 28, which is an appropriate delivery schedule for the eventual clinical application of this active agent. A subsequent release assay conducted in triplicate confirmed the accuracy of the model prediction, lending support for this type of analysis.

For formulations without sustained release profiles, extrapolative predictions can also aid in setting the dosing frequency for subsequent animal studies and the duration of sampling in future release assays. An example of how predictions can aid in setting dosing schedules is represented by the analysis illustrated in Figure 5. The *in vitro* release data from this formulation shows 60% of superoxide dismutase SOD being delivered over 8 days, implying that a once-weekly dosing schedule would seem logical based on the experimental data. Yet, mathematical predictions suggest a secondary burst of SOD is imminent at 30 days following the start of release. Based on this information alone, the results may warn a user that repetitive administration at 1 week intervals could lead to unexpected release of drug that is over 5 times the desired dosing of SOD beginning at day 60. Retrospective analysis suggests (alternatively) that adjusting the dosing interval to once every 8 weeks and repeating the release experiments could potentially minimize the simultaneous delivery of SOD from repeat administration while still producing the desired effect. An example of how predictions can aid in setting release assay duration is represented by the analysis in Figure 6. This *in vitro* data set could suggest that the rate of release slows from day 15 until the assay's close at day 30. In contrast, model predictions suggest that release may actually accelerate between days 20 and 30 instead of decelerating. This behavior would warrant collection of data until day 40 when closure is predicted, if it was necessary to experimentally determine this formulation's maximal rate or duration of release. These information-loss examples are representative of release assays conducted on a number of other protein and peptide formulations. This suggests that extrapolative mathematical prediction may prove extremely useful when developing formulations for a wide variety of applications and in particular for the delivery biologics or poorly soluble molecules, which are noted for often having lengthy release profiles.[8a,12]

However, it is important to note some limitations of the current model for this type of analysis. For instance, the current set of model equations do not provide a way to predict the rate (as opposed to the magnitude) of the initial burst, which is correspondingly overpredicted in 10 of 19 simulations herein. Existing mathematics[29] (or future work on the current model construct) describing dissolution limitations or electrostatic interactions with the polymer matrix may serve to account for any systematic, overpredicted release in these cases. Cumulative release was also overpredicted during the last 10−20% of drug delivery in 6 out of the 10 simulations that include data is this region. This systemic deviation might be due to the model's approximation of pore formation as a cumulative normal distribution.[8a] A comparison to experimental data reveals that this function begins to overestimate the rate of erosion (mass loss from the polymer matrix, responsible for pore formation) when just 20% of original mass remains.[28b] A more physically accurate description of erosion, perhaps accounting for crystallinity among oligomers, might correct this systemic overestimation of final release.[30]

Regardless, this retrospective analysis (producing *in silico* data for 20 real-world *in vitro* release assays) is, to our knowledge, the first instance of such use of mathematical modeling technology for biodegradable matrices. There are similarities of this kind of analysis to previous nascent methods to compute specific pharmacokinetic drug properties (e.g., bioavailability and clearance route) in the 1960s.[9] Since then, pharmacokinetic analysis has grown substantially in both the scope of its mathematics and the impact of its results. PK modeling analysis now regularly makes predictions for the processes of adsorption, metabolism, distribution, and excretion (ADME). It is also now an essential component of the US regulatory approval process.[10] Furthermore, PK modeling has most recently grown to encompass molecular simulations of drug−protein binding in order to predict ADME processes during drug discovery.[31] In contrast, the role of predictive and data modeling has significant room for expansion in controlled release formulation development. Indeed, since Higuchi first demonstrated the utility of mathematics for describing solubility limited drug release from a matrix system, models have been developed for a number of specific biodegradable polymer-based drug delivery systems.[24b,32] In practice though, the systematic or statistical design of experiments (DoE) is still considered state of the art and is significantly faster than the trial-and-error approaches used in past decades.[33] Augmenting DoE with existing models of release would be a simple, inexpensive, and rapid way to achieve greater efficiency in the formulation development process. In the future, even further gains could be derived from harnessing molecular scale simulations, as done in drug discovery, to predict drug−polymer interactions thought to influence release.[34] We are

currently exploring such methods to add power of prediction at some expense to the generalizability of the results produced.

CONCLUSIONS

Predictive modeling is of extreme potential value for the analysis of *in vitro* release data in much the same way that modeling is now considered an integral part of PK studies. The *in vitro* and *in silico* data presented herein demonstrate predictive modeling as a key for interpreting *in vitro* release assays and planning subsequent studies, either *in vitro* or *in vivo*. By interpolating and extrapolating with predictions, the necessary duration of an assay can be determined, the timing of sample points can be set, and the suitability of different detection techniques can be evaluated. These benefits are of interest not only during formulation design but also during the establishment of quality by design (QbD) manufacturing routines, when connections between a formulation's attributes and performance are established.

ASSOCIATED CONTENT

Supporting Information

Brief model synopsis, glossary of variables, and figures for systems tabulated in Tables 1 and 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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