



The reliable targeting of specific drug release profiles by integrating arrays of different albumin-encapsulated microsphere types

Wonjae Lee^a, Meredith E. Wiseman^b, Nam-Joon Cho^{b,c}, Jeffrey S. Glenn^{c,d,*}, Curtis W. Frank^{b,**}

^a Department of Mechanical Engineering, Stanford University, Stanford, CA 94305, USA

^b Department of Chemical Engineering, Stanford University, Stanford, CA 94305, USA

^c Department of Medicine, Division of Gastroenterology and Hepatology, School of Medicine, Stanford University, CCSR Bldg, Rm 3110, 269 Campus Dr., Stanford, CA 94305, USA

^d Veterans Administration Medical Center, Palo Alto, CA 94304, USA

ARTICLE INFO

Article history:

Received 15 July 2009

Accepted 30 August 2009

Available online 22 September 2009

Keywords:

Controlled drug release

Microsphere

Microencapsulation

Biodegradation

ABSTRACT

Biodegradable polymer microspheres have been successfully utilized as a medium for controlled protein or peptide-based drug release. Because the release kinetics has been typically controlled by modulating physical or chemical properties of the medium, these parameters must be optimized to obtain a specific release profile. However, due to the complexity of the release mechanism and the complicated interplay between various design parameters of the release medium, detailed prediction of the resulting release profile is a challenge. Herein we suggest a simple method to target specific release profiles more efficiently by integrating release profiles for an array of different microsphere types. This scheme is based on our observation that the resulting release profile from a mixture of different samples can be predicted as the linear summation of the individually measured release profiles of each sample. Hence, by employing a linear equation at each time point and formulating them as a matrix equation, we could determine how much of each microsphere type to include in a mixture in order to have a specific release profile. In accordance with this method, several targeted release profiles were successfully obtained. We expect that the proposed method will allow us to overcome limitations in controlling complicated release mechanisms so that drug delivery systems can be reliably designed to satisfy clinical demands.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Over the past decades, many clinically useful peptides or proteins have been discovered, characterized, and commercialized. The scope of their activity in physiological and biological processes extends to ligands for signaling, enzymes for biotransformation reactions, and antibodies in the immune system. However, incorporation of these drugs in successful pharmaceutical treatments is sharply dependent on methods of administration. This is because most biomolecules easily lose their therapeutic potency at gastric pH levels or in the presence of proteolytic enzymes [1], and have specific plasma levels for optimal clinical efficacies [2], which

usually has been maintained by repeated administration. Drug delivery systems, therefore, have been designed to deploy medications intact through a protective medium that can also control the rate of drug release.

Biodegradable polymer microspheres have been successfully utilized as drug carriers due to their biocompatibility, protein or peptide-based drug compatibility, and convenience in processing and delivery. Because the release rate is dependent on physical or chemical properties of the releasing medium, researchers have proposed various design parameters that can be modulated to achieve desired release profiles. These parameters include polymer composition [3], molecular weight [4], particle size [5,6], physical configuration [7], level of protein loading [8], and polymer concentration in the organic solvent during microsphere preparation [9]. It has also been reported that stable release from biodegradable microspheres could be successfully obtained by conjugating the drug to the polymer matrix [10], or by providing an outer layer that surrounds the core unit containing the drug [11].

These studies suggest many variables that could, in principle, be engineered to control the kinetics of drug release, but do not offer

* Corresponding author. Division of Gastroenterology and Hepatology, School of Medicine, Stanford University, CCSR Bldg, Rm 3110, 269 Campus Dr., Stanford, CA 94305, USA. Fax: +1 650 723 3032.

** Corresponding author. Department of Chemical Engineering, Stanford University, 381 North-South Mall, Stauffer III, Stanford, CA 94305, USA. Fax: +1 650 723 9780.

E-mail addresses: jeffrey.glenn@stanford.edu (J.S. Glenn), curt.frank@stanford.edu (C.W. Frank).

an efficient, systematic scheme to target specific release profiles. In practice, therefore, a trial-and-error process is required to optimize these various parameters to achieve a specific drug release profile for actual clinical applications. The overall release kinetics is determined from the intricate combination of (1) initial release of free drug from a polymer surface, (2) release through pores within a polymer matrix, (3) diffusion through an intact polymer membrane, which depends on spatial feature of network structure, or swelling ratio in aqueous environments, and (4) polymer erosion and bulk degradation [12]. It is thus very difficult to anticipate what the result of manipulating a single design parameter would be and to tune it to target a specific release profile. In addition, the complicated interplay between various parameters makes it hard to isolate them and appreciate their contribution to a resulting release profile. For example, changing polymer composition in a typical double-emulsion synthesis of polymer microspheres can modify the degradation rate of the polymer. However, the change also affects the size of the microspheres due to the incidental change in the polymer viscosity, hence changing the overall release rate in unanticipated ways. This uncertainty makes it experimentally cumbersome to develop specific release profiles required in various clinical applications.

Herein we suggest a more efficient method to target specific release profiles. The proposed scheme was developed to overcome limitations in controlling the complicated release mechanisms and difficulties in modulating the interconnected design parameters of the release medium. We adopted a previously described approach for controlling the release rate by using a cocktail of microspheres [6,13,14], because this method provides a simple platform to design controlled drug release systems without directly regulating design parameters of the release medium. Our analysis of release profiles of mixtures showed that they could be predicted as a linear summation of the individually measured release profiles of each sample. This implies that release kinetics of any one sample in a mixture is independent of the others. This independence allows one to modulate the overall release rate by determining how much of each microsphere type is present in a mixture. By applying the proposed scheme using BSA (bovine serum albumin)-encapsulated poly(lactide-co-glycolide acid) (PLGA) microspheres, we could directly match a targeted release profile without the need to change any design parameters of the drug release medium, and successfully obtained a variety of different targeted release profiles experimentally. This simplified development process could enable drug delivery systems to be conveniently and reliably designed to satisfy clinical demands.

2. Materials and methods

2.1. Preparation of PLGA microspheres by a double emulsion process

The model protein bovine serum albumin (BSA, Sigma–Aldrich) was encapsulated in PLGA microspheres by the double emulsion process (water-in-oil-in-water (w/o/w)). Poly(lactide-co-glycolic acid) (PLGA, LACTEL) was dissolved in a non-aqueous organic solvent, dichloromethane (DCM) at designated concentrations (10%, 20%, or 30% (w/v)). 50/50 PLGA (composed of 50/50 molar ratio of glycolide units and lactide units, 85 k molecular weights), 65/35 PLGA (95 k molecular weight), 75/25 PLGA (75 k molecular weight), and 85/15 PLGA (80 k molecular weight) were utilized. BSA was dissolved in Dulbecco's Phosphate Buffered Saline (DPBS, Cellgro) with 0.05% (w/w) Synperonic® PE/F68 (Sigma–Aldrich) at 50 mg/ml. Aqueous BSA solution was then added to the non-aqueous PLGA solution at the designated ratios (10%, 20%, or 30% (v/v)) and then emulsified for 2 min at approximately 30,000 rpm using a homogenizer (PRO200 Laboratory, Pro Scientific). The emulsion was poured into 100 times its volume of DPBS with 0.05% (w/v) poly(vinyl alcohol) and stirred at 1,000 rpm for overnight at room temperature and atmospheric pressure to allow the DCM to evaporate. The resulting solid microspheres were collected by centrifugation and incubated in DPBS for 1 day with stirring and refreshing of DPBS to remove unincorporated free albumin. This washing step allowed reducing the effect of the initial burst drug release, so that the drug release profile could be modulated from the early

incubation period. The microspheres were then collected by centrifugation, lyophilized, and stored at -20°C .

2.2. Measurement of protein encapsulation efficiency

Approximately 10 mg of microspheres were dissolved in acetone, and the albumin precipitate was collected by centrifugation. The collected precipitate was dissolved in 1 ml of 1 M sodium hydroxide (NaOH), and the solution was neutralized with hydrochloric acid and sodium hydroxide by use of a pH meter (Corning). Protein concentrations were determined by a micro-BCA (bicinchoninic acid) protein assay kit (PIERCE). The drug encapsulation efficiency was calculated by comparing the actual amount of protein encapsulated to the theoretical amount of protein loaded in PLGA microspheres during the microsphere preparation.

2.3. Determination of release profile

A predetermined amount (~ 10 mg) of microparticles was mixed with 1 ml DPBS buffer and incubated in a thermomixer (Eppendorf) at 37°C with 1,200 rpm mixing speed. At the designated time points, the supernatant was collected after centrifugation and replaced with fresh PBS. The collected supernatants were stored at -20°C until analysis. The albumin in the collected supernatant was measured by a micro-BCA protein assay kit (PIERCE), and then the amount of released drug per mass of PLGA was calculated. The amounts of albumin released from mixed samples were determined under the same conditions.

3. Results

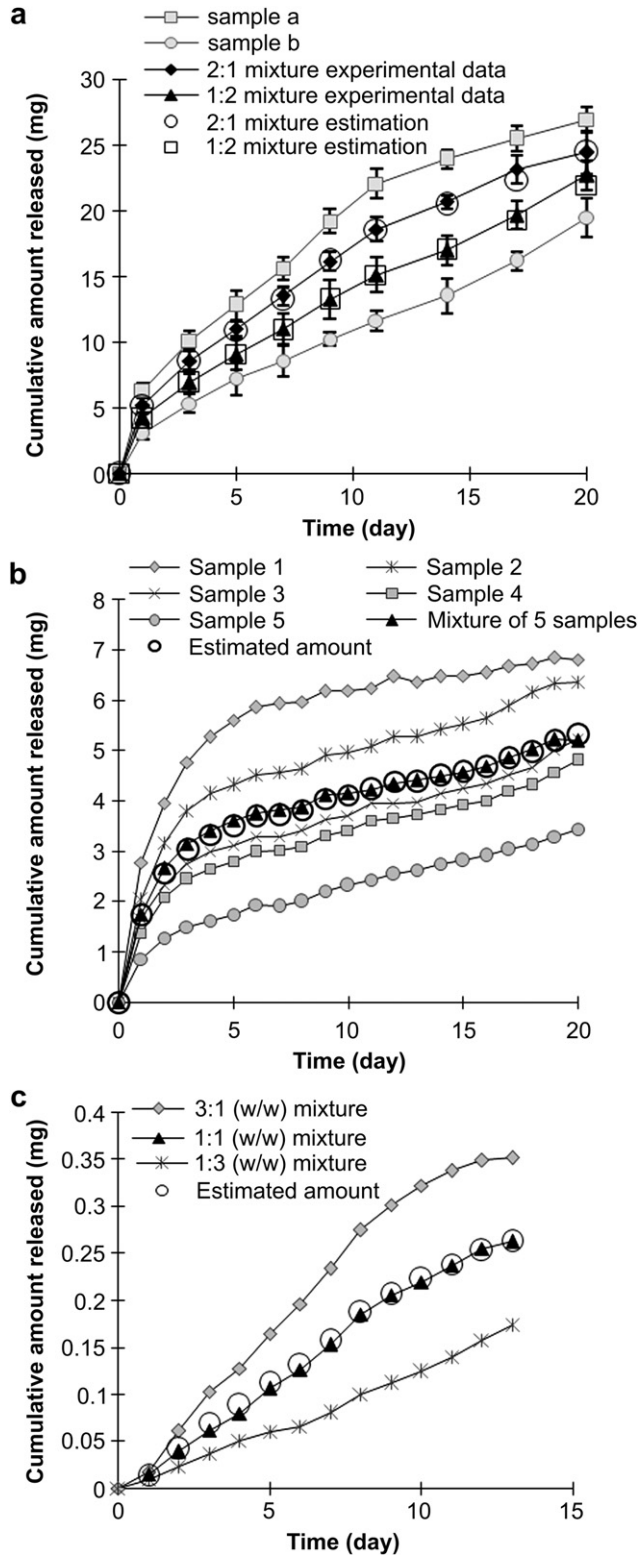
3.1. Principle of the proposed scheme

3.1.1. Independence of release kinetics of each microsphere type in a mixture

First, we attempted to identify the characteristic response of release profiles of mixtures of different microsphere types. We prepared BSA-encapsulated microspheres with 50/50 PLGA (85 k molecular weights, sample a) and 75/25 PLGA (75 k molecular weight, sample b). The release profiles of the two samples and the mixtures at 2:1 and 1:2 ratios were measured (Fig. 1), and we found that the release profile of a mixture of different samples could be predicted as the linear summation of their individually measured release profiles. The overall amounts of released BSA at each time point could be calculated as the linear summation of released BSA amounts from each sample, weighted by their relative amounts (Fig. 1a, for the 2:1 mixture, p -value = 0.96, for 1:2 mixture, p -value = 0.97, ANOVA).

This linear independence of release profiles is seen elsewhere in the literature. To demonstrate this, we adopted the release profiles reported in the paper of Siepmann et al. (2004) [6] and Berkland et al. (2002) [14] with permission. Their profiles were digitized using Graph Digitizer Scout (ByteScout) and reproduced in Fig. 1b and c. Siepmann investigated the effect of the size of biodegradable microparticles on the release rate of an incorporated drug [6]. In their paper, the drug release profile of a five-sample mixture was reported, and we digitized each sample profiles and calculated the linear summation of five drug release profiles. We found that there were statistically no differences between their experimental data and our calculation (p -value = 0.98, ANOVA).

We also analyzed drug release profiles in the paper of Berkland et al. [14]. In this study, they attempted to obtain constant (zero-order) drug release by controlling microsphere size distribution and mixing of uniform microspheres [14]. Assuming linearity, we used the profiles of 3:1 and 1:3 mixtures of two sample types to predict the release profile of a 1:1 mixture (Fig. 1c). When we compared our estimation with their experimental data, there was no statistically significant difference (Fig. 1c, p -value: 0.92, ANOVA). Because the release profile of mixtures could be predicted as the linear summation of the individually measured release profiles of each sample, weighted by their relative amounts, we conclude that



the release kinetics of different microsphere types in a mixture are independent.

3.1.2. Employing a matrix equation to determine the relative amounts of each microsphere type for targeting a specific release profile

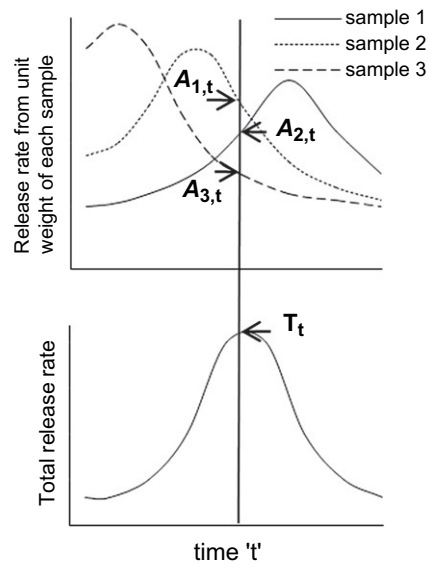
Herein we describe a scheme to target a specific release profile by employing a matrix equation, as depicted in Fig. 2. Since release rates are linearly independent, the total release rate of mixtures at a certain time point could be obtained by summing up the contributions from the constitutive sample groups. This can be expressed as the following linear equation:

$$T_t = \sum A_{t,i} \cdot a_i$$

T_t : total release rate at time point, t

$A_{t,i}$: release rate from unit weight of i th sample at time point, t

a_i : amount of i th sample present



$$T_t = a_1 \cdot A_{1,t} + a_2 \cdot A_{2,t} + a_3 \cdot A_{3,t}$$

T_t : total release rate at time point, t

$A_{i,t}$: release rate from i th sample at time point, t

a_i : amount of i th sample

For $t = 1, 2, 3$

$$\begin{pmatrix} T_1 \\ T_2 \\ T_3 \end{pmatrix} = \begin{pmatrix} A_{1,1} & A_{2,1} & A_{3,1} \\ A_{1,2} & A_{2,2} & A_{3,2} \\ A_{1,3} & A_{2,3} & A_{3,3} \end{pmatrix} \cdot \begin{pmatrix} a_1 \\ a_2 \\ a_3 \end{pmatrix}$$

$$T = A \cdot a$$

Fig. 2. Employing a matrix equation to determine mixture composition. Due to the independence of release kinetics for each sample in a mixture, a matrix equation could be employed to determine the amounts of each sample in a mixture for targeting a specific profile. At one particular time point, a specific release rate could be achieved by summing up released contributions from different sample groups, which can be formulated as a linear equation. Multiple linear equations generated at several time points constitute a matrix equation. The vectors $[A_{1,1}, \dots, A_{i,n}]$ represent release profiles of i th sample and the vectors T is a target. If the release profiles of all the samples were known, the vector a for a given target profile T could be achieved, and it is determined how much each sample should be mixed to achieve the specific target.

Fig. 1. Independence of each sample's drug release kinetics in a mixture. (a) The drug release profiles of the 2:1 and 1:2 mixtures of the sample 'a' and 'b' were predicted as the linear summation of the individually measured drug release profiles of each sample, weighted by their relative amounts. When our estimation was compared with experimental data, there was no statistical difference (p -value = 0.96 for 2:1 mixture and p -value = 0.97 for 1:2 mixture, ANOVA). (b,c) Literature support for the independence of drug release kinetics in a mixture. When the mixtures' drug release profiles were estimated based on our assumption, there were also no statistical differences between our estimation and experimental data (p -value = 0.98, ANOVA in (b) and p -value = 0.92, ANOVA in (c)). The data were adopted from the papers of (b) Siepmann et al. (2004) [6] and (c) Berklund et al. (2002) [14] with permission.

In practice, the release profiles are discretized into a series of n time points. This allows us to write a matrix equation to describe the release of an array of m different particle types:

$$\begin{pmatrix} T_1 \\ \vdots \\ T_n \end{pmatrix} = \begin{pmatrix} A_{1,1} & \cdots & A_{m,1} \\ \vdots & \ddots & \vdots \\ A_{1,n} & \cdots & A_{m,n} \end{pmatrix} \cdot \begin{pmatrix} a_1 \\ \vdots \\ a_m \end{pmatrix}$$

$$T = A \cdot a$$

In this equation, the time-discretized release profiles of each sample and the desired profiles are represented as vectors $[A_{i,1}, \dots, A_{i,n}]$ for the i th sample and a vector T for a target. If the matrix A , representing the release profiles of all the samples, were known, we could, in principle, solve for the vector a which represents the amounts of each microsphere type to be mixed to achieve T . However, because A is not invertible in many cases and, due to physical constraints, a cannot admit negative coefficients, it is impractical to directly solve the equation to obtain a . Instead, we could find the solution vector a which minimizes $\|A \cdot a - T\|$ subject to the constraint of non-negative coefficients. This has been previously solved as a non-negative least squares problem, implemented in MATLAB as the function *lsqnonneg* using the algorithm developed by Lawson and Hanson [15]. However, because this algorithm treats all time point equally, there are some experimental cases where assigning equal weight does not give the most useful solution, such as when certain time points are more critical than others or when having equal weights causes certain qualitative features of the fitted profile to be lost. For these cases, critical time points can be weighed more importantly in the computation. We use this method to solve for a ‘best-fit’ vector a to find the best formulation of polymer microspheres to reproduce a desired release profile.

3.2. Verification of proposed scheme

In order to verify the validity of this proposed scheme, we prepared BSA-encapsulated poly(lactic-co-glycolic acid) (PLGA) microspheres using a well-established double emulsion technique [16]. By modulating well-known parameters, we produced an array of samples such that each has a distinct release profile (Table 1). We utilized 50/50 PLGA (composed of 50/50 molar ratio of glycolide units and lactide units), 65/35 PLGA, 75/25 PLGA, and 85/15 PLGA for preparing samples 1–4. Because the degradation time of PLGA increases as the composition ratio of glycolide units over lactide units decreases, the peak time points of release rates were delayed from sample 1 to sample 4 (Fig. 3a). We also encapsulated BSA into samples with different PLGA concentration, or water-in-oil ratio,

but these conditions did not dramatically change the trend of released BSA (Fig. 3b,c).

By selecting 9 time points and 10 samples, we formulated a 9×10 matrix, A , which describes the release profiles of each sample. We designed four arbitrary target release profiles with very different characteristic shapes and represented them as a matrix T ; the first one had a stable concentration level of released BSA (T_1), and the second, the third, and the fourth had only one peak level of released BSA at distinct time periods (T_2 , T_3 , and T_4). The obtained matrix, A , and the determined targets are as follows:

$$A = \begin{pmatrix} 3.6 & 2.3 & 1.9 & 1.4 & 2.7 & 2.6 & 2.3 & 2.7 & 2.9 & 2.9 \\ 3.9 & 2.2 & 1.3 & 1.2 & 3.0 & 2.8 & 1.9 & 2.8 & 2.2 & 1.5 \\ 3.6 & 2.5 & 1.7 & 0.8 & 2.9 & 2.5 & 1.5 & 3.6 & 2.4 & 1.3 \\ 2.4 & 3.3 & 1.4 & 0.7 & 2.6 & 2.0 & 1.4 & 2.9 & 1.9 & 0.8 \\ 2.6 & 3.4 & 1.9 & 1.0 & 2.1 & 1.6 & 1.5 & 1.9 & 1.5 & 0.8 \\ 2.2 & 2.7 & 2.7 & 0.9 & 1.7 & 1.8 & 1.5 & 1.6 & 1.5 & 0.7 \\ 2.0 & 1.4 & 3.3 & 1.0 & 1.5 & 1.8 & 1.3 & 1.5 & 1.7 & 0.9 \\ 1.1 & 1.7 & 2.7 & 1.0 & 1.7 & 1.6 & 1.3 & 0.9 & 1.8 & 1.3 \\ 1.1 & 1.6 & 2.5 & 0.9 & 1.7 & 1.5 & 1.2 & 1.6 & 1.4 & 1.2 \end{pmatrix}$$

$$T = (T_1, T_2, T_3, T_4) = \begin{pmatrix} 20 & 20 & 10 & 10 \\ 20 & 20 & 10 & 10 \\ 20 & 20 & 15 & 10 \\ 20 & 15 & 20 & 10 \\ 20 & 10 & 20 & 15 \\ 20 & 10 & 15 & 20 \\ 20 & 10 & 10 & 20 \\ 20 & 10 & 10 & 20 \\ 20 & 10 & 10 & 15 \end{pmatrix}$$

The numbers in the A matrix represent released BSA amount in μg from one mg of microspheres during 24 h, and the numbers in the T matrix mean the targeted BSA amount in μg during 24 h. Using the previously described mathematical method, we obtained the ‘best-fit’ solution matrix, ‘ a ’, where each number represents mg of each sample in a mixture.

$$a = \begin{pmatrix} 0.0 & 0.0 & 0.0 & 0.0 \\ 1.2 & 0.0 & 5.5 & 0.9 \\ 3.2 & 0.0 & 0.4 & 5.5 \\ 0.0 & 0.0 & 0.0 & 0.0 \\ 2.3 & 4.8 & 0.0 & 0.0 \\ 0.0 & 0.0 & 0.0 & 0.0 \\ 0.0 & 0.0 & 0.0 & 0.0 \\ 0.0 & 1.3 & 0.0 & 0.0 \\ 2.8 & 0.0 & 0.0 & 0.4 \\ 0.0 & 1.2 & 0.0 & 0.0 \end{pmatrix}$$

Table 1

| | PLGA composition | Molecular weight | PLGA concentration in DCM (w/v) (%) | BSA solution ratio to PLGA solution (v/v) (%) | Encapsulation efficiency (%) |
|-----------|------------------|------------------|-------------------------------------|---|------------------------------|
| Sample 1 | 50/50 | 85 k | 20 | 20 | 38 ± 1 |
| Sample 2 | 65/35 | 95 k | 20 | 20 | 37 ± 2 |
| Sample 3 | 75/25 | 75 k | 20 | 20 | 42 ± 4 |
| Sample 4 | 85/15 | 80 k | 20 | 20 | 46 ± 2 |
| Sample 5 | 50/50 | 85 k | 10 | 20 | 36 ± 1 |
| Sample 6 | 50/50 | 85 k | 20 | 20 | 37 ± 2 |
| Sample 7 | 50/50 | 85 k | 30 | 20 | 44 ± 1 |
| Sample 8 | 50/50 | 85 k | 20 | 10 | 43 ± 3 |
| Sample 9 | 50/50 | 85 k | 20 | 20 | 37 ± 0.4 |
| Sample 10 | 50/50 | 85 k | 20 | 30 | 33 ± 1 |

PLGA composition is the molar ratio between glycolide and lactide units.

PLGA concentration in DCM is the dissolved concentration of PLGA in non-aqueous solvent, dichloromethane.

BSA solution ratio to PLGA solution is the aqueous BSA solution ratio to non-aqueous PLGA solution for double (water-in-oil-in-water) emulsion.

BSA encapsulation efficiency was calculated by comparing the actual amount of protein encapsulated over the theoretical loaded protein in PLGA microspheres during the microsphere preparation.

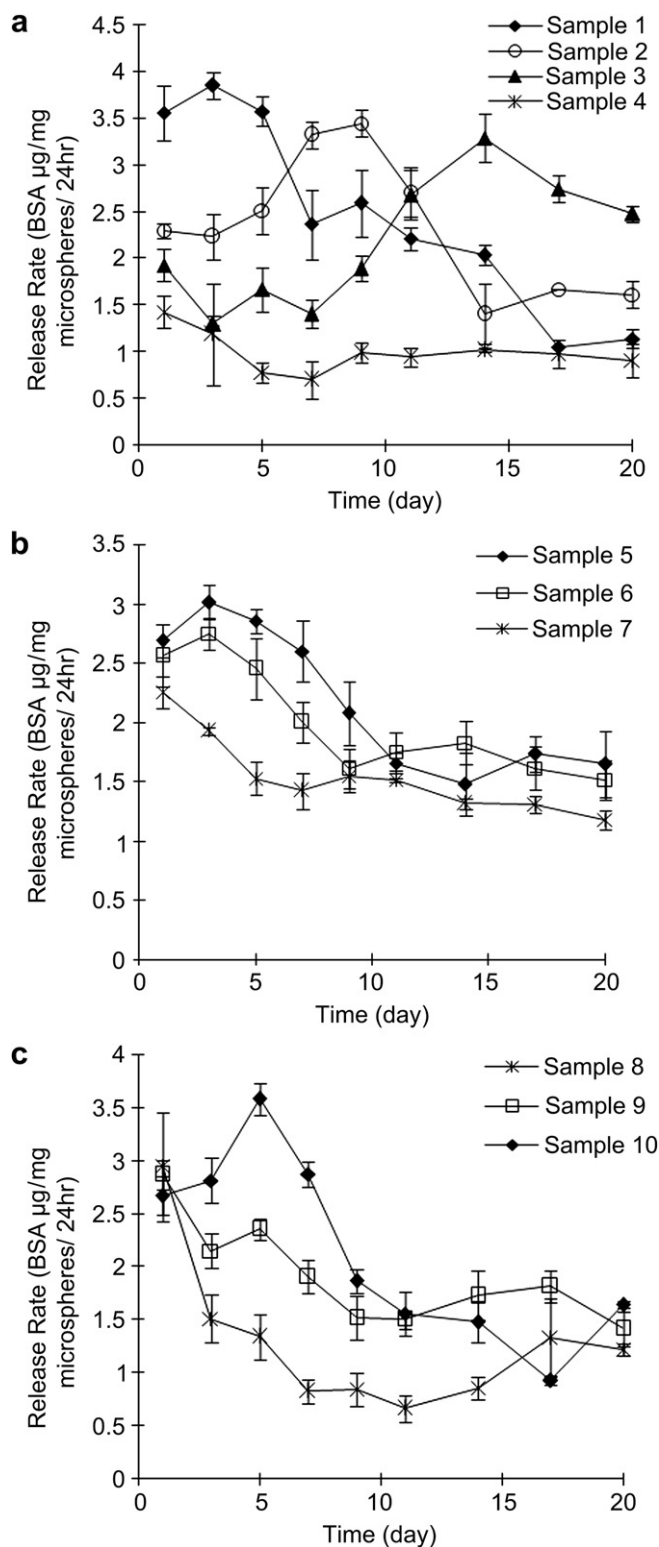


Fig. 3. Albumin release profiles of different microsphere types. (a) Samples 1–4 were prepared by changing the PLGA composition ratio between glycolide units and lactide units. Sample 1 was prepared from 50/50 PLGA with the shortest degradation time, and sample 4 was prepared from 85/15 PLGA with the longest degradation time. The peak time points of released drug were delayed more as the material had longer degradation times. (b) Sample 5–7 were prepared by changing PLGA concentration in DCM from 10% (w/v) to 30% (w/v). (c) We loaded different amount of BSA solution from 10% (v/v) to 30% (v/v) for Sample 8–10. As the PLGA concentration in DCM decreased, or the loaded BSA solution increased, the released BSA amounts at each time point increased in general.

We then mixed samples in accordance with the amounts specified in vector \mathbf{a} and measured the released BSA amount from the mixtures at designated time points. The targeted release profiles matched quite closely (Fig. 4). The statistical analyses using ANOVA at 9 time points were performed by comparing the targeted release profiles and our experimental data. The comparison between the experimentally obtained data and the first target vector t_1 has the p -value of 0.56 (Fig. 4a), and the second, the third, and the fourth have the p -values of 0.58 (Fig. 4b), 0.82 (Fig. 4c), and 0.86 (Fig. 4d), respectively, from ANOVA analysis, indicating the four targets were successfully achieved by our scheme.

4. Discussion

In this study, we sought a way to target specific release profiles by integrating an array of different microsphere types. Our preliminary finding of the independence of each sample's release kinetics within a mixture was a cornerstone to developing this method. Because of this linear independence, we were able to target specific release profiles by employing a matrix equation to determine how much of each different microsphere type should be mixed. We successfully achieved a constant release rate (Fig. 4a), which could be applied to diverse treatments, such as cancer, viral and bacterial infections, birth control, and AIDS, where drugs should be continuously administered for a certain period of time [12]. We also showed that our method could cover clinical situations in which multiple biomolecules exhibit sequential distinct plasma levels (Fig. 4a,b, and c), such as regeneration processes of blood vessels [17], bone [18], and cartilage [19], as well as the differentiation processes of stem cells [20].

The accuracy of this scheme is strictly dependent on how effectively the basis set of samples "spans" the space of possible target drug release profiles. The best way to achieve this experimentally is to have the basis samples display well-defined sequential peak levels of releasing activity. It has been reported that the gradient or the time of peak drug release could be modulated by changing polymer molecular weight [21], blending of structurally different polymers [22], and modulating porosity [23]. Recently, Pitukmanorom et al. proposed a useful method to achieve distinct peak time points of released drug concentration without initial drug burst by improving the method of covering a drug-encapsulated core unit with an outer layer [24]. If these methods are applied to compose the basis set of samples, more complicated drug release profiles could be precisely achieved by our proposed scheme.

The advantage of our scheme is that one would only have to modulate particle design parameters once, and only roughly, to generate a good basis set of samples. After this, one could produce virtually any drug release profile without having to modify the particles themselves. Therefore, this scheme provides an efficient way of utilizing previously developed drug delivery technology to achieve different release profiles in the clinical or "end-user" setting by passing over the time-consuming trial-and-error process of optimizing the physical design parameters.

5. Conclusion

In this study, we attempted to develop a simplified method for generating drug release profiles required in various clinical applications using biodegradable microspheres. It was motivated by the fact that targeting specific release profiles is experimentally burdensome due to the complexity of the release mechanism and the complicated interplay between various design parameters of the release medium. We found that the resulting release profile of mixed samples is simply the linear summation of the individually measured release profiles of reference

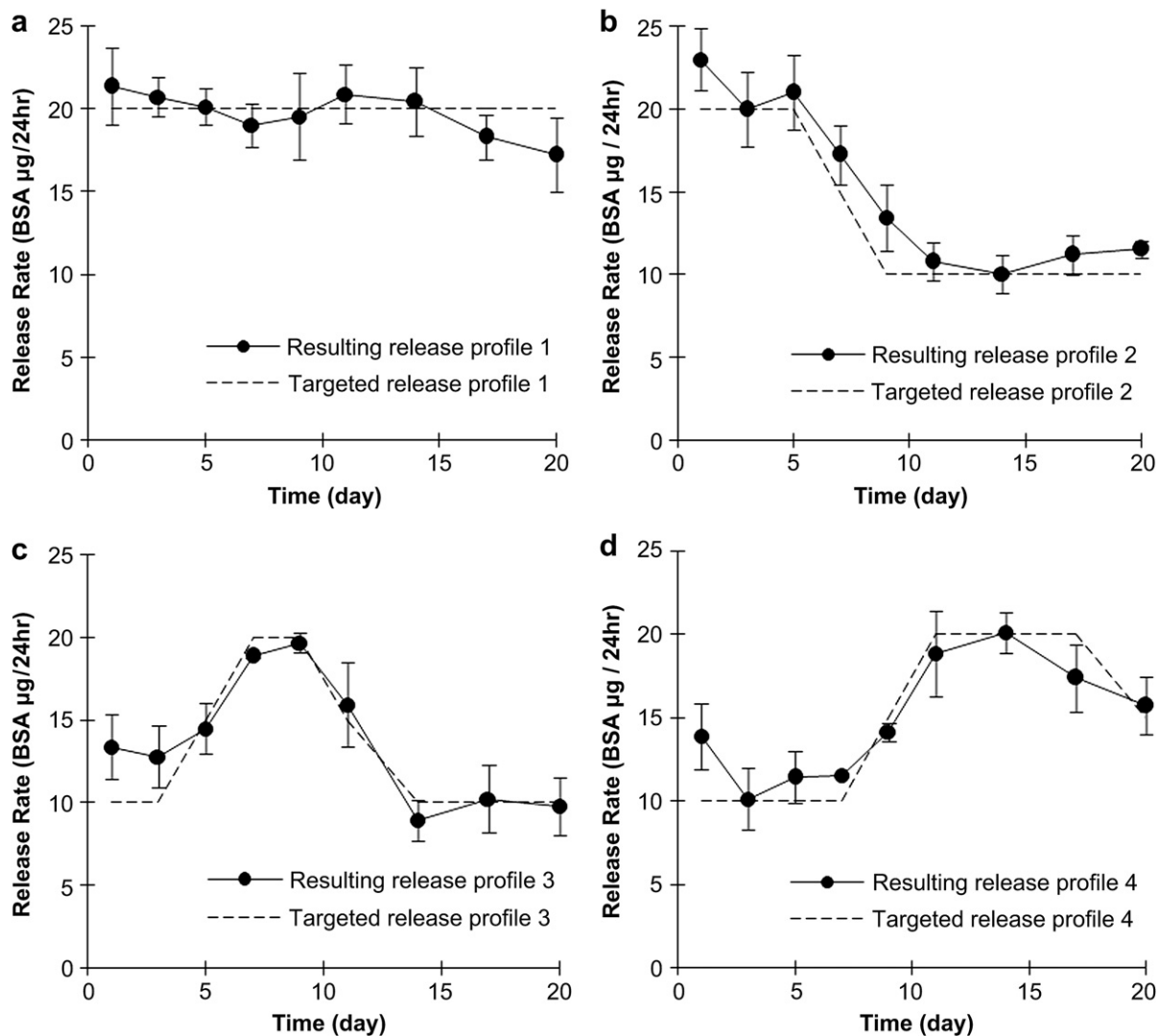


Fig. 4. Targeting of four arbitrary release profiles by preparing combinations of different microsphere type with our proposed scheme. The experimentally measured release rates were compared with the targets. The first result in (a) has a p -value of 0.56, and the second in (b), the third in (c), and the fourth in (d) have p -values of 0.58, 0.82, and 0.86, respectively. It indicates that there are no statistical differences between the targeted profiles and the actually achieved experimental data, and the four targets were successfully obtained.

samples, which allows design of controlled drug release systems without the direct manipulation of design parameters of the release medium. By employing a matrix equation, we could determine the amount of each microsphere type to include in a mixture so as to have a specific release profile. The proposed method was applied to BSA-encapsulated PLGA microspheres, and arbitrary target profiles with a continuous release rate and pulsatile release rate at distinct time periods were successfully obtained. We expect this simplified development process will permit drug delivery systems to be conveniently and reliably designed to satisfy clinical demands.

Acknowledgment

The authors gratefully acknowledge funding from Beckman ITRP AWARD (1097820 -104-GHAPQ), a Burroughs Wellcome Fund Clinical Scientist Award in Translational Research (to JSG), and the Center for Translational Research in Chronic Viral Infections. N.J.C. is a recipient of the American Liver Foundation Postdoctoral Fellowship Award and a Stanford Dean's Postdoctoral Fellowship.

References

- [1] Prausnitz MR, Mitragotri S, Langer R. Current status and future potential of transdermal drug delivery. *Nat Rev Drug Discovery* 2004;3(2):115–24.
- [2] Langer R. New methods of drug delivery. *Science* 1990;249(4976):1527–33.
- [3] Sanders LM, Kell BA, McRae GI, Whitehead GW. Prolonged controlled-release of nafarelin, a luteinizing hormone-releasing hormone analogue, from biodegradable polymeric implants: influence of composition and molecular weight of polymer. *J Pharm Sci* 1986;75(4):356–60.
- [4] Capan Y, Woo BH, Gebrekidan S, Ahmed S, DeLuca PP. Preparation and characterization of poly (D, L-lactide-co-glycolide) microspheres for controlled release of poly (L-lysine) complexed plasmid DNA. *Pharmacol Res* 1999;16(4):509–13.
- [5] Sansdrap P, MoES AJ. Influence of manufacturing parameters on the size characteristics and the release profiles of nifedipine from poly (DL-lactide-co-glycolide) microspheres. *Int J Pharm* 1993;98(1–3):157–64.
- [6] Siepmann J, Faisant N, Akiki J, Richard J, Benoit JP. Effect of the size of biodegradable microparticles on drug release: experiment and theory. *J Controlled Release* 2004;96(1):123–34.
- [7] Abidian MR, Kim DH, Martin DC. Conducting-polymer nanotubes for controlled drug release. *Adv Mater* 2006;18:405–9.
- [8] Gabor F, Ertl B, Wirth M, Mallinger R. Ketoprofen-poly (D, L-lactic-co-glycolic acid) microspheres: influence of manufacturing parameters and type of polymer on the release characteristics. *J Microencapsulation* 1999;16(1):1–12.
- [9] Chen W, Lu DR. Carboplatin-loaded PLGA microspheres for intracerebral injection: formulation and characterization. *J Microencapsulation* 1999;16(5):551–63.

- [10] Ringsdorf H. Structure and properties of pharmacologically active polymers. *J Polym Sci Polym Symp* 1975;51:135–53.
- [11] Huang YY, Chung TW, Tzeng TW. A method using biodegradable polylactides/polyethylene glycol for drug release with reduced initial burst. *Int J Pharm* 1999;182(1):93–100.
- [12] Sinha VR, Trehan A. Biodegradable microspheres for protein delivery. *J Controlled Release* 2003;90(3):261–80.
- [13] Narayani R, Panduranga Rao K. Gelatin microsphere cocktails of different sizes for the controlled release of anticancer drugs. *Int J Pharm* 1996;143(2):255–8.
- [14] Berkland C, King M, Cox A, Kim K, Pack DW. Precise control of PLG microsphere size provides enhanced control of drug release rate. *J Controlled Release* 2002;82(1):137–47.
- [15] Lawson CL, Hanson RJ. Solving least squares problems. Englewood Cliffs, New Jersey: Prentice-Hall; 1974.
- [16] Gunatillake PA, Adhikari R. Biodegradable synthetic polymers for tissue engineering. *Eur Cell Mater* 2003;5(1):1–16.
- [17] Richardson TP, Peters MC, Ennett AB, Mooney DJ. Polymeric system for dual growth factor delivery. *Nat Biotechnol* 2001;19(11):1029–34.
- [18] Raiche AT, Puleo DA. In vitro effects of combined and sequential delivery of two bone growth factors. *Biomaterials* 2004;25(4):677–85.
- [19] Martin I, Suetterlin R, Baschong W, Heberer M, Vunjak-Novakovic G, Freed LE. Enhanced cartilage tissue engineering by sequential exposure of chondrocytes to FGF-2 during 2D expansion and BMP-2 during 3D cultivation. *J Cell Biochem* 2001;83(1):121–8.
- [20] Weissman IL. Stem cells units of development, units of regeneration, and units in evolution. *Cell* 2000;100(1):157–68.
- [21] Park T. Degradation of poly (D, L-lactic acid) microspheres: effect of molecular weight. *J Controlled Release* 1994;30(2):161–73.
- [22] Edlund U, Albertsson A. Morphology engineering of a novel poly (L-lactide)/poly (1, 5-dioxepan-2-one) microsphere system for controlled drug delivery. *J Polym Sci Part A Polym Chem* 2000;38:786–96.
- [23] Jeyanthi R, Thanoo BC, Metha RC, Deluca PP. Effect of solvent removal technique on the matrix characteristics of polylactide/glycolide microspheres for peptide delivery. *J Controlled Release* 1996;38(2–3):235–44.
- [24] Pitukmanorom P, Yong TH, Ying JY. Tunable release of proteins with polymer-inorganic nanocomposite microspheres. *Adv Mater* 2008;20(18):3504–9.