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A method of elevated temperatures coupled with magnetic stirring to predict real time release from long acting progesterone PLGA microspheres

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\section*{ABSTRACT}

The object of the study was to develop a quick and reproducible accelerated in vitro release method to predict and deduce the function of the real time (37 °C) release for long acting PLGA microspheres. The method could be described in several steps. First, the release of the microspheres were studied using the sample and separate method at 37 °C with normal orbital shaking and elevated temperatures with magnetic stirring to further accelerate the release. Second, the most similar profile at elevated temperatures with the real time release was chosen with the help of the \( n \) value in the fitted Korsmeyer-Peppas Function. Third, the Weibull function and conversion ratio were used to deduce the function of real time release according to the chosen profile at elevated temperatures. The key point in this study was to provide a quick and precise method to predict the real time release for long acting progesterone PLGA microspheres. So the elevated temperatures coupled with magnetic stirring were used to accelerate the release further, and when there have many similar release profiles with the real time release at elevated temperatures, releasing time at elevated temperatures and the \( R^2 \) of the final deduced function will be used to help choosing the most similar release profile with the real time release. Four different types of progesterone PLGA microspheres were used to verify the method, and all the deduced function correlated well with the real time releases, for \( R^2 = 0.9912, 0.9781, 0.9918 \) and 0.9972, respectively.

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1. Introduction

Poly(lactic-co-glycolic acid) (PLGA) microspheres as a traditional sustained release systems have been deeply studied and there already have been many products on the market [1–3]. One of the generalities of the products is that each of them has a long duration of action, which ranges from weeks to several months [4–7]. However, this generality not only greatly improved patients' compliance [8] but also brings...
many “high risk” [8,10], because these microspheres contain a large amount of active drugs, and any unexpected changes in the release will lead to toxicity or severe side effects [11]. So, there is a great need to assure the release profile of these microspheres [9]. However, studying the release profiles will be both time-consuming and expensive, especially in the preliminary stages of formulation development and the quality control phases [12], because it will take a lot of time to finish the release and any minor changes may greatly influence the release profiles. Therefore, a quick and easy method to predict the drug release in a short time would be very helpful [13,14], and this was also recommended by the American Association of Pharmaceutical Scientists/International Pharmaceutical Federation (AAPS/FIP) [15].

It was well-known that there were many parameters, such as temperature, presence of temperature, presence of enzymes and surfactants and pH, could accelerate the rate of PLGA hydration and degradation or enhance the drug diffusion to accelerate the drug release [16]. Stirring rate could also increase the releasing rate in a way. So in this study, the temperatures coupled with magnetic stirring were used to further accelerate the releasing rate.

Nevertheless, during the past few years, there have been many articles talking about elevated temperature accelerated release to predict the real time release. For example, Janagan et al. [17] and Shen et al. [18], they both use elevated temperatures to accelerate the microspheres release, the same accumulated releases were achieved at different temperatures. When the great correlation between the profile of accelerated release and the real time release was obtained, the accelerated release profile could be used to predict the real time release. However, how to predict and how to really describe the real time release in a more clearly way were not mentioned.

Arrhenius equation was also widely used to predict the real time release, and the equation was $k = A \exp(-E_a/(RT))$, where $k$ is the rate constant, $A$ is the pre-exponential factor, $E_a$ is the energy of activation. $R$ is the gas constant, and $T$ is the absolute temperature. For example, Zolnik et al. [19], in their study, the activation energy of the microspheres were calculated based on release data at the elevated temperatures ($53 \degree C$, $60 \degree C$, $70 \degree C$), and the calculated activation energy was used to calculate the rate constant at real time release ($37 \degree C$), they found the predicted rate constant was in agreement with the real rate constant at $37 \degree C$, so they announced that these elevated temperatures($53 \degree C$, $60 \degree C$, $70 \degree C$) can be used to predict the real time release. In articles mentioned above, one of the most important questions should be discussed. The release rate of the microspheres could not be the same from the beginning to the end, so the rate constant of a period could not describe the release profile integrally. However, there are still some articles using the same method [12], they calculated the release rate separately to describe the real time release. In this way, it seems more accurate. But it still can’t describe the real time release integrally and also can’t describe it in a clear and easy way. Therefore, in this study, aiming at the shortcomings, an easy and quick method was developed.

Here, progesterone was chosen as a model drug and the traditionally solvent evaporation method was used to make the progesterone-PLGA microspheres. The elevated temperatures coupled with magnetic stirring were used to greatly accelerate the releasing rate, compared with the releasing rate at $37 \degree C$ with orbital shaking. Release characteristics at different conditions were studied. And the data were deeply analyzed by origin 8.0. The Korsmeyer-Peppas equation and Weibull function were also used to describe the release profiles and to deduce the function of the real time release.

2. Materials and methods

2.1. Materials

Poly(d,l-lactide-co-glycolide) with a lactide: glycolide ratio 65:35, noted as PLGA RG653H (inherent viscosity = 0.321 dl/g in CHCl3 at 25 °C) was kindly donated from Evonik, Ltd. Poly(d,l-lactide-co-glycolide) with a lactide: glycolide ratio 65:35, noted as PLGA 65:35 (inherent viscosity = 0.386 dl/g in CHCl3 at 25 °C), and (d,l-lactide-co-glycolide) with a lactide: glycolide ratio 75:25, noted as PLGA 75:25 (inherent viscosity = 0.401 dl/g in CHCl3 at 25 °C) was purchased from Jinan Daigang Biomaterial Company, Ltd. (Shandong, China). Progesterone(99.0%) was purchased from BeiErKa Biological Medicine Co Ltd (Wuhan, China). Poly(vinyl alcohol) (PVA, MW30000) was purchased from Kuraray China Co, Ltd. HPLC grade ethyl formate and methylene chloride were obtained from Yuyang Company (Shandong, China).

2.2. Methods

2.2.1. Preparation of microspheres

Traditionally solvent evaporation method was used to make the progesterone-PLGA microspheres. And in this method, ethyl formate [20,21] was used to substitute methylene dichloride to make environmentally friendly [22], 10% max.

First, 20 mg progesterone and 200 mg PLGA RG653H (0.321 dl/g, Evonik) were dissolved in 3.2 ml ethyl formate, and then they were poured into 8 ml of 1% PVA aqueous solution. After the addition, the emulsion was continuously stirred for 220 min. After that, the microspheres were collected by filtration and washed with distilled water for 3 times and dried overnight under vacuum [22].

2.2.2. Encapsulation efficiency of the progesterone PLGA microspheres

About 20 mg of dried microspheres were completely dissolved in 3 ml methylene dichloride, and then 18 ml methanol was poured into it to precipitate PLGA. The final suspension was filtered through a 0.22 μm membrane. The amount of progesterone in the filtrate was analyzed by HPLC. The Diamond C18 (4.6 mm × 25 cm, 5 μm) was used as an analytical column with a mobile phase consisting of methanol, acetonitrile and water (45:30:25, v/v) at a flow rate of 1 ml/min. The UV detector was set at 254 nm, and the encapsulation efficiency (EE) and drug loading (DL) was defined below.

$EE\% = (M_a/M_d) \times 100\%$, where “$M_a$” means the actual amount of progesterone loaded in the microspheres, “$M_d$” means the theoretical progesterone loaded in the microspheres.

$DL\% = (n/m) \times 100\%$, where “$n$” means the amount of progesterone in the microspheres, “$m$” means the weight of the progesterone microspheres.
2.2.3. The characteristics of progesterone microspheres
The diameters of the progesterone-PLGA microspheres were measured by Sympatec Laser Particle Size Analyzer. The microspheres were also observed by scanning electron microscope (SEM). (Hitachi S-3400; Hitachi High Technologies, Kyoto, Japan).

The differential scanning calorimeter (Shimadzu, Japan) was used to determine the glass transition temperature (Tg) of the microspheres [23]. The samples of the microspheres were sealed in aluminum pans with pinhole lids, and were heated to 150 °C at 20 °C/min and rapidly cooled to −10 °C. The second cycle was used to determine the glass transition temperature [13,23].

2.2.4. In vitro release testing
The progesterone microspheres were investigated using the traditional sample and separate method [10,24,25]. In this method, about 20 mg of the microspheres was dispersed in 40 ml 0.1M sodium phosphate buffer at pH7.4 Tween 80 (2%, w/v) and NaN₃ (0.1%, w/v) were also added to increase the stability of the release medium. They were incubated at 37 °C under orbital shaking at 100 rpm. At each time-point, 4 ml release medium without microspheres was taken out and 4 ml fresh medium was added. The concentration of progesterone at each time-point was quantified by HPLC method as in encapsulation efficiency study. And the release at elevated temperatures were also did in the same way but under magnetic stirring to obtain a faster release rate compared with orbital shaking at 37 °C.

2.2.5. Data analysis
The accumulated releases of progesterone-PLGA microspheres at different temperatures were deeply analyzed using the Origin 8.0. In addition, the Korsmeyer-Peppas equation and Weibull function were used to describe the release profile, and to help reveal the relationship between the real time release profile and accelerated release profile, so as to predict the real time release.

As for biodegradable erosion systems, drugs were dispersed in the polymer evenly, and the Korsmeyer-Peppas equation could be used to explain the release mechanisms simply. For Korsmeyer-Peppas equation [26,27] \( M_t/M_\infty = k t^n \), where the \( M_t/M_\infty \) was the fraction of drug released at time \( t \), \( k \) was the rate constant, and \( n \) was the release exponent related to the releasing mechanism. For microspheres, the correlations between the \( n \) value and release mechanisms are: \( n < 0.43 \), non-fickian diffusion, and \( n = 0.43 \), Fickian diffusion, and \( 0.43 < n < 0.85 \), anomalous diffusion, and \( n > 0.85 \), case II diffusion [17].

And Weibull Function was used to describe the release profiles for its widely use in microspheres, and it was also recommended [28] by American Association of Pharmaceutical Scientists/International Pharmaceutical Federation (AAPS/FIP) workshop. The Weibull function [29-31] mentioned here is: \( Y = 1 - \exp(-a t^b) \), where \( Y \) means the percent of the drug release from the microspheres, and \( a \) is related to the apparent rate constant, and \( b \) is corresponding to the shape of the release profile [29-31]. Weibull Function [30] is suitable for sigmoidal drug release profiles, and it is used to model release profiles [30] that show zero to minimal initial burst release, zero to minimal diffusion-mediated release rate [30] or erosion-dominated process coupled with minimal diffusive release rates [30].

In addition, the detailed method of the elevated temperatures to predict real time release from progesterone-PLGA microspheres was deeply discussed in the results and discussion part.

2.2.6. Reproducibility testing
The reproducibility of this method to predict the real time release from progesterone-PLGA microspheres was investigated. The PLGAs which have the different inherent viscosity, different lactide/glycolide ratio, and purchased from different companies were used to make progesterone-PLGA microspheres. Not only the ethyl formate was used as the organic phase to make the microspheres, but also the methylene chloride was used. Different drug loading rates were obtained by changing the grams of progesterone and PLGA in the preparations.

Different release mediums including “0.5% SDS-pH 7.4 PBS-0.1% NaN₃” and “1% SDS-pH 7.4 PBS-0.1% NaN₃” were used to do the reproducibility testing. When use these release medium, it should be noted that: first, 10 mg of the microsphere were suspended in 10 ml release medium; second, at each timing point, the samples were centrifuged at 4500 rpm for 5 min, then 9 ml of the samples was removed and 9 ml fresh medium was added to maintain the sink condition.

In all, the predicting method of ethyl formate-progesterone-PLGA (65:35, Daigang) microspheres, methylene chloride-progesterone-PLGA (65:35, Daigang) microspheres and methylene chloride-progesterone-PLGA (75:25, Daigang) will be discussed in the next part.

Table 1 – The DL, EE and Tg of the microspheres.

| Variable                                      | DL (%)  | EE (%)  | Tg (°C) |
|-----------------------------------------------|---------|---------|---------|
| Ethyl formate-progesterone-PLGA (RG653H, Evonik) | 9.32 ± 0.15 | 98.20 ± 1.6 | 45.3 ± 0.61 |
| Ethyl formate-progesterone-PLGA (65:35, Daigang) | 12.7 ± 0.17 | 98.01 ± 1.0 | 53.2 ± 1.1 |
| Methylene chloride-progesterone-PLGA (65:35, Daigang) | 10.1 ± 0.19 | 97.93 ± 1.8 | 52.9 ± 0.99 |
| Methylene chloride-progesterone-PLGA (75:25, Daigang) | 31.2 ± 0.52 | 90.81 ± 1.4 | 57.4 ± 0.87 |

3. Results and discussion

3.1. Physicochemical properties of progesterone microspheres

The physicochemical properties of progesterone-PLGA microspheres were listed in Tables 1–3. The SEM images were shown in Fig. 1.
Table 2 – The particle size distribution of the microspheres.

| Variable                                      | D10 (μm)   | D50 (μm)   | D90 (μm)   |
|-----------------------------------------------|-----------|-----------|-----------|
| Ethyl formate-progesterone-PLGA (RG653H, Evonik) | 1.32 ± 0.020 | 4.03 ± 0.071 | 9.69 ± 0.17 |
| Ethyl formate-progesterone-PLGA (65:35, Daigang) | 3.98 ± 0.069 | 7.52 ± 0.13 | 14.73 ± 0.18 |
| Methylene chloride-progesterone-PLGA (65:35, Daigang) | 7.89 ± 0.11 | 19.98 ± 0.29 | 28.31 ± 0.46 |
| Methylene chloride-progesterone-PLGA (75:25, Daigang) | 10.12 ± 0.19 | 23.58 ± 0.37 | 37.24 ± 0.58 |

Table 3 – The intrinsic viscosity of PLGA and the release medium of the microspheres.

| Variable                                      | Intrinsic viscosity of PLGA (dl/g) | Release medium                        |
|-----------------------------------------------|-----------------------------------|---------------------------------------|
| Ethyl formate-progesterone-PLGA (RG653H, Evonik) | 0.321                             | 40 ml 2% Tween80-pH 7.4 PBS −0.1% NaN3 |
| Ethyl formate-progesterone-PLGA (65:35, Daigang) | 0.386                             | 10 ml 0.5% SDS-pH 7.4PBS −0.1% NaN3    |
| Methylene chloride-progesterone-PLGA (65:35, Daigang) | 0.386                             | 40 ml 2% Tween80-pH 7.4 PBS −0.1% NaN3 |
| Methylene chloride-progesterone-PLGA (75:25, Daigang) | 0.401                             | 10 ml 1% SDS-pH 7.4 PBS −0.1% NaN3    |

Table 4 – The results of Korsmeyer-Peppas Equation for the release of ethyl formate-progesterone-PLGA (RG653H, Evonik) microspheres (Tg = 45.3 ± 0.61).

| Temperatures (°C) | k      | n      | R²   |
|-------------------|--------|--------|------|
| 37                | 0.1276 | 0.3162 | 0.9968 |
| 43                | 0.1369 | 0.2812 | 0.9749 |
| 47                | 0.5917 | 0.2164 | 0.9971 |
| 52                |        |        |      |

3.2. In vitro release testing

The progesterone-PLGA microspheres (noted as ethyl formate-progesterone-PLGA (Evonik) microspheres) were made by the solvent evaporation method, and the organic phase was ethyl formate. The intrinsic viscosity of PLGA was 0.321 dl/g. Fig. 2 shows the in vitro release of the microspheres at 37 °C and elevated temperatures.

3.3. Data analysis

The release curves at different temperatures were fitted by the Korsmeyer-Peppas function [32], and results were shown in Table 4. At 37 °C, n = 0.3162, it seems that the progesterone released from the PLGA microspheres followed the non-Fickian diffusion. However, as a poor water soluble drug, progesterone usually can’t follow the non-Fickian diffusion. And this question will be deeply discussed here. The mechanism of drug release from PLGA was very complex. There have two main release mechanisms, which are diffusion and degradation/erosion [33]. The release rate is often said to be diffusion-controlled initially [30,33,34] and degradation/erosion controlled during the final stage of the release period [30,33,34]. Here, some of the release mechanisms of different stages were listed in the Table 5. And in Fig. 3, the relationship between release mechanisms and different releasing processes was deeply described. So the simply use of Korsmeyer-Peppas equation to determine the release mechanisms was a bit arbitrary. Furthermore, the Korsmeyer-Peppas equation should be used to describe the profile when the drugs were dispersed in the polymers evenly. In short, the Korsmeyer-Peppas equation could not be totally appropriate to describe the real release mechanism, especially when the release profile has a significant initial burst part. And when the initial burst of the release profile at 37 °C was excluded, the function will be: $y = 0.04519e^{0.4634}$, $R^2 = 0.9822$, and $n = 0.4634$, which means the mechanism of the release profile at 37 °C was anomalous transport.

It also implies n values in these functions which describe the release profiles with the large initial burst at elevated temperatures could not totally describe the real release mechanism at elevated temperatures. And it should be noted that in this study, the stirring rate at elevated temperatures was faster than that at 37 °C to obtain a quicker release rate. This also explained why the n value changes with temperature irregularly.
Fig. 1 – The SEM of the progesterone-PLGA microspheres. (A) Ethyl formate-progesterone-PLGA (RG653H, Evonik) microspheres, (B) Ethyl formate-progesterone-PLGA (65:35, Daigang) microspheres, (C) Methylene chloride-progesterone-PLGA (65:35, Daigang) microspheres, (D) Methylene chloride-progesterone-PLGA (75:25, Daigang) microspheres.

The initial burst release in the release profile at 37 °C was because of the low inherent viscosity of the choosing PLGA (RG653H, 0.321 dl/g, Evonik) and the solvent evaporation method. And the burst release was mostly because of the accumulation of the loaded drug at the surface of the microparticles [35]. In addition, the release profile of progesterone-PLGA (RG653H, 0.321 dl/g, Evonik) microspheres at 37 °C exhibited similar shape and initial burst with other literatures [35–37] which also described the release profile of progesterone-PLGA microspheres.

As a matter of fact, Peppas and Sahlin [38] have already deduced functions to describe the diffusion and erosion mechanism separately.

\[
\frac{M_t}{M} = k_1 \cdot t^m + k_2 \cdot t^{2m} \\
F = k_1 \cdot t^m \\
R = k_2 \cdot t^{2m} \\
R/F = \left(\frac{k_2 \cdot t^{2m}}{k_1 \cdot t^m}\right)
\]

When the value of \(R/F\) was larger, the erosion mechanisms contributed larger. When the value of \(R/F\) was smaller, the diffusion mechanism contributed larger [38]. The Peppas and Sahlin function could be much more appropriate to describe the complex release mechanism.

Nevertheless, in this study, the Korsmeyer-Peppas equation was still used to describe the release profiles, the \(n\) value...
Table 5 – Explanation of the origins of the phases observed during drug release [33].

| Phase I                                      | Phase II                                      | Phase III                                      | Reference                        |
|----------------------------------------------|-----------------------------------------------|------------------------------------------------|----------------------------------|
| Burst                                        | Slow diffusion-controlled release              | Rapid erosion-controlled release                | Loo et al. [39]                  |
| No burst                                     | Slow diffusion-controlled release              | On-set of degradation.                         | Alexis et al. [40]              |
| Diffusion-controlled release of drug molecules at the surface or in pores initially connected to the surface | Dependent on diffusion and erosion             | Dependent on diffusion and erosion             | Zolnik et al. [13]              |
| Similar to the row above                     | Lag-phase, as the first and second phase did not overlap | Second phase, erosion-controlled release        | Johnson et al. [41]             |
| Similar to the row above                     | Slow and minimal release                       | Rapid release. Rapid water absorption associated with sudden mass loss. | Duvvuri et al. [42]             |
| Similar to the row above Burst. Drug molecules on or with access to the surface | Degradation and erosion.                      | Onset of bulk degradation                      | Capen et al. [43]               |
| Burst                                        | Diffusion governed by water absorption and swelling | Erosion phase at which degradation occurs       | Chen and Ooi [44]               |
| Burst                                        | Diffusion due to hydration                     | Faster diffusion due to erosion. The onset of this phase depends on the rate of hydration | D’Souza et al. [12]             |
| Burst. Surface-bound and poorly encapsulated drugs may diffuse through pores and cracks | Slow diffusion, which may be attributed to binding of the drug to the polymer | Faster diffusion through the eroding matrix. Decrease in the polymer M_w increases the gaps in the matrix | Janoria and Mitra [46]          |
| Burst. Solvent penetration and glass transition | Limited drug dissolution. Polymer degradation and relaxation | Diffusion through water-filled pores           | Lao et al. [47]                 |

Fig. 3 – The complex picture of physico-chemical processes taking place within PLGA matrices, leading to drug release. The influence of processes on drug release and on other processes is illustrated by arrows. Note that some arrows point in both directions. (Modified from Fig. 2 in Reference [33].)
here was not used to identify the release mechanism but to describe the shape of the release profile, and to predict the real time release. Since the n value in the Korsmeyer-Peppas equation could be used to clearly describe the shape of the relapse profile, the n values at 43 °C and 47 °C were closest to 37 °C which were shown in Table 4, it means the profile at 43 °C and 47 °C exhibited the similar release profile with the profile at 37 °C. So the release profile at 43 °C and 47 °C could be used to predict the real time release (37 °C). The n value at 52 °C was not mentioned in Table 4, because 52 °C was higher than Tg (45.3 ± 0.61) of the microspheres. When the temperature was near or above the Tg, the chain of the polymer will be more mobility so the drugs will be released from the polymer in a quicker way. In addition, the high magnetic stirring rate used in this study also increased the releasing rate from the polymer. So, at 52 °C, the release was finished in a very short time. Few available timing points could be fitted by the Korsmeyer-Peppas equation and Weibull function. This was also the reason that part of the n value was not mentioned in Tables 6–8 in the “reproducibility testing” part.

The comparison of the release profile at 37 °C and 43 °C were shown in Fig 4A. Although the two profiles have different x-axis scales, they share the similar shape which means they have the similar functions. With this amazing finding, we just need to convert the x-axis scales to deduce the function of the real time release. So the profile of the 43 °C was used to predict and deduce the function of the release profile of progesterone PLGA microspheres at 37 °C.

In the Fig. 4A, it could be seen clearly that the microspheres released about 40% at 2 h at 43 °C and need about 30 h to reach the same amount at 37 °C, this was the conversion ratio of the x-axis between the release profile at 37 °C and 43 °C. And the conversion ratio was 2:30 = 1:15. The profile at 43 °C were fitted by the Weibull function as Y = 1-exp(-((0.08828 h)0.4345)), R² = 0.9322. By using of the conversion ratio, the function of the release profile at 37 °C was deduced as Y = 1-exp((0.08828 h/15)0.4345), R² = 0.9921.

It should be noted that, in both the Korsmeyer-Peppas equation and the Weibull function, the value of the M/M was

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from 0 to 1, but in the figures, the y-axis means the accumulated release (100 M/M), so all the deduced functions were multiplied by 100% to show in the figures.

As mentioned above, the release profile of the microspheres at 47 °C could also be used to deduce the function of the real time release. The comparison of the release profile between 37 °C and 47 °C, and the correlation between the deduced function and the function of real time release were shown in Fig. 4B.

The Weibull function of the release profile at 47 °C was: 
\[ Y = 1 - \exp(-0.8362t^{0.4627}), R^2 = 0.9830. \]
From Fig. 4B, the microspheres released about 50% of its contents within 0.5 h at 47 °C, and it need about 71.5 h to reach the same amount at 37 °C, and this was the conversion ratio of the x-axis between the release profile at 37 °C and 47 °C. Since the conversion ratio was 0.5:71.5 = 1:143, the deduced function of the release profile at 37 °C was 
\[ Y = 1 - \exp(-0.8362t/143^{0.4627}), R^2 = 0.9912. \]
But thinking about the releasing time at 43 °C and 47 °C, the release profile at 47 °C was a great choice to deduce the function of the real time release.

In summary, with this kind of method, the shape of the real time release could be predicted and deduced successfully and the method was simple. After a series in vitro release at elevated temperatures were done, the most similar profile with the real time release profile was picked, and the conversion ratio was used to deduce the release profile.

3.4. **Reproducibility testing**

The physicochemical properties of these progesterone microspheres made by different types of PLGA were elaborately listed in Tables 1–3. And the SEM images were shown in Fig. 1.

3.4.1. **Data analysis of ethyl formate-progesterone-PLGA (65:35, Daigang) microspheres**

For ethyl formate (organic phase)-progesterone-PLGA (65:35, 0.386 d/l/g, Daigang), the in vitro release profile at different temperatures were shown in Fig. 5A, and described by the Korsmeyer-Peppas equation were listed in Table 6. The n value of these functions showed that the release profile at 55 °C was the most similar to the real release profile at 37 °C.

The Weibull function was used to describe the release profile at 55 °C as 
\[ Y = 1 - \exp(-(0.6425t)^{0.6533}), R^2 = 0.8408. \]
In the Fig. 5B, the conversion ratio between the two release profile at x-axis was 0.5:150 = 1:300. Therefore, the deduced function of the real time release was: 
\[ Y = 1 - \exp(-(0.6425t/300)^{0.6533}), R^2 = 0.9781. \]

It should be discussed that, in the Weibull function of the release profile at 55 °C, the value of R² was a little small. From the Fig. 5B, the shape of the release profile at 55 °C looks more like a line than “S” shape. So, when using the Weibull function to describe the profile, the value of R² would be a little small. But it can still describe the profile at 55 °C. When the temperature was much higher than the Tg, the releasing rate will be greatly increased, and this will leads to huge burst release at the first timing point, and the release profile integrally will looks like a straight line, so as to lose the opportunity to exhibit the similar shape with the release profile of the real time release. And this method can’t be used to deduce the function of the real time release. This was also the reason that some of the n values were not mentioned in Tables 4, 6–8. So it was not the higher temperature the better. The choosing elevated temperatures should be used to exhibit the shape of the release profile.

3.4.2. **Data analysis of methylene chloride-progesterone-PLGA (65:35, Daigang) microspheres**

The methylene chloride (organic phase)- progesterone-PLGA (653H, 0.386 d/l/g, Daigang) was also made to help test the reproducibility of the predicting method. The in vitro release profile at different temperatures were shown in Fig. 6A and described by the Korsmeyer-Peppas equation were listed in Table 7. The n value of these functions showed that the release profile at 51 °C was the most similar to the release profile at 37 °C.
The Weibull Function was used to describe the release profile at 51 °C, as
\[ Y = 1 - \exp(-0.5393t/160^{0.6195}) \], \( R^2 = 0.9796 \). In Fig. 6B, the conversion ratio between the two release profiles at x-axis was 0.580 = 1:60. Therefore, the deduced function of the real time release was:
\[ Y = 1 - \exp(-0.5393t/160^{0.6195}) \], \( R^2 = 0.9918 \).

3.4.3. Data analysis of methylene chloride-progesterone-PLGA (75:25, Daigang) microspheres

The in vitro release profiles at different temperatures were shown in Fig. 7A. The Korsmeyer-Peppas equation was used to describe the release profiles and was listed in Table 8. The n value of these functions showed that the release profile at 60 °C was the most similar to the release profile at 37 °C. According to the Fig. 7B, the conversion ratio between the two release profiles at x-axis was 1:400. The Weibull Function of the release profile at 60 °C was:
\[ Y = 1 - \exp(-0.6394t/400^{0.7850}) \], \( R^2 = 0.9819 \). Therefore, the deduced function of the real time release would be:
\[ Y = 1 - \exp(-0.6394t/400^{0.7850}) \], \( R^2 = 0.9972 \). It also exhibited a great correlation between the deduced function and the profile of the real time release.

It should also be noted that the different drug loading rates and particle size distributions were obtained by using different types of PLGA and organic phases. This was because it was difficult to change one of these factors with the similar preparing method. For example, in order to increase the drug loading rate, the encapsulation efficiency usually decreased and maybe other factors like particle size distribution will also changes. So in this study, different types of PLGA and different organic phases were used to obtain the different drug loading rate, particle size distribution and even using different release mediums. However, in this way, many different factors were

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mixed and tested to help verify the reproducibility of the predicting method.

Luckily, from these results, even with different kinds of drug loading rate, particle size distribution, different release medium and different preparing method with different types of PLGA, the shape of the release profiles were similar, and there were many other literatures [35–37] deeply studied the progesterone-PLGA microspheres with many different parameters, and they all exhibited the similar shape of the release profile as mentioned in this study. In all, the reproducibility of the predicting method was great, and this method could be a potential good choice to predict the real time release.

However, the method still has some shortcomings and it could be seen in figures describing the correlation between the deduced function and the real time release. The final part of the profile of the real time release didn’t show perfect correlation with the deduced function. In the very final part, the microspheres were already ruptured because of the bulk erosion, so it could not release drugs in the controlled manner which was the same with the former part.

In addition, the Weibull function could only be used to describe erosion-dominated process coupled with minimal diffusive release rates. So, the method in this study couldn’t perfectly describe the very final part of the profiles, but it can successful describe the profile in general.

4. Conclusion

The method of elevated temperatures coupled with magnetic stirring to predict real time release from long acting progesterone PLGA microspheres could be described briefly in several steps. First, obtain release profiles at 37 °C with orbital shaking and elevated temperatures with magnetic stirring. Second, the Korsmeyer-Peppas equation was used to depict the release profiles at different temperatures to help finding the most similar release profiles from the elevated temperatures with the profile of real time release. Third, the Weibull function was used to describe the chosen elevated temperature, and the function of the real time release (37 °C) was calculated with the help of Weibull function and conversion ratio. With the use of this method, the function of the real time release could be easily deduced according to the release profile at elevated temperatures.

Different types of PLGA (BG653H (Evonik), 65:35(0.386, Daigang), 75:25 (0.401, Daigang)) and different organic phases (ethyl formate and methylene chloride) were used to make the progesterone PLGA microspheres with different drug loading rate, particle size distribution and even different release mediums were used to help verify the predicting method. All the deduced functions exhibited a great correlation with the profile of the real time release in this study with $R^2 = 0.9912, 0.9781, 0.9918$ and 0.9972, respectively.

However, this elevated temperatures predicting method has limitations. Because the Weibull function was only suitable for sigmoidal drug release profiles, and it was used to model release profiles that showed zero to minimal initial burst release, zero to minimal diffusion-mediated release rate or erosion-dominated process coupled with minimal diffusive release rates [30]. So the very final part of the real time release was not perfectly described by the deduced function. But this method was still a great choice to predict and deduce the function of real time release in general. It also provided an inspiration in predicting the release profile of long acting microspheres.

Declaration of interest

No conflict of interest exists in the manuscript, and the article is approved by all the authors.

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