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RESEARCH PAPER

Injectable Sustained-Release Depots of PLGA Microspheres for Insoluble Drugs Prepared by hot-Melt Extrusion

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ABSTRACT

Purpose Progesterone (PRG) was selected as a model drug to develop a long-acting injection system for poorly water-soluble drugs.

Methods Microspheres with high density-low porosity were prepared by hot-melt extrusion (HME) combined with wet-milling as the representative formulation, and a microcrystal suspension was also studied as a comparison. The morphology, particle size and distribution, polymorphism, drug distribution, density and porosity were characterized by scanning electron microscopy, laser diffraction particle size analyzer, power X-ray diffraction and DSC respectively. The *in vivo* performance of the different formulations within 7 days after intramuscular injection was evaluated in male SD rats.

Results The drug-loading rate of the microspheres could be as high as 40%. The average initial burst release of the microspheres (PLGA lactide:glycolide = 75:25) was only 6.7% much lower than that of the microsuspension (25.7%) and a sustained release was exhibited for at least 7 days. The release mechanism was speculated to be as follows. The microspheres are a drug depot with drug microcrystals in the PLGA matrix which is a layer by layer honeycomb structure.

Conclusions Microspheres prepared by HME combined with wet-milling could achieve a long-term sustained release effect as a novel long-acting formulation strategy.

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KEY WORDS HME · *in vivo* · microsphere · PLGA · progesterone

ABBREVIATIONS

API	Active pharmaceutical ingredient
ART	Assisted reproductive technology
$AUC_{0-\infty}$	Area under the plsma concentration-time curve
C_{max}	Maximum plasma drug concnetration
CSD	Cambridge structural database
DSC	Differential scanning calorimetry
FDA	Food and Drug Administration
HME	Hot-melt extrusion
HPMC	Hydroxypropyl methylcellulose
HSP	Hansen solubility parameters
i.m.	Intramuscular
LC–MS/MS	Liquid chromatography–mass spectrometry/mass spectrometry
PBS	Phosphate buffered saline
PLGA	Poly d,l-lactic-co-glycolic acid
PMC	Progesterone microcrystal suspension
PMS	Progesterone microspheres
PRG	Progesterone
PXRD	Powder x-ray diffraction
SD	Sprague-dawley
s.c.	Subcutaneous
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy
SP	Span value
$T_{1/2}$	Plasma half-life
T_{max}	Peak time

INTRODUCTION

Currently, the number of diseases, which require long-term drug treatment, such as hormone-dependent conditions (1)

and mental disorders (2) is increasing gradually. Thus, sustained-release drug delivery system that can exert long-term efficacy are of concern. The most readily available drug delivery systems that can realize long-term drug release processes is parenteral injection, by which dosages are mainly administered via subcutaneous (s.c.) and intramuscular (i.m.) routes (3).

At present, oily solutions, *in situ* forming drug depot systems, drug microcrystal suspensions and drug-loaded microspheres are the most extensively used injectable dosage forms for achieving sustained release of active pharmaceutical ingredients (API) (4–6). Simply speaking, oil depots of water-insoluble lipophilic drugs are broadly used as sustained release parenteral depot formulations (4), such as haloperidol decanoate for mental disorders (7). Another interesting injectable sustained-release system *in situ* forming depot systems (8,9) are low-viscosity injectable polymers or suspensions before administration, which then solidify into semi-solid depots after injected into the muscle (10). In addition, formulating poorly-soluble drugs into micro- or nano-suspensions can rescue them from 'brickdust' candidates (11). In drug microcrystal suspension depots, the components are simply API microcrystals and stabilizers, so the dosage form is simple and inexpensive to prepare. The microcrystal suspension depots can achieve long-term therapeutic effects, since poorly soluble API dissolves from the drug crystals into body fluids slowly. When a suspension cannot attain the desired release rate, a microsphere preparation is the most important long-term method of administration. Microspheres based on polylactic acid-glycolic acid (PLGA) have been intensively investigated as parenteral delivery systems for the sustained release (12–14) and they are now one of the most successful complex parenteral drug products on the market. PLGA is the most commonly used biodegradable polymer for sustained release formulations that can achieve long-term release from weeks to months, and the polymer has been approved by the FDA for parenteral use because of excellent biocompatibility and biodegradability (15–18).

Conventional techniques for preparing drug-loaded PLGA microspheres include emulsification-solvent evaporation (19), spray drying and inkjet printing. Water-insoluble drug molecules are typically encapsulated in PLGA microspheres by oil/water solvent evaporation or extraction (20). Generally, the drugs and the polymers are dissolved in the organic phase firstly, and the organic phase is added to the aqueous phase containing the emulsifier under the mechanical force in order to prepare the O/W droplets. Then the organic phase is separated by solvent evaporation or spray drying so that the primary droplets shrink and are turned into solid polymeric particles in the end. The microspheres prepared by these methods have a common problem, that the solvent removal process by evaporation or spray drying leads to holes and channels in the microspheres. Hence, drug diffuses through the pores and cracks in the polymer matrix. Such behaviors lead to unwanted dosage rapid release in the initial phase. The other concerns about microspheres are the drug loading limitations, and potential toxic solvent remainders inside the microspheres.

Hot-melt extrusion (HME) is drawing more attention as an alternative (21). Compared to both emulsification and spray drying processes, this technique's major advantage is that no solvent is used and no pores and cracks are caused by solvent migration during the preparation process. Additionally, drug loading is not restricted by the formation of microcrystals in the matrix. This method has only a few steps, and is a continuous and highly reproducible process (22). By controlling the extrusion temperature and the rotational speed, the carrier melts so that the drug microcrystals are uniformly dispersed in the PLGA matrix. Then, the solid dispersion forms nearly spherical sustained-release microspheres by using the wet-milling method. The microspheres have a honeycomb layer by layer structure. With the incremental erosion of PLGA layers, the drug in the corresponding grid is released gradually. Progesterone(PRG) secreted by the ovarian corpus luteum is essential for luteal phase support in assisted reproductive technology (ART) (23). It was selected as a model drug because of its poor solubility and its need for long-term administration. Besides, PRG is very potent and stable at high temperatures. It is, therefore, often incorporated in polymer matrices using a melting process for extended drug release.

Progesterone microspheres (PMS) prepared by HME were chosen as representative formulations for PRG long-acting injections for luteal phase support in ART. Simultaneously, a progesterone microcrystal suspension (PMC) was also studied as a comparator. Sample PMC(F1) is the representative of the first formulation strategy studied, and microcrystal suspensions were obtained by particle size reduction. F2 and F3 represent the second formulation strategy in which the microspheres of PRG are dispersed uniformly in the PLGA matrix by HME. Here, the PLGA composition's influence was evaluated by comparing a PLGA with a lactide: glycolide ratio of 50:50 (PLGA50:50) (F2) to another PLGA with a lactide: glycolide ratio of 75:25 (PLGA75:25) (F3). The present study provides an insight into the preparation of high density-low porosity microspheres by hot melt extrusion combined with wet grinding method. This will be a novel option for long - acting injections.

MATERIALS AND METHODS

Materials and Animals

PLGA co-polymers (lactide:glycolide molar ratio of 50:50, Mw 10,000 Da, i.v. = 0.12 dL/g and lactide:glycolide molar ratio of 75:25, Mw 10,000 Da, i.v. = 0.12 dL/g) were

obtained from Jinandaigang Biomaterials (Shandong, China). The API was progesterone (PRG) provided by Zhejiang Xianju Pharmaceutical Co., Ltd.(China). Mannitol was obtained by Tianjin Guangcheng Chemical Agent Co., Ltd.(China). Hydroxypropyl methyl cellulose (HPMC E5) were kindly donated by DOW (Midland, Michigan). All reagents and chemicals used were of analytical or chromatographic grade.

Animal experiments were performed with male Sprague-Dawley rats (200 ± 10 g) which were provided by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). All the animal experiments carried were assessed and approved by the University Ethics Committee for the use of laboratory animals and in compliance with the Guidelines for the Care and Use of Laboratory Animals.

Composition of Formulations

Table I provides an overview of the formulation and preparation method of three formulations tested. The formulations will be demonstrated by the code F1-F3 as shown in Table I.

Preparation of a Progesterone Microcrystal Suspension(PMC)

The PMC was prepared using a wet-milling (Minizate, NETZSCH-Feinmahlechnik GmbH, Staufen, Germany) technique. The API was first dispersed in water containing a stabilizer, and the ultimate suspension was ground in a recycle mode with a milling medium (diameter of 0.1 mm zirconia ground beads). The processing temperature was maintained below 30°C by the cooling water circulation in the outer sheath. The particle size was measured using laser particle size analyzer (BT-9300S, Bettersize Co., Ltd., Dandong, China). The particle size distribution was measured while grinding, at a rotation frequency of 1500 rpm until the particle size was reduced to the target particle size and then the suspension was took out. Due to the uneven size distribution of the initial suspension, particle size screening was required to obtain PMC that meets the particle size distribution requirements. The initial suspension was standed in a beaker for 5 min, and suspensions without large particles were collected by pouring the supernatant suspension carefully into another beaker. The suspension was placed in a centrifuge tube and centrifuged at 1000 rpm for 3 min. The supernatant was then discarded and the lower layer was re-dispersed in water to obtain a homogeneous suspension without any large particles nor small particles. The suspensions consisted of 4% drug in pH 7.4 PBS containing 0.2% HPMC E5. Finally, the particles were freeze dried then stored at room temperature.

Preparation of Progesterone Microspheres(PMS)

Micronization of APIs

PRG was wet-milled to reduce the particle size to about 1 μm, and the chamber was filled with zirconia milling beads with a diameter of 1.0 mm. The milling medium was coated with zirconia to provide high-energy efficient milling and low contamination from the metal parts during the attrition process. The particle size was measured using a laser particle size analyzer and the PRG particles were collected by lyophilization.

Hot Melt Extrusion and Medium Milling

The required ratio of PRG and polymer were weighed into a sealed polyethylene bag and hand blended for about 10 min. The mixture was extruded on a HME (ATS ZE-16 twin-screw extruder) with a 1 mm diameter die, giving matrices 1.1–1.2 mm in diameter. The screw rotation speed was set at 20 rpm and the residence time was about 10 min. The extruder parts temperature was set as follows: zone 1 (40°C), zone 2 (60°C), zone 3 (80°C), zone 4 (80°C), zone 5 (80°C), zone 6 (80°C) and die (60°C). The extrudate was cut to5 mm length segments, then microrods were shattered with a mini-type pulverizer and passed through a 100 mesh sieve. Finally, the solid dispersion powder and stabilizer were dispersed in an aqueous-based solution, and PMS were also prepared with the same method by media milling and particle size screening as in the case of the microsuspensions. The overall PMS preparation process is shown in Fig. 1.

Characterization of PMS and PMC

Particle Size Determination

The particle size of the PMS and PMC were determined with a Laser Particle Size Analyzer (BT-9300S, Bettersize Co., Ltd., Dandong, China). The particle size distribution was evaluated by d_{50} and span, the particle size was calculated from the volume distribution with the Mie model, and the

Table I Overview of Formulation Composition and Manufacturing Method

Formulation	Dosage form	Composition (wt.%)	Manufacturing method
Formulation 1 (F1)	microcrystal suspension	API	Media milling
Formulation 2 (F2)	Microsphere	API/PLGA(50:50/i.v. = 0.12dL/g) 40/60	hot melt extrusion
Formulation 3 (F3)	Microsphere	API/PLGA(75:25/i.v. = 0.12dL/g) 40/60	hot melt extrusion

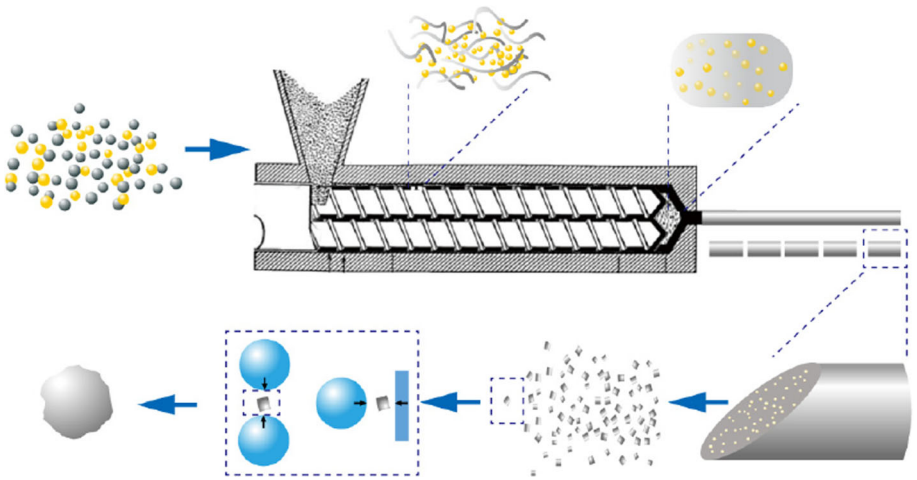

Fig. 1 The complete process and possible mechanism of formation of PMS, where PLGA is gray, PRG is yellow and Grinding beads are blue.

SP (span value) = $(d_{90}\text{-}d_{10})/d_{50}$, where d_{90} is the particle diameter at 90% volume percentiles, where d_{90} is the particle size cumulative distribution at 90% by volume, d_{10} is at 10%, and d_{50} is at 50%. The SP is a measure of how wide is the size distribution or the flatness of the distribution curve (24). For span values ≤ 5, the size distribution is considered to be narrow (25). The measurements were performed 3 times for each sample.

Microphotograph and Scanning Electron Microscopy (SEM)

The morphological evaluation of PMS and PMC were examined using an optical microscope (COIC-500, Chongqing COIC Instrument Co., Ltd., China) and a scanning electron microscope (JEOL, Peabody, MA, USA). Freeze-dried PMS and PMC were laid on double-sided conductive tape pre-attached to the copper strip. After coated with a gold layer, the samples were observed by SEM.

Differential Scanning Calorimetry (DSC)

The thermal properties of crude PRG, PLGA, physical mixture and extruded product were analyzed using a Thermal Analyzer-60WS, DSC-60 (Shimadzu, Japan). The powder sample was placed in a sealed aluminum plate equipped with a porous cap and heated from room temperature to 200°C at a heating rate of 5°C/min under nitrogen flow protection with the rate of 50 ml/min.

Powder X-ray Diffraction (PXRD)

Powder X-ray diffraction (PXRD) patterns were performed with Cu Ka radiation, and standard runs at a voltage of 40 kV and current of 40 mA, a scanning rate of 2°/min over a 2θ range from 5° to 60° using a Panalytical Empyrean diffractometer (PANanalyical, Almelo, The Netherlands).

Density and Porosity

The apparent density of the hot melt extruded rod (ρ_g) was calculated from its weight and volume. The true density of progesterone and PLGA can be obtained from literature studies, the true density of the extruded rod (ρ_t) was calculated by the proportion of progesterone and PLGA contained in the extrudate. The porosity can be calculated by Eq. (1).

$$Porosity = 1 - \frac{\rho_g}{\rho_t} \quad (1)$$

Solubility Determination

Excess amount of PRG was added to different pH PBS in sealed glass vials. The vials were agitated at 100 rpm for 72 h in the shaker water bath (ZWY-110X30 reciprocal shaking water bath, Zhicheng Inc., China) at 37°C, then still standing 24 h and centrifuged (HC-3018R, Anhui USTC Zonkia Scientific Instruments Co., Ltd., China) at 10,000 rpm for 10 min at 4 C to remove the undissolved drug. The concentration of PRG was analyzed by HPLC at 241 nm. The experiments were performed in triplicate.

In vitro **Drug Release**

The rate and extent of diffusion from muscle into blood of PRG from microparticles were simulated using a Franz diffusion cell system (26) (RYJ-12B, Huanghai Medicine&Drug Testing Instruments Ltd., Shanghai, China). The Franz Cell system was maintained at a constant temperature of 37 ± 0.5°C in a thermostatic bath, while the receptor medium was stirred at 100 rpm during the experiments. For the dissolution studies, a 0.22 μm synthetic microporous membrane was used as the separation medium, and each membrane was carefully placed at the interface between the donor and receptor compartments. Microparticles of

different formulations equivalent to about 5 mg PRG were dispersed in 1 ml of water which was directly added to the donor cell, and the receptor cells were filled with 7 mL PBS (pH 7.4) containing 0.5% SDS as the receptor fluid to maintain sink conditions. At predetermined intervals, receptor buffer was withdrawn via the sampling port and replaced by an equal volume of fresh medium. The samples were passed through a 0.45 μm filter and the concentration of PRG was measured by spectrophotometry at 241 nm (UV-2800A, UV–Visible spectrophotometer, UNICO, Shanghai China). Each sample was measured in triplicate.

In vivo Performance

Intramuscular Drug Administration

Animal experiments were performed in male SD rats (200 ± 10 g) and randomly divided into three groups, each of four animals. The rats were fasted overnight before injection but but were free to drink water throughout the study. Before administration, the three formulations were suspended in normal saline, and each group received the suspension by intramuscular injection. For three formulations, the drug dose administered was 14.58 mg/kg. After mild anesthesia, approximately 0.5 ml of blood samples were collected from the retro-orbital plexus to the micro-centrifuge tubes containing heparin at 0.5, 1, 2,3, 4, 8,12, 24, 48, 72, 96, 120, 144 and 168 h. The blood samples were centrifuged at 4000 rpm for 10 min at 4°C to separate the erythrocytes, plasma was obtained and stored at −20°C until analyzed.

Plasma Processing

The PRG concentration in plasma was determined by liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS). The PRG in 20 μL plasma was extracted with 1 ml extraction solvent (normal hexane: dichloromethane: isopropanol = 100:50:5). The organic layer was removed and evaporated to dryness under nitrogen at 40°C (27)And the residue was reconstituted in 100 μL methanol \r to analysis using an LC-MS/MS system (28).

Pharmacokinetic Data Analysis

Pharmacokinetic data analysis was performed using DAS 2.0. software (Mathematical Pharmacology Professional Committee of China, Shanghai, China). The area under the plasma concentration time curve $AUC_{0-\infty}$, C_{max}, $T_{1/2}$ and T_{max} were calculated. Statistical differences were examined using Student's t-test with $p < 0.05$ as the minimum level of significance. Burst release was identified based on the plasma concentrations obtained within 4 h after injection, at this point, all plasma concentrations had reached a maximum, so this time point was selected. The percentage of drug released during this time was calculated by Eq. (2).

$$Initial\ burst = 100 \times \frac{AUC_{0-4h}}{AUC_{0-\infty}} \quad (2)$$

RESULTS AND DISCUSSION

Characterization of Microparticles

Particle Size Distribution Analysis

The mean particle size of the bulk drug was about 90 μm, and that of the PRG particles after micronization was approximately 1 μm. For the preparation of formulations, due to the uneven size distribution of the initial suspension, particle size screening was required to obtain PMS or PMC that meet the particle size distribution requirements. Firstly, the initial suspension was placed in a beaker for 5 min and, when the suspension was left to stand, large particles rapidly settled naturally, in contrast to small particles. This phenomenon follows Stokes' Law, and the suspensions were collected by pouring off the supernatant suspension carefully, thereby removing large particles. Then, the suspension was placed in a centrifuge tube and centrifuged at 1000 rpm for 3 min, at a low speed for a short period very small particles remained suspended in the upper layer and the supernatant was discarded to remove the small particles. So, PMS and PMC presented as a monomodal population with very similar diameters, d_{50} ranging from 8.118 μm to 9.714 μm (Table II) and there were no particles larger than 35 μm. It was well known that the particle size and its distribution will significantly affect the *in vitro* and *in vivo* release in microsphere systems. The difference in release rate

Table II Mean Particle Size (μm) and Span Value of Bulk PRG, Micronized PRG and Microparticles of Three Formulations

Formulation	d_{10}	d_{50}	d_{90}	Span value [a]
Bulk drug	21.66	90.36	287.1	2.937
Micronized drug	0.543	1.101	2.466	1.745
F1(PMC)	3.169	8.264	16.09	1.564
F2(PMS PLGA50–50)	2.685	8.118	17.89	1.873
F3(PMS PLGA50–50)	1.968	9.714	19.40	1.795

[a] The mean particle size of the microparticles was measured using a Laser Particle Size Analyzer. Span value = $(d_{90}-d_{10})/d_{50}$

due to dosage form factors is more intuitive with approximate particle size distribution of three formulations.

Morphology of PRG Microparticles

Regarding morphology, the optical microscope images of PMS and PMC exhibited block particles with a uniform particle size (Fig. 2). As illustrated in Fig. 3a, PMC exhibited a spherical, non-porous surface in the SEM image, and the surface morphology of PMS prepared by hot melt extrusion combined with wet-milling is shown in Fig. 3b where it exhibits an irregular shape with a uniform size distribution, and the surface of the microspheres was very smooth. The PMS prepared by the hot melt extrusion grinding method were different from the microspheres prepared by emulsification or spray drying, and there were no signs of drug and polymers dissolving in organic solvent to form primary droplets. The nearly spherical and rough surface of the PMS were formed by collision and milling of the solid dispersion particles produced by grinding beads, and it was difficult to obtain a spherical and smooth surface. Also, this situation did not affect the sustained release of PMS, and the reason for this will be given later. In addition, the particle size observed by SEM was consistent with that obtained from microscopy and Laser Particle Size Analyzer examinations.

Study of the Physicochemical Properties

Thermal Analysis

In Fig. 4, the sharp melting peak at 129°C (onset values) indicated the crystalline nature of the original PRG powder. Comparing the endothermic peak of PRG in the PMS (Fig. 4-f) and extruded product (Fig. 4-e), with that of the physical mixture (Fig. 4-d), it was clear that the crystalline nature of PRG in the extruded product had been maintained throughout the preparation process. PLGA is non-crystalline without any endothermic peak. The state of PLGA changed when the melting point of PRG was reached, due to the increase in temperature during the DSC analysis. Therefore, the crystalline state of the drug was further analyzed by XRD pattern.

Powder X-ray Diffraction (PXRD)

PXRD were undertaken to the analyzed crystallinity of PRG with PMS and PMC. The XRPD patterns of APIs were studied and compared with the Cambridge Structural Database (CSD) crystallographic data (PROGSTERONE, form I), which suggested that it was in a highly crystalline state and was Form I of Progesterone. The PXRD patterns for the PRG, PLGA, extrusion millrods and the PRG-loaded PMS are showed in Fig.5. The PLGA polymer and PRG controls (Fig. 5a) demonstrate that PRG has numerous sharp distinct characteristic peaks at a scattering angle of 2-theta, which suggested that it is highly crystalline, while the PLGA was presumed to be an amorphous structure pattern with a smooth PXRD pattern. The PXRD patterns for the extrusion millrods and PRG-loaded PMS produced by HME are parallel to the PRG controls, with many sharp high intensity diffraction peaks associated with PRG (Fig. 5a and b). It would suggest that the PRG is dispersed in the form of crystals. This is due to the millirods being produced at approximately 80°C, which is above the glass transition temperature of PLGA and below the melting temperature of PRG (128°C) and, consequently, PRG remains in its crystalline form and is dispersed into the softened PLGA by hot-melt extrusion. For the sustained formulation, progesterone form1 was found to have excellent crystallization with a higher stability, and a lower solubility and dissolution rate (29). XRD diffractograms of PMS, PRG and cryoprotectant are shown in Fig. 5. Clearly, lyophilized PMC was Form I of PRG.

Density and Porosity

According to the measurements carried out, the apparent density of the extruded rods was 1.1654 g/cm^3. The true density of the extrudate can be calculated from the true density and proportion of progesterone and PLGA.

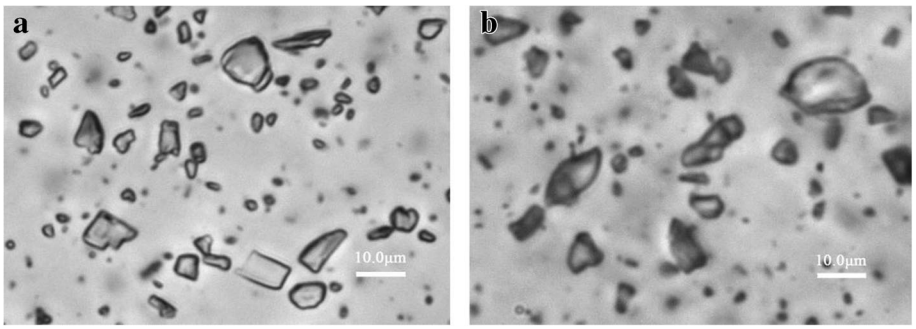

Fig. 2 Optical microscope images of PMS (**a**) and PMC (**b**).

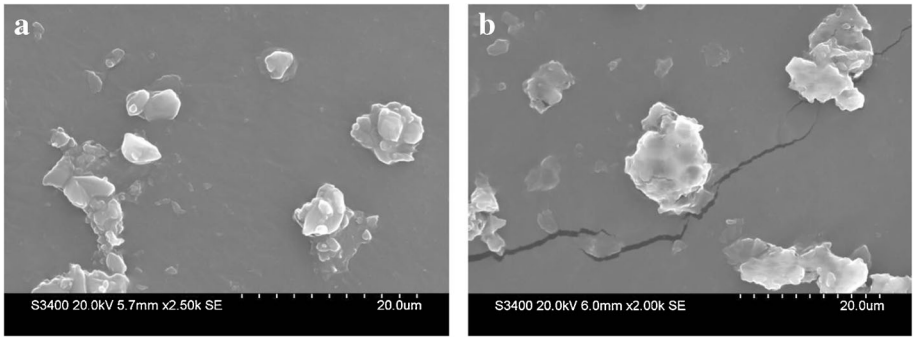

Fig. 3 SEM images of PMS (**a**) and PMC (**b**).

The true density of progesterone was 1.13 g/cm^3, while that of PLGA was 1.30 g/cm^3, and the ratio of progesterone to PLGA was 4:6 in the extrudate. Therefore, the true density of the extruded rods was calculated to be 1.2320 g/cm^3, and the ratio of the apparent density to the true density was 0.9460. The literature shows that the ratio of apparent density to true density of microspheres prepared by emulsification or electrospraying is less than 0.90, or even lower. On the other hand, the porosity calculated by Eq. 2 was only 5.40%, which was also lower than the porosity reported in the literature (30,31). During hot melt extrusion, the drug and matrix were compacted tightly with few pores, under high pressure and no pores and cracks were formed by solvent migration during the process. Therefore, the apparent density was very close to the true density.

In Vitro Release Study

A method simulating drug release from the muscles was used to compare the release behavior of the three different formulations. As is well known, intramuscular injections are administered by the extravascular route, and the desired pharmacological response was produce until the drug entered the systemic circulation from the site of injection. After administration, the drug will be released from the microparticles and dissolve in the interstitial fluid, then diffuse into the capillary circulation which mainly depends on passive drug diffusion.

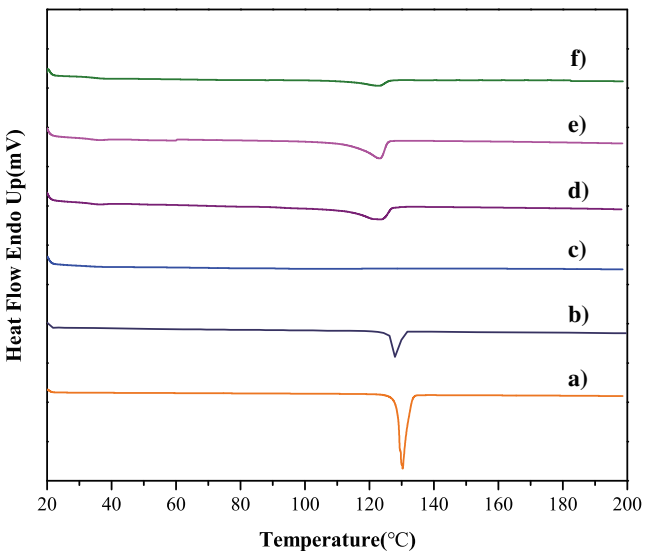

Fig. 4 The DSC curve of PMC and PMS PRG (**a**), PMC (**b**), PLGA (75:25) (**c**), physical mixture (**d**), extrusion product (**e**), PMS (**f**).

In the release system, the donor cells are equivalent to muscle tissue, the receptor cells can be compared with the systemic circulation, and the microporous membrane is the capillary wall between the donor and receptor compartments. When the drug particles are released, a high concentration of PRG is formed in the receptor cells. Due to the concentration gradient, the drug molecules diffuse into the receiving cells passively through the microporous membrane. The receptor cells were filled with PBS (pH 7.4) containing surfactant to maintain sink conditions as release medium to simulate the systemic circulation. Differences *in vitro* release were analyzed between the different model formulations (Fig. 6).

The *in vitro* release of the formulations was tested up to 7d in a Franz Diffusion Cell system (Fig. 6). The release of PMS (F2 and F3) during the entire course of the experiment (0–7 days) was lower than that of PMC(F1) and both showed similar release behavior. From the release curves (Fig. 6), PMC (F1) exhibited immediate and complete drug release with approximately 90% drug release after 24 h, due to the large surface area of PRG exposed to the release medium. It appeared that the cumulative release of PMC(F1) was approximately three times that of PMS (F2 and F3) over a period of 24 h. The diffusion of PMC simply followed Fick's first law, and the release of the drug from the microparticles followed the Noyes-Whitney equation. Drug dissolved from the microcrystals and spread from high to low concentrations in the tissue fluid and then diffused into the blood circulation. However, the drug release in PMS was more complex. Firstly, the PRG at surface of the microspheres was released, resulting in some burst release. Then, with PLGA

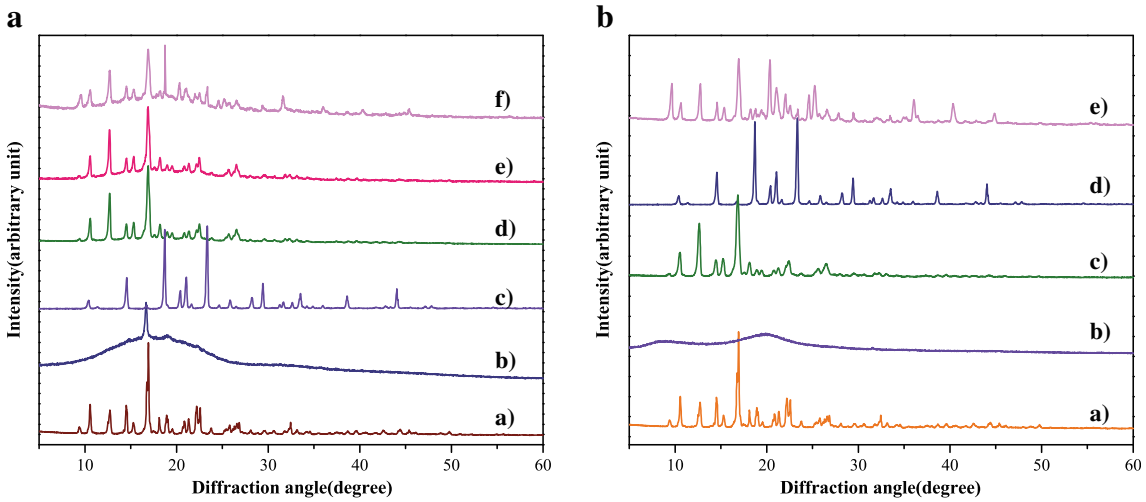

Fig. 5 **a** The PXRD curve of PMS crude PRG (**a**), PLGA (75:25) (**b**), cryoprotectant mannitol(**c**), physical mixture(**d**), extrusion product(**e**), and freeze-dried PMS(**f**) **b.** The PXRD curve of PMC crude PRG (**a**), stabilizer HPMC E5 (**b**), physical mixture (**c**), cryoprotectant mannitol (**d**), and freeze-dried PMC (**e**).

gelation and degradation in the microspheres, the drug was further released by diffusion and exposure.

Compared with PMS (F2 PLGA50:50), PMS (F3 PLGA 75:25) was released rather more slowly. This phenomenon may be explained by the degradation rate and solubility of the polymers. Generally, the degradation rates of the different microparticles follow the expected order: PLGA (lactide:glycolide = 50:50) > PLGA (lactide:glycolide = 75:25), and release rates are found to decrease with an increasing lactic content of the polymer. Since PLGA (75:25) degrades slowly, the drug release was relatively gentle in PMS(PLGA75:25). Also, the solubility parameter may be another reason. Hansen solubility parameters (HSP) have an irreplaceable use in assessing chemical interactions between solvent and polymer (32). This gives us the classic formula for Hansen Solubility Parameters where for the total parameter as in Eq. (3), δ is broken into δ_d, δ_p and δ_h for Dispersion, Polar and Hydrogen-bonding, such as in Eq. (4)–(6) (33):

$$\delta t^2 = \delta d^2 + \delta p^2 + \delta h^2 \tag{3}$$

$$\delta_d = \frac{\sum_i Fd_i}{\sum_i V_i} \tag{4}$$

$$\delta_p = \frac{\left(\sum_i F_{p_i}^2\right)^{0.5}}{\sum_i V_i} \tag{5}$$

$$\delta_h = \frac{\left(\sum_i E_{h_i}\right)^{0.5}}{\sum_i V_i} \tag{6}$$

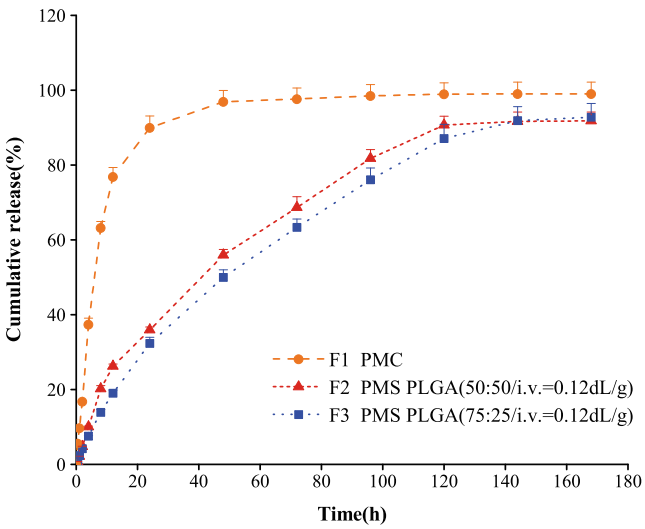

Fig. 6 Cumulative release of PMS and PMC *in vitro*.

The calculations of HSPs and molar volume for PRG and two different polymer PLGA were obtained from the CRC handbook of solubility parameters (34). The results were as follows: δ_t of PRG was equal to 20.74, δ_t of PLGA 75:25 was equal to 22.99, and δ_t of PLGA 50:50 was equal to 23.84. PLGA microspheres act as a drug depot, and PRG microcrystals are dispersed in the PLGA matrix. During the release process, with the gelatinization of PLGA, PRG is released from the inside of the gel driven by diffusion of the drug. Compounds with similar δt values are likely to be miscible, and dissolved drugs are more likely to remain in polymers. In relative terms, the Δδ between PRG and PLGA 75:25 was smaller than it between PRG and PLGA 50:50. Thus, during the release process, the dissolved PRG molecules were more easily to detain in the diffusion path of the PLGA 75:25 microspheres and are released comparatively slower.

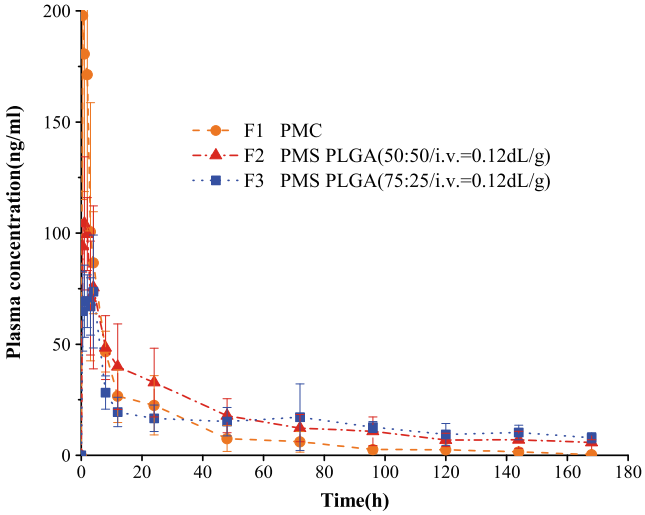

Fig. 7 Plasma concentration-time profiles up to 7 days after IM administration of the three formulations F1–F3 (mean ± SD, $n = 4$).

In Vivo Studies

In these studies, three groups of SD rats were treated with PMC(F1), PMS(F2) and PMS(F3) at a dose of 14.58 mg/kg. Fig. 7 shows the plasma concentration-time profiles of PRG within 7 days after intramuscular injection of the three formulations and Fig. 8. shows these profiles up to 12 h after administration. The main pharmacokinetic parameters, such as $AUC_{0-\infty}$, T_{max}, C_{max} and burst release, are listed in Table III.

Plasma Concentration-Time Profiles

Between 0.5 and 4 h post-administration PMC(F1) demonstrates faster plasma concentration-time curve decline (Fig. 8), in contrast to the more stable curves of PMS (F2 and F3). Compared with the PMS(F2) and PMS(F3) groups, the rats

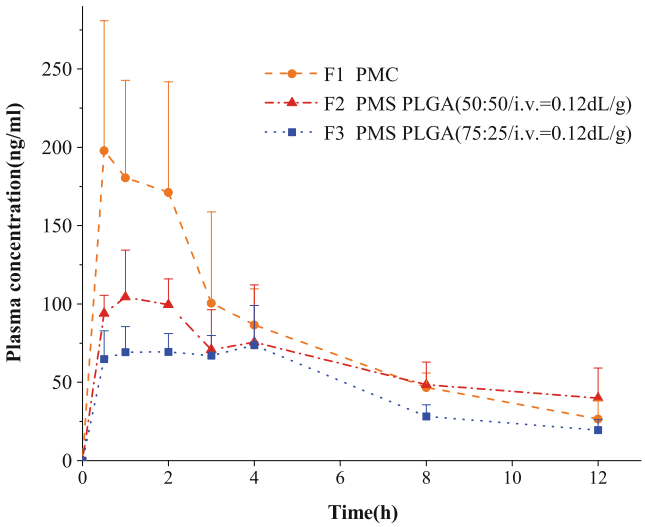

Fig. 8 Plasma concentration-time profiles up to 12 h after IM administration of the three formulations F1–F3 (mean ± SD, $n = 4$).

treated with PMC(F1) showed higher PRG concentrations at the early time points but lower PRG concentrations at the terminal time points(Fig. 7). The bioavailability of each formulation was calculated using DAS 2.0 by the plasma concentrations. The $AUC_{0-\infty}$ of PMS(F2) and PMS(F3) was 3403.79 and 3846.58 h·ng/ml respectively, while the $AUC_{0-\infty}$ of PMC(F1) was approximately half that.

Table III indicates the time required for each formulation to reach the maximum plasma concentration (T_{max}). The T_{max} was expressed as progressive, PMC(F1) was the fastest, followed by PMS(F2), and finally PMS(F3), indicating that PMC reached a C_{max} very quickly. Simultaneously, the C_{max} of PMS(F3) (84.03 ng/ml) was much lower than that of PMS(F2) (184.45 ng/ml) and PMC(F1) (216.20 ng/ml).

Burst Release

Sustained release refers to a better therapeutic effect by delaying the release rate of the drug from the formulation and reducing the rate of drug entry into the body. For plenty of the sustained release formulations, an initial large amount of drug is released when the preparation was just placed in the release medium, before reaching the stable release phase. This phenomenon is typically known as 'burst release' (35). Compared with other formulations, the formulation PMC (F1) showed a distinctly higher initial burst release as represented in Fig. 8 with high plasma concentrations being already reached 30 min after intramuscular injection. For each formulation, the initial burst release was calculated by Eq. (2) and shown in Table III. The results show the percentage of drug released of the total amount of drug released during the first four hours post-administration, namely burst release. The average initial release of F1 accounted for 25% of the total drug release, compared with 10.9% for F2 and 6.7% for F3. Therefore, the burst release of F1 was almost four times of F3, was twice of F2.

Formulation Strategy of Microcrystal Suspensions

For poorly soluble drugs, microsuspensions are commonly used as methods for developing sustained release formulations. Solubility test results showed that the solubility of PRG in PBS at pH 5.0, pH 7.4 and pH 8.0 was 2.94, 4.22 and 5.31 μg / ml, respectively. Due t the low solubility of API, resulting in a relatively low dissolution rate and sustained release, which was the approach investigated here using PMC(F1). PMC showed an initial high burst release of PRG both *in vivo* and *in vitro*. After intramuscular injection for 0.5 h, the PMC reached the maximum plasma concentration, and 25% of the total amount of drug had already been released 4 h post- administration (Fig. 7), and then the plasma concentration decreased rapidly, which was consistent with the *in vitro* release. The above data demonstrate that microcrystal

Table III Pharmacokinetic Parameters ($n = 4$, mean ± SD) After Intramuscular Administration of PMC(F1), PMS(F2) and PMS(F3) at a Dose of 14.58 mg/kg in SD Rats

Parameter	F1(PMC)	F2(PMS)	F3(PMS)
T_{max}(h)	0.8 ± 0.3	1.9 ± 1.5	2.5 ± 1.3
$T_{1/2}$(h)	38.3 ± 17.8	63.2 ± 43.8	64.2 ± 38.7
C_{max}(ng/ml)	216.2 ± 68	184.5 ± 152.1 [a]	84 ± 21.5 [a]
AUC_{0-t} (h·ng/ml)	1865.2 ± 487.6	2918.4 ± 238 [a]	2605.3 ± 601.3 [a]
$AUC_{0-\infty}$ (ng·d2/ml)	2142.8 ± 630.6	3403.8 ± 547.5 [a]	3846.6 ± 991.7 [a]
Burst release	25.7% ± 9.3%	11.0% ± 3.8% [a]	6.7% ± 1.1% [a]

All the data were presented in the form of mean ± S.D

[a] Statistically significant compared with the PMC ($p < 0.05$)

suspensions of PRG are not suitable as long-acting sustained-release preparations.

For injectable suspensions, there are two categories depending on the nature of the drug and the incidence of onset. If the particles rapidly dissolve in the blood, the pharmacokinetics will be similar to those of solution formulations (36,37) and, thus, the suspension will produce a relatively fast onset of action as an immediate release formulation. Alternatively, if the drug solubility is extremely low, depot delivery via subcutaneous, intramuscular achieves long-term drug release,as a sustained release formulation. This is reflected in the production of commercially successful formulations like Invega® Sustenna(Janssen) (38), with an aripiprazole solubility of 0.2 μg/ml (39), and 2 μm aripiprazole particles can be slowly released for 1 month.

However, for some insoluble drugs with a solubility which is not low enough, they cannot be released for a long period simply as PMC. For example, PRG particles 10 μm in size with a solubility of about 5 μg/ml were released rapidly. PRG in microcrystal suspensions did not exhibit any retardation, resulting in a large surface area of PRG being exposed to tissue fluid directly. The rich capillary network around muscle tissue allows dissolved PRG to spread into the capillaries quickly, and enter the systemic circulation and produce corresponding plasma concentrations. PRG release from the microparticles follows Fick's first law, and the faster drug release can be attributed to the relatively high solubility, large surface area and reduced particle size. This phenomenon was consistent with the trend of *in vitro* release. We concluded that for an insoluble drug with a slightly higher solubility, it is not suitable to develop microcrystal suspensions as a sustained-release formulation.

The Release Process of PMS

Fig. 8 shows the more stable and sustained plasma concentration-time profiles (0.5–4 h after injection) of PMS (F2 and F3) compared with PMC (F1). The initial burst of F2 accounted for 10.97% of the total drug release, while the burst release of F3 accounted for only 6.7%. This means that the drugs are more constant released and more suitable for prolonged release in F3. This conclusion was consistent with the *in vitro* release, and the conclusion can be explained by the solubility parameter in the "*in vitro* release".

Overall, PMS was beneficial for both the amount of drug release and the sustained release of drug. The solid dispersion was manufactured at approximately 80°C by hot-melt extrusion, which is above the glass transition temperature of PLGA. PLGA is soft above the glass transition temperature at which the PLGA molecules enter each gap between PRG particles under agitation and extrusion to form uniformly distributed millirods with a high density-low porosity and, finally, a high pressure at the exit. From the formation process of PMS, we can speculate that the soft PLGA was filled between PRG particles with a diameter of 1 μm during the extrusion process under high pressure. At the exit, the soft PLGA became cool and hardened to form a skeleton structure with a drop in temperature. Hence, the solid dispersion formed a honeycomb structure, in which PLGA acted as a grid skeleton and the PRG microparticles filled the spaces within each mesh, there were moreover a fraction of the drug existed in a solubilized state in the PLGA matrix, due to the compatibility of PRG and PLGA. We believe that the release mechanism is as follows: After administration, PRG exposed to the surface of the PMS is released firstly, and then the PLGA under the layer of PRG is corroded, and the PRG in the next grid is exposed to the medium and obtain the opportunity to release. Although the surface of PMS prepared by HME combined wet-milling is not smooth enough, the drug release is still sustained with a low burst release. This can be explained by the structure of the PMS and properties of PLGA. PRG is distributed in each grid and separated by PLGA in the PMS, and the microspheres have a layer by layer honeycomb structure. Firstly, the release of the surface drug produces a certain initial burst, but the PRG under PLGA layer cannot be reached. With the erosion of the PLGA layer from thesurface to the centre layer by layer, drug existed in a solubilized state in the PLGA matrix is released, then the drug in the corresponding

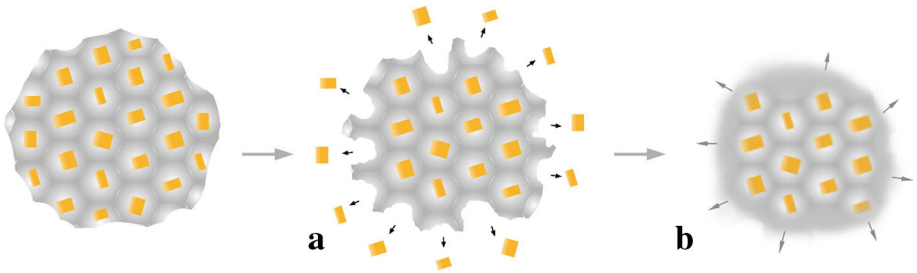

Fig. 9 The structural evolution of a PMS when exposed to a release environment, where A = release of the surface drug and B = gelatinization of PLGA. PLGA in gray, PRG microcrystal in yellow.

gridis released gradually. Then, with the gelatinization of PLGA, PRG is released from the inside of the gel driven both by diffusion of PRG and gel degradation itself to achieve a long-term sustained-release (Fig. 9).

On the basis ofthe structure of PMS prepared by the hot melt extrusion-media milling method, addition of porogen can be used in this preparation as one of the ways of adjusting the release rate. Changing the proportion of drug and matrix, and he molecular weight of PLGA are common methods of various technologies, but only the addition of porogen can be used in this process. However, the morphology and surface state of the microspheres prepared by this method are not easily controlled, and the uniform particle size distribution also requires further screening at a later stage, which requires further exploration and improvement for the HME combined wet-milling method.

CONCLUSIONS

Physicochemical characterization, and *in vitro* and vivo evaluation of different formulation strategies for controlled release injectable of a poorly soluble PRG were performed. Compared with PMC, *in vitro* and *vivo* evaluation demonstrated that the release of PMS was sustained with a low burst release, despite the surface of the PMS was not smooth enough. This was explained by the structure of the microspheres where the PRG was distributed in each grid and separated by PLGA in the PMS. Hence, drug deeply dispersed in the matrix is not affected by the surface. In addition, the microsphere structure suggests that the release rate can be controlled by changing the lactide-glycolide molar ratio of PLGA by polymer. This study provides a novel long-acting formulation strategy for relatively poorly soluble drugs, namely, combining hot melt extrusion and wet milling methods to produce high density-low porosity microspheres.

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