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A novel risperidone-loaded SAIB–PLGA mixture matrix depot with a reduced burst release: effects of solvents and PLGA on drug release behaviors in vitro/in vivo

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Abstract The purpose of this study was to develop an in situ forming SAIB (sucrose acetate isobutyrate)-PLGA (poly (d, lactide-co-glycolide)) mixture matrix depot for sustained release of risperidone. The factors affecting the risperidone release kinetics were investigated to obtain further insight into the drug release mechanisms. The burst release in vitro was significantly reduced (4.95%) by using DMSO as solvent. And, increasing the PLGA content from 2 to 10% w/w decreased the initial release from 6.95 to 1.05%. The initial release in vivo decreased with increasing PLGA content (2.0% w/w PLGA, $C_{max} = 1161.7 \pm 550.2$ ng ml^{-1}; 10% w/w PLGA, $C_{max} = 280.3 \pm 98.5$ ng ml^{-1}). The persistence ($AUC_{4-20\ days}$) over 20 days increased from 76.8 ± 20.7 to 362.8 ± 75.0 ng d ml^{-1} by inclusion of 10% PLGA compared with the PLGA-free depot. These results demonstrate that the SAIB–PLGA mixture matrix depot could be useful as a sustained delivery system for risperidone.

1 Introduction

During recent decades, injectable in situ forming systems have received considerable attention due to their distinct advantages [1, 2]. These systems can be manufactured straightforwardly even for sensitive molecules, and can be injected easily with a syringe into the body in a minimally invasive manner. Once injected, the liquid systems solidify to form a semisolid depot/gel to sustained release the drug. Furthermore, their lower specific surface area after solidification, compared to microsphere systems, may reduce burst release. So far, the researches broadly fall into aqueous based systems and systems using organic solvents. Aqueous based systems often have a disadvantage of rapid drug release due to the high percentage of water. In situ precipitation systems formed with PLGA copolymers have attracted a great deal of attention in recent years due to the regulatory approval of Eligard® [3]. However, the local and systemic toxicity produced by the large amounts of solvent (>50% NMP) and the acidic metabolites remains a major concern [1].

Another interesting sustained-release injectable depot is a sucrose acetate isobutyrate (SAIB)-based system [4, 5]. SAIB is a highly hydrophobic, water insoluble and fully esterified sucrose derivative, which exists as a very viscous liquid. After mixing with small amounts of pharmaceutically acceptable solvent, the high viscosity SAIB is formulated as a low viscosity solution [4]. Upon injection, the SAIB/solvent system forms a high viscosity depot from which the drug is released slowly. The period of sustained and controlled drug release can be adjusted from a few days to 3 months or more [6]. The amount and duration of drug release from the SAIB depot can be controlled through a number of formulation variables such as the drug loading, type and amount of solvent, the type and amount of additive, and the morphology of any crystals used [7–10].

The SAIB system has the advantages of requiring less organic solvent, easy administration, easy manufacture and a flexible sustained release period. However, there is an obvious burst release for the SAIB system due to the lag between the injection and the formation of the depot. Despite the fact that the fast drug release in the initial

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release stage is used in certain drug administration strategies, the negative effects of the burst can be pharmacologically dangerous and economically inefficient. Therefore, it is essential to control the burst release in sustained release systems.

Several strategies have been described in the literature to reduce the burst release effect of SAIB systems. For example, PLA has been dissolved in the SAIB solution as a release modifier, leading to a continuous release of native recombinant human growth hormone (rhGH) from the SAIB depot with minimal initial release [7]. Similar effects on the release of risperidone from the SAIB depot have also been reported by Lu et al. [10]. Although the inclusion of 10% (w/w) PLA could significantly reduce the in vitro burst release of risperidone to 3.5%, the resulting SAIB/PLA/EtOH exhibited high viscosity, which makes administration by injection difficult. In addition, a great deal of effort has been devoted to the drug release kinetics from the SAIB depot. Nevertheless, there is little published data on the physical properties of the SAIB depot, which limits the accurate prediction of the drug release behavior of SAIB systems. For the present study, risperidone was chosen as model compound to develop a novel SAIB–PLGA complex depot system and to carry out a systematic assessment on the depot.

In this investigation, the effects of different solvents and additives on the physical characterizations, in vitro release and in vivo pharmacokinetics of SAIB depots were evaluated. To help further understand the release kinetics of risperidone from SAIB systems, a systematic assessment on the SAIB depot characteristics was carried out, including the viscous properties, compatibility, phase inversion, solvent diffusion and morphology of the depot. On the basis of the above investigations, a novel SAIB–PLGA mixture matrix depot was developed to modulate the release of risperidone.

2 Materials and methods

2.1 Materials

SAIB was provided by Sigma Aldrich (density of 1.146 g ml^{-1} at 25°C, St. Louis, MO). Poly (lactide-co-glycolide) 75/25 (10 kDa, inherent viscosity of 0.13 dl g^{-1} in $CHCl_3$ at 25°C) and poly-D,L-lactic acid (10 kDa, inherent viscosity of 0.18 dl g^{-1} in $CHCl_3$ at 25°C) were obtained from Shandong Institute of Medical Instruments (Jinan, China). Risperidone was provided by the Beijing Fengde Chemical Science Company (Beijing, China). DMSO, NMP and EtOH were obtained from the Tianjin Chemical Reagent Company (Tianjin, China). All other chemicals and solvents were of chromatographic grade.

2.2 Methods

2.2.1 Determination of risperidone solubility

The apparent solubility of risperidone in release buffer (10 mM PBS pH 7.4, 0.02% NaN_3), EtOH, DMSO, NMP, ethyl acetate and ethyl lactate was measured by the equilibrium method. In this, 10 ml samples of solvents containing excess risperidone in bottles were rotated for 3 days at 37°C. The saturated risperidone solutions were passed through 0.22 μm membrane. These samples were analyzed by reverse phase HPLC systems (Hitachi, L-7000 series, Japan) equipped with a 5 μm C_{18} column (200 × 4.6 mm i.d., HiQil®), and eluted with a mobile phase consisted of ammonium acetate aqueous solution (5 g l^{-1}) and methanol (30:70, v/v) at a flow rate of 1 ml min^{-1}. The injection volume was 20 μl and the wavelength of the detector was 278 nm.

2.2.2 Compatibility investigations

Compatibility investigations of SAIB with additives, such as PLA and PLGA, were performed by analyzing the glass transition temperature (T_g) by differential scanning calorimetry (DSC) to evaluate the long-term physical stability of the in situ depot systems. The T_g values of SAIB, PLGA, PLA, the SAIB/PLA (70:10, w/w) mixture and the mixtures of SAIB and PLGA at three mass ratios, 78:2, 75:5 and 70:10, were measured using a Mettler-Toledo DSC822^e (Mettler Toledo Gmbh, Giessen, D), and the data were evaluated using the Mettler-Toledo STAR^e SW 9.30. Samples were placed in 40 μl hermetically sealed aluminum pans and an empty aluminum pan was used as the reference. The investigations were performed by heating from −40 to 70°C at a rate of 1°C min^{-1}. The glass transition was determined to be the onset of a heat capacity change in the reversing signal. The SAIB and PLGA mixtures at three ratios were prepared by melting at 60°C and mixing to obtain transparent blends. The SAIB/PLA (70:10, w/w) mixture was obtained in the same manner.

2.2.3 Rheological properties

The dynamic viscosities of the various SAIB solutions were evaluated using an AR2000 Rheometer (TA-Instruments, Leatherhead, United Kingdom) in the flow mode with a plate geometry of 40 mm diameter. The shear rate sweeps were measured with a linear shear rate increasing from 0 to 500 s^{-1} in 2 min and, after a constant shear rate of 500 s^{-1} for 10 s, the shear rate was decreased linearly to 0 s^{-1} in 2 min at the controlled temperature of 25°C. The temperature sweeps were performed in rate controlled

mode from 20 to 40°C with a heating rate of 5°C min^{-1} at a constant shear rate of 100 s^{-1}.

2.2.4 Construction of ternary phase diagrams

The dynamics of the interactions between SAIB solutions and non-solvent (buffer solution) for the formation of in situ depots were evaluated by the construction of ternary diagrams. This measurement was performed as reported by Shively et al. [11, 12]. EtOH, NMP and DMSO were used as solvents to prepare a series of SAIB solutions with different concentrations according Table 1. Different concentrations of SAIB/PLGA (70:10, w/w) in DMSO were also prepared (Table 1). Vials containing SAIB solutions and a vial of PBS (10 mM PBS pH 7.4, 0.02% NaN_3) were placed in a ZHWY-110X reciprocating water bath shaking incubator (Shanghai Zhicheng Analytical Instrument Manufacturing CO., LTD, Shanghai, China) at 37°C. PBS was then added dropwise to the SAIB solution vials under stirring. When the visual precipitate no longer re-dissolved within 30 min, the total mass (calculated by the reducing weight method) of PBS added was recorded. This experiment was carried out in triplicate for each solution, and all determined values were plotted in ternary phase diagrams.

2.2.5 Solvent diffusion studies

The solvent diffusion kinetics was investigated by determining the refractive index at predetermined time [11]. About 0.3 g (accurately to 0.0001 g) of the analyzed depot solutions was injected directly into a 5 ml vial filled with 3 ml PBS buffer at pH 7.4 (equilibrated at 37°C) with a 1 ml syringe. The vial was then kept in the reciprocating water bath shaking incubator (Shanghai Zhicheng Analytical Instrument Manufacturing CO., LTD, Shanghai, China) at 100 rpm and 37°C. Since the resulting shape was close to spherical, the surface area of the resulting precipitate was approximately 2.1 cm^2. Then, 100 μl samples were taken after 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 h and replaced with 100 μl fresh medium.

All experiments were performed in triplicate. The refractive index was determined by a 2WAJ Abbe refractometer (Shanghai, China). And the solvent concentration was calculated from a calibration curve over the range from 1 to 30 mg ml^{-1}.

2.2.6 Optical microscopy of the formed depots

The morphology of the formed depot allows an insight into their effect on drug release characteristics. The analyzed solutions were injected into 3 ml PBS buffer. After incubating at 37°C for 4 days, the PBS buffer was removed for analysis. The sample was examined under a biomicroscope (DMBA450, Motic China Group CO., LTD., Xiamen, China). The magnification is ×100.

2.2.7 Porosity of the formed depots

After injection of SAIB solution into the aqueous phase, solvent rapidly diffused out of the system into the water phase, while the external water diffused into the depot. This led to porous networks of water-rich domains surrounded by an SAIB-rich matrix. The porosity was determined by testing the volume of water diffused into the depot. About 0.3 g (W_1, accurately to 0.0001 g) of the analyzed solutions was injected into 3 ml water and was incubated at 37°C. At predetermined time (4 and 10 day), water was removed. And then the depots were lyophilized to remove the absorbed water. By measuring the difference in the water level before and after lyophilization, the volume of water absorbed by the depot can be readily determined. Thus, the porosity can be calculated as:

Table 1 Formulations of SAIB solutions

Formulation	SAIB/EtOH solution		SAIB/NMP solution		SAIB/DMSO solution		SAIB–PLGA(70:10, w/w)/DMSO solution	
	SAIB (% w/w)	EtOH (% w/w)	SAIB (% w/w)	NMP (% w/w)	SAIB (% w/w)	DMSO (% w/w)	SAIB–PLGA (% w/w)	DMSO (% w/w)
I	85	15	85	15	85	15	85	15
II	80	20	80	20	80	20	80	20
III	75	25	75	25	75	25	75	25
IV	70	30	70	30	70	30	70	30
V	65	35	65	35	65	35	65	35
VI	60	40	60	40	60	40	60	40
VII	55	45	55	45	55	45	55	45
VIII	50	50	50	50	50	50	50	50
IX	40	60	40	60	40	60	40	60
XI	30	70	30	70	30	70	30	70

$$p = \frac{(W_2 - W_3)/\rho_1}{W_1 \times C/\rho_2 + (W_2 - W_3)/\rho_1} \times 100\% \quad (1)$$

where p is the porosity, W_1 is the weight of SAIB solution, W_2 is the weight of water absorbed depot, W_3 is the weight of lyophilized depot, C is the concentration of SAIB in SAIB solution, ρ_1 is the density of water and ρ_2 is the density of SAIB. This experiment was carried out in triplicate for each solution.

2.2.8 *Preparation of risperidone-loaded in situ forming depot systems*

Risperidone-loaded in situ forming depot systems were prepared as described in the literature [10]. Briefly, A 20 mg ml^{-1} solution of risperidone containing 1% acetic acid was spray-dried using an EYELA SD 1000 spray drier (Tokyo Rikakikai, Japan). The following operating conditions were used: inlet temperature of 130°C, a drying air flow rate of 0.64 m^3 min^{-1}, an atomizer pressure of 190 kPa, and a liquid feed of 7.0 ml min^{-1}, an outlet temperature of approximately 80°C. Then disperse the spray-dried risperidone powders in different SAIB systems followed by vortexing to form a uniform suspension with a drug loading of 25 mg g^{-1}.

2.2.9 *In vitro drug release studies*

Risperidone-loaded SAIB in situ forming depot systems (0.1 g) were injected into 5 ml tubes along with 3 ml release buffer (10 mM PBS pH 7.4, 0.02% NaN_3). Then, the tubes were incubated in a reciprocating water bath shaking incubator at 100 rpm and 37°C [10]. Samples were removed completely at every sampling point and replaced with fresh release buffer. All experiments were performed in triplicate. The drug concentration was analyzed by HPLC.

2.2.10 *In vivo studies*

Pharmacokinetics of risperidone-loaded SAIB/PLGA depots in rats was investigated by comparing with that of the risperidone-loaded SAIB depot. Wistar rats weighting 180–220 g (Experimental Animal Center of Shenyang Pharmaceutical University, Shenyang, China) were allowed to acclimate for a week. Animals were randomly divided into four groups (six rats (three male and three female) per group) and fasted overnight with access to water ad libitum prior to experiment. The four groups were treated with 25 mg g^{-1} risperidone-loaded SAIB/PLGA depots with different contents of PLGA at single intramuscular dose of 12.5 mg kg^{-1}. Blood samples (about 0.3 ml) were collected into heparinized centrifuge tubes by retro-orbital puncture at predetermined time points. The samples were immediately centrifuged at 4,000 rpm for 10 min and plasma samples were collected and stored at −80°C until analysis. The experimental protocol was evaluated 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. The risperidone and 9-OH-risperidone serum levels were determined by UPLC–MS/MS ((Waters Corp., Milford, MA) [13]. Pharmacokinetic results were generated using non-compartmental analysis.

3 Results

3.1 The selection of solvents

The solubility of risperidone in several solvents was investigated to choose the most suitable solvents for preparing depot systems. The apparent solubility of risperidone at room temperature in release buffer, EtOH, DMSO, NMP, ethyl acetate and ethyl lactate was 0.22, 19.75, 16.12, 95.27, 138.33 and 335.28 mg ml^{-1}, respectively. As reported in previous research [10], a key factor influencing the burst release was the nature of the solvent used. The higher the drug solubility in the solvent, the more free-drug soluble in the depot system, leading to a greater burst release. In addition, in situ implant formulations (polymer dissolved in either NMP or DMSO) were also investigated in rhesus monkeys and considered acceptable for use as injectable implant systems [2]. Thus, EtOH, NMP and DMSO were selected as the organic solvents for the preparation of SAIB systems.

3.2 Compatibility of SAIB and additives

As shown in Fig. 1a, the T_g value of pure SAIB was −8.58°C, while that of pure PLGA was 18.66°C. The melting-method prepared SAIB/PLGA mixtures at three mass ratios, 78:2, 75:5 and 70:10, all exhibited a single T_g at −8.18, −7.85 and −5.04°C, respectively. The compatibility of mixtures was determined according to the Fox equation [14]:

$$1/T_g = W_1/T_{g1} + W_2/T_{g2} \quad (2)$$

where T_g is the glass transition temperature of the blend, T_{g1} is that of SAIB, T_{g2} is that of PLGA, and W_1 and W_2 are the weight fractions of SAIB and PLGA, respectively. The theoretical T_g values for the SAIB/PLGA 78/2, 75/5 and 70/10 (w/w) blends were −7.96, −7.03 and −5.46°C, respectively. These were in good agreement with the experimental values of SAIB/PLGA mixtures prepared by the melting method.

For the SAIB/PLA melting mixture, two T_g were observed, one at −12.29°C and the other at 22.13°C (Fig. 1b). The theoretical T_g value was calculated as −4.12°C. The deviation of the actual value from the theoretical one for SAIB/PLA was up to 8°C. Moreover, a rapid delamination occurred with the SAIB/PLA melting mixture at room temperature. Thus, PLA showed a poor compatibility with SAIB compared with PLGA. Consequently, PLGA with a molecular weight of 10 kDa was mixed with SAIB by the melting method to obtain stable and homogeneous mixture matrix depot systems.

3.3 Rheological characterization of SAIB solutions

As reported in the literature [15], the solvent diffusion rate is affected by the viscosity of the depot solution during phase inversion. Thus, the rheological characteristics of solutions is an important factor that influences the depot-formation kinetics and initial drug release behavior [16].

Figure 2a shows that the shear stress increased with an increasing shear rate in a linear fashion. SAIB in EtOH, DMSO and NMP can be described as Newtonian fluids. The dynamic viscosity decreased in the following order DMSO > NMP > EtOH.

Figure 2b shows that the change in apparent viscosity with temperature generally fitted the Arrhenius model: $\eta = \mathrm{A}\exp^{(\mathrm{Ea/RT})}$, where A is the frequency factor (Pa s), Ea is the activation energy (kJ mol^{-1}), R is the gas constant (kJ mol^{-1} K^{-1}) and T is the absolute temperature (K). The activation energy represented the energy barrier that must be overcome before the elementary fluid molecular flow process can occur [17]. The Ea values of SAIB in EtOH, NMP and DMSO were 29.30, 37.58 and 42.89 kJ mol^{-1}, respectively. This indicated that the SAIB/DMSO solution exhibited a higher intermolecular force than SAIB/EtOH and SAIB/NMP solutions at the same concentration.

Fixing the concentration of DMSO at 20% (w/w), the solution dynamic viscosity and Ea value increased with the increasing content of PLGA and all solutions exhibited Newtonian fluid properties (Fig. 2b). This indicated that SAIB and PLGA both existed in their molecular form in DMSO, resulting in a homogenous solution. On increasing the amount of PLGA, the intermolecular force (Ea) of the systems also increased.

3.4 Construction of ternary phase diagrams

Ternary phase diagrams can characterize the phase inversion of matrix solutions, and the interactions among matrix, solvent and buffer solution (non-solvent) [18]. This could affect the phase inversion dynamics and morphology of the depot, and lead to different drug release profiles [19].

Ternary phase diagrams were constructed by adding PBS into different SAIB solutions at different concentrations (Fig. 3). The straight line in the diagrams was drawn

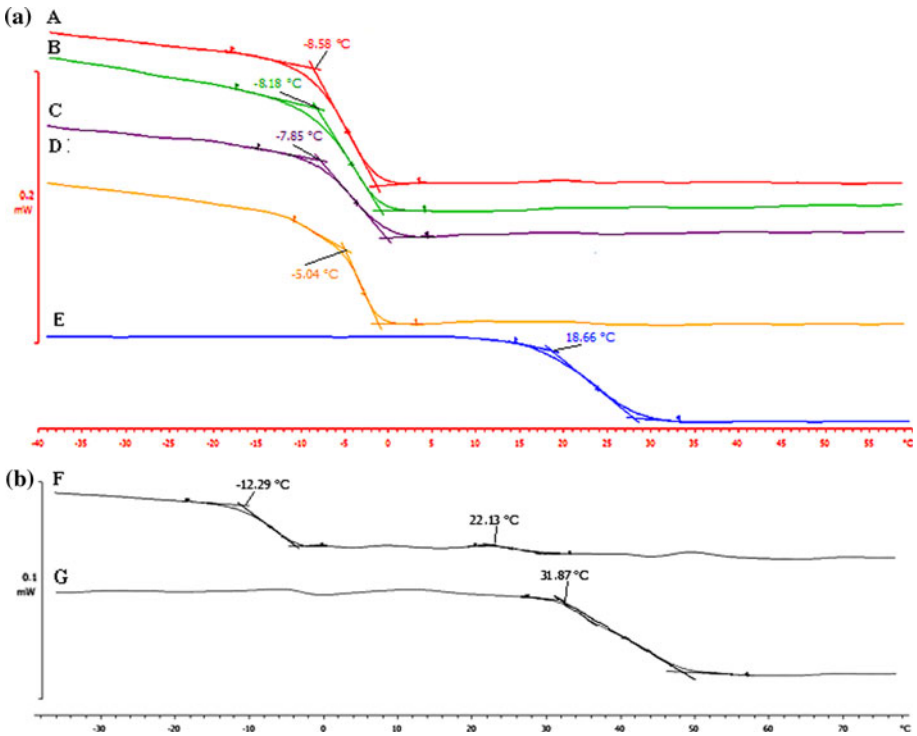

Fig. 1 DSC thermograms of SAIB and/or additives. Key: *A* SAIB, *B* SAIB/PLGA (78/2, w/w) prepared by the melting method; *C* SAIB/PLGA (75/5, w/w) prepared by the melting method, *D* SAIB/PLGA (70/10, w/w) prepared by the melting method, *E* PLGA, *F* SAIB/PLA (70/10, w/w), *G* PLA

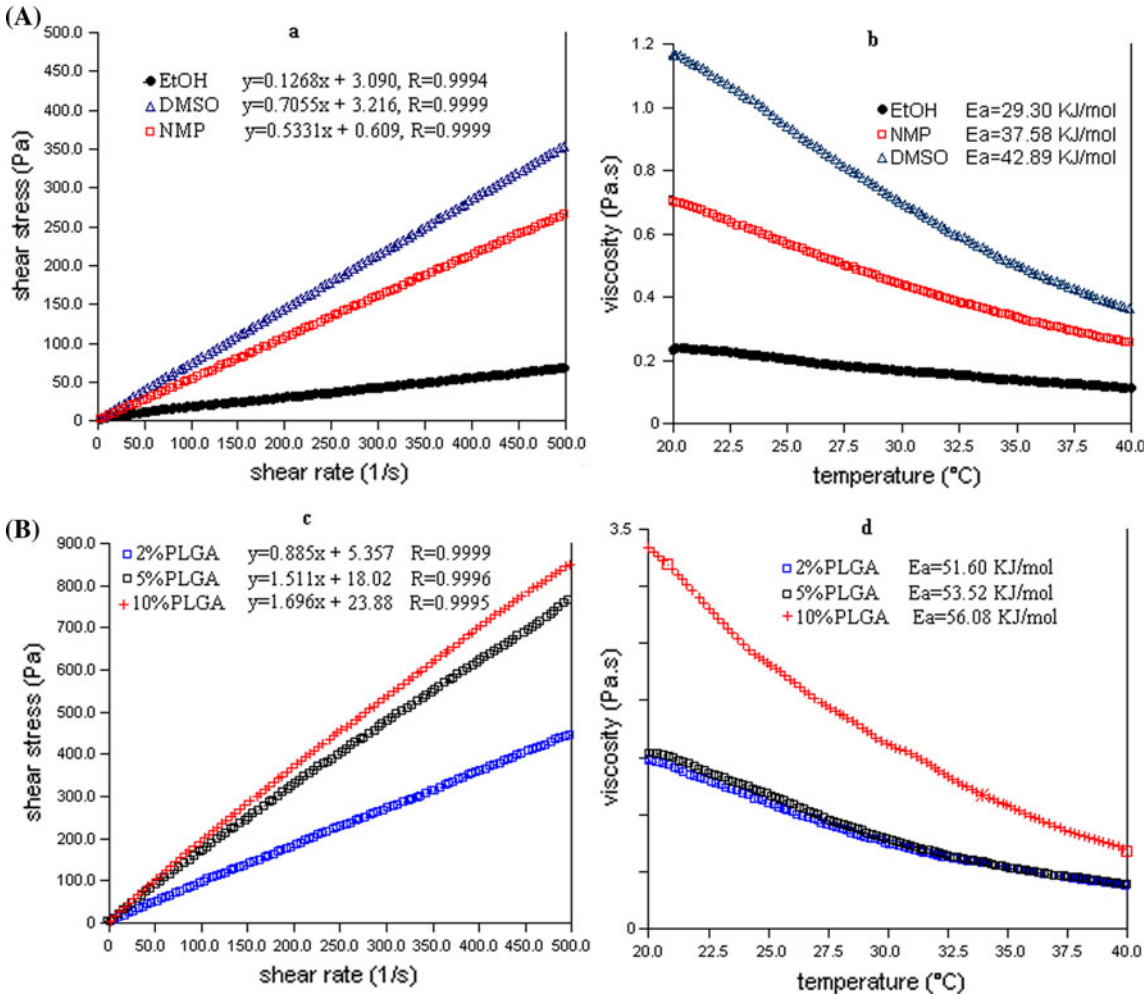

Fig. 2 The rheological properties of SAIB systems. **a** The effects of solvents on the rheological properties (*a*) and the activation energy Ea (*b*) of 80% (w/w) SAIB/solvent systems. **b** The effects of additive (PLGA) on the rheological properties (*c*) and the activation energy Ea (*d*) of SAIB/DMSO systems, with a DMSO concentration of 20% (w/w)

from 100% matrix and divided the area into the upper homogenous three-component system and a binary system (phase separation) below the line. Precipitation occurred in the presence of 2.89% water for a solution of 80% SAIB in EtOH, 1.07% water for 80% SAIB/DMSO, and 0.94% water for 80% SAIB/NMP. These results indicate that EtOH systems require almost twice amount of water to initiate precipitation compared with NMP and DMSO systems. By adding 10% PLGA to the SAIB/DMSO systems, only 0.69% water was required for the precipitation of SAIB/PLGA/DMSO (70:10:20, w/w/w). Practically, this has important implications with regard to the in vitro and in vivo release characteristics of depots. As reported, the quantity of water in the subcutaneous and intramuscular environment was only 8.9% ($N = 23$, w/w) [12]. Hence, the less water was required for the precipitation of the SAIB system, the more rapid was the solidification rate.

3.5 Solvent release rate from injectable in situ forming depot of SAIB

As reported, the solvent diffusion dynamics displayed an important role in the formation of depot and the initial drug release [16]. The solvent release rates from different systems were investigated to obtain further insight into the underlying drug release mechanisms.

Figure 4a illustrates the solvent diffusion kinetics of the depot, injected into buffer. In the first 15 min, 44.65% of NMP was released from the 80% SAIB/NMP depot, and 45.72% EtOH for the 80% SAIB/EtOH depot. After 24 h, both NMP and EtOH systems displayed complete solvent release into the buffer. The solvent diffusion profile of the 80% SAIB in DMSO showed a lower initial solvent release of 24.36% in the first 15 min, and the release of DMSO was 80.65% after 24 h. Using 20% (w/w) DMSO as the solvent, a marked reduction in the solvent release rate was

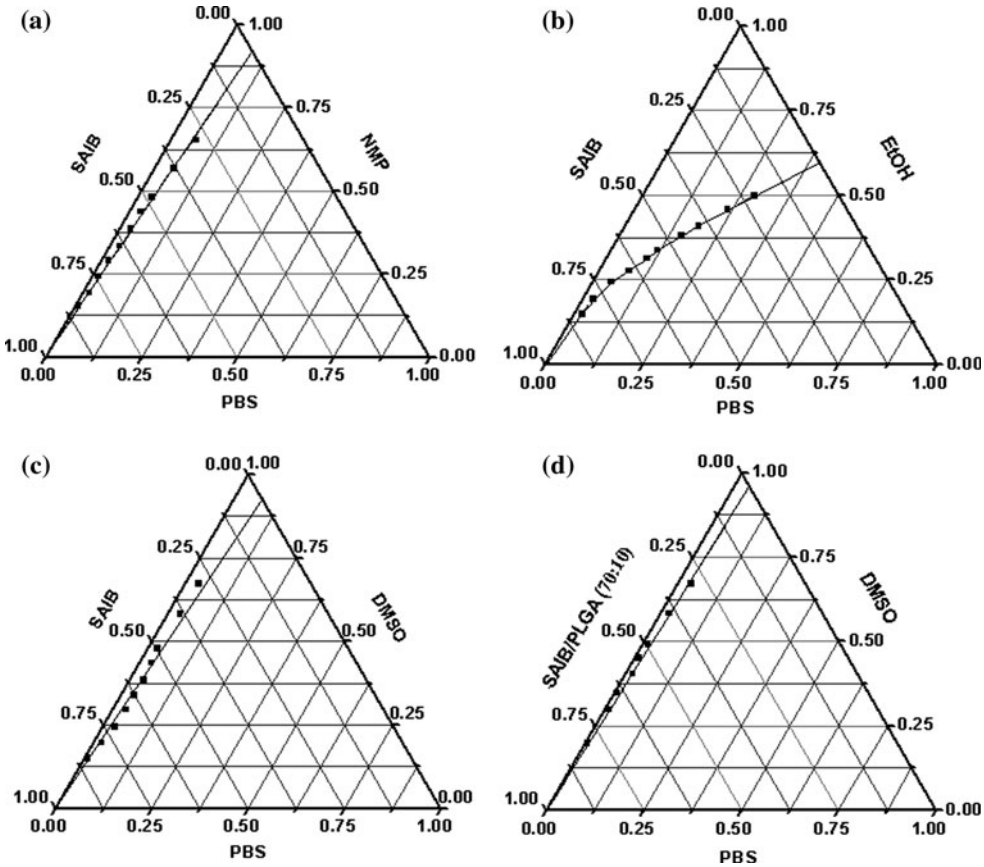

Fig. 3 Ternary phase diagrams at 37°C for SAIB/NMP/PBS (**a**), SAIB/EtOH/PBS (**b**), SAIB/DMSO/PBS (**c**) and SAIB–PLGA (70:10, w/w)/DMSO/PBS mixtures. A homogenous system was indicated *above the straight line* and a binary mixture *below the line*

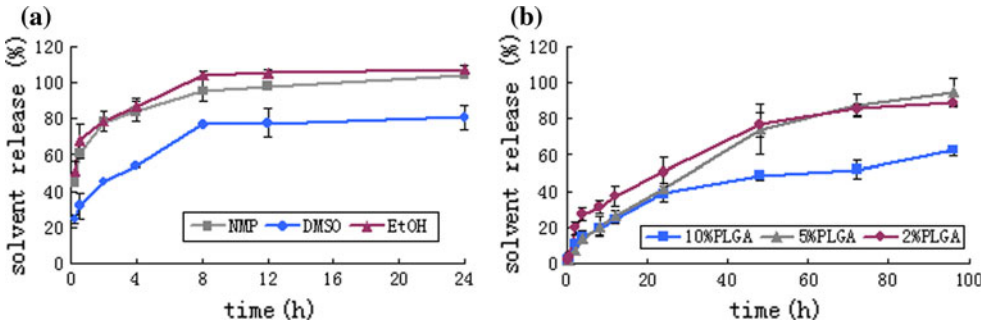

Fig. 4 The effects of solvents (**a**) and PLGA (**b**) on the solvent release kinetics of SAIB systems in release buffer (10 mM PBS pH 7.4, 0.02% NaN_3) at 37°C, with a limited surface area of approx. 2.17 cm^2. Each point represents the mean ± SD; $n = 3$

obtained after the inclusion of PLGA (Fig. 4b). The solvent release rate of decreased with increasing PLGA concentration. When 10% PLGA was added to SAIB/DMSO solutions, only 2.14% of the DMSO diffused into the environment in the first 15 min, then 24.47% after 12 h, and up to 62.38% at 96 h.

3.6 Morphology and porosity of SAIB in situ formed depots

The morphology and porosity of depot was investigated to obtain an insight into their effects on drug release characteristics.

As a semi-solid depot was formed after the phase inversion, optical microscopy method was applied for the morphology investigation instead of conventional scanning electronic microscopy method. As shown in Fig. 5, the two-phase morphology of the depots can be described as a matrix-rich phase and a water-rich phase, except that the SAIB/EtOH system exhibited only a dense matrix-rich phase. After injection of 80% SAIB/EtOH into the aqueous phase, EtOH rapidly diffused out of the system into the water phase. Meanwhile, the internal EtOH steadily diffused into the water–organic interface. Since more water was required to dissolve SAIB in EtOH (Fig. 3), there was no obvious precipitation took place on the surface of the SAIB/EtOH system. The consequent slower phase inversion kinetics led to a dense structure of the SAIB/EtOH depot. However, because less water was needed to initiate precipitation of SAIB in the NMP or DMSO system (Fig. 3),

phase inversion took place within minutes of the injection. This led to highly porous networks of solvent–nonsolvent-rich domains surrounded by an SAIB-rich matrix. Since there was a higher solvent release rate for NMP than for DMSO (Fig. 4), the SAIB/NMP depot displayed a higher exchange rate between the internal solvent and the external water. Subsequently, a higher overall porosity (32.58 ± 2.57% after 4 days and 17.24 ± 2.12% after 10 days) was observed for SAIB/NMP system (Table 2).

For the SAIB–PLGA/DMSO systems, a semi-solid shell was formed on the surface of the depot as soon as the system was injected into the buffer. The shell created an effective barrier to restrict mass transfer into the interior of the depots. As a result, more solvent was locked in the depot and exchanged with water steadily. Thus, more water diffused in after the solvent diffused out completely, compared with the depot without PLGA. Meanwhile, a higher PLGA concentration resulted in a lower overall porosity (Fig. 5). The porosity of different depots was listed in Table 2. Since the drug dispersed in the matrix-rich phase diffused into the release buffer through the water-filled pore structure, the porosity of the depot might affect the overall drug release behavior.

3.7 In vitro release of risperidone

3.7.1 The effects of solvent on drug release

The in vitro release profiles of risperidone from 80% (w/w) SAIB in different solvents are shown in Fig. 6a. Drug release from depots was biphasic, characterized by risperidone being released initially due to diffusion, followed by a constant release period. The cumulative release of drug after 1 day was 17.37% for EtOH, 15.74% for NMP, and 4.95% for DMSO. A significantly reduced burst release was obtained for the SAIB/DMSO system compared with the SAIB/EtOH and SAIB/NMP systems. This indicates that more drugs diffuse into the buffer during the depot forming of SAIB solutions in NMP or EtOH. In addition, a Higuchi model was used to characterize the drug release due to a diffusion process [20].

$$Q = 2C_0\sqrt{Dt/\pi} \tag{3}$$

where Q represents the drug released amount, C_0 represents the initial drug concentration, D represents the diffusion coefficient of the drug, and t represents the time. The cumulative drug release was plotted against the square root of the time (Table 3). For 80% (w/w) SAIB in different solvents loaded with risperidone, the initial release rates were high, followed by a slower drug release to 60 days. From day 0 to 4, there was a high rate of release for the 80% (w/w) SAIB/NMP system. And during the initial stage, the initial release rate was reduced in the order of NMP > EtOH > DMSO.

3.7.2 The effects of PLGA on drug release

Due to the lower burst release in the DMSO system, the effects of PLGA on the drug release were further evaluated

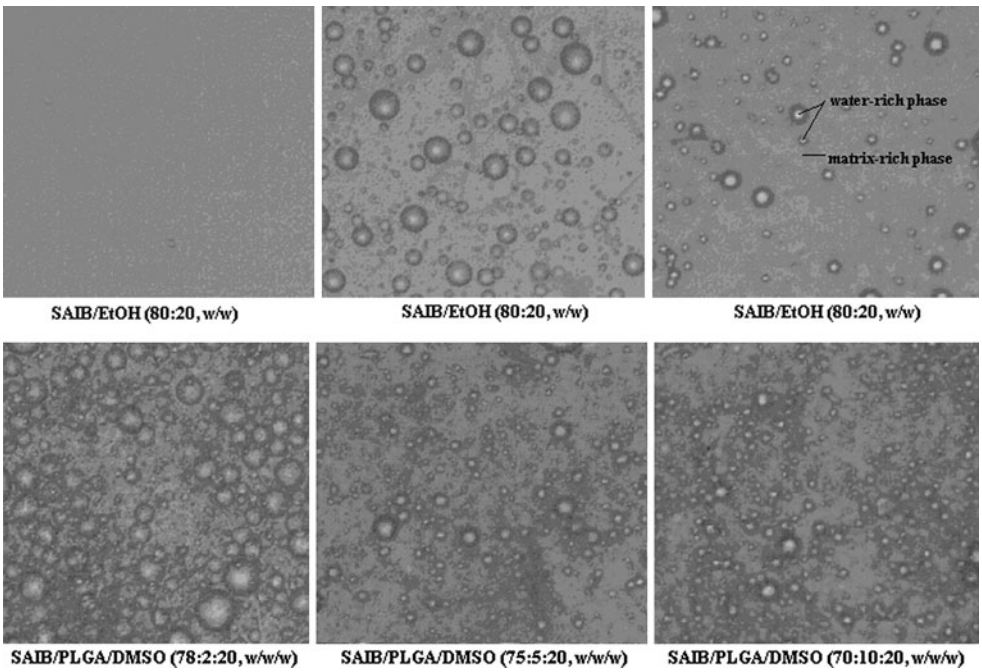

Fig. 5 Morphologies of SAIB and SAIB/PLGA depots after incubating in release buffer (10 mM PBS pH 7.4, 0.02% NaN3) at 37°C for 4 days. The magnification was ×100

Table 2 The porosity of different SAIB depots and SAIB-PLGA depots (mean ± SD, $n = 3$)

Formulation (%)	SAIB/EtOH (80/20, w/w)	SAIB/NMP (80/20, w/w)	SAIB/DMSO (80/20, w/w)	SAIB/PLGA/DMSO (78/2/20, w/w)	SAIB/PLGA/DMSO (78/5/20, w/w)	SAIB/PLGA/DMSO (78/10/20, w/w)
4 day	6.82 ± 1.08	32.58 ± 2.57	27.91 ± 2.76	45.01 ± 2.83	32.03 ± 3.23	23.54 ± 1.73
10 day	8.05 ± 1.34	17.24 ± 2.12	15.25 ± 2.03	29.32 ± 2.02	30.88 ± 2.77	27.32 ± 2.23

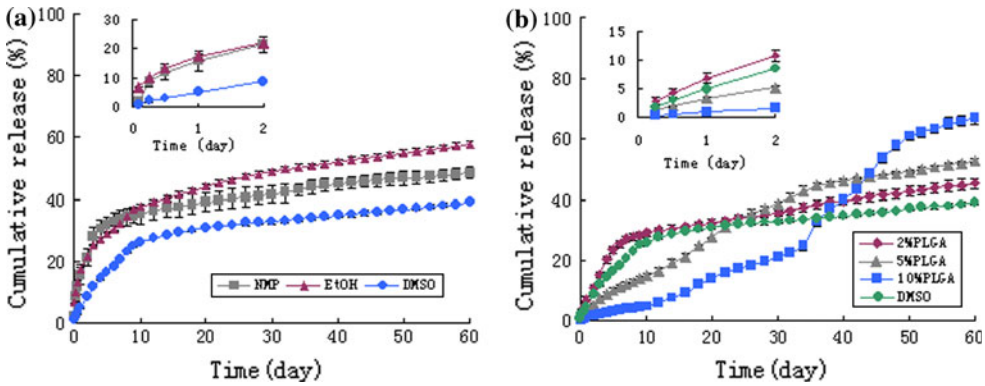

Fig. 6 The effects of solvents (**a**) and PLGA (**b**) on the release of risperidone from SAIB systems with a drug loading of 25 mg g^{-1} into release buffer (10 mM PBS pH 7.4, 0.02% NaN_3) at 37°C. Each point represents the mean ± SD; $n = 3$

Table 3 Evaluation of drug release kinetics of different risperidone formulations according to the Higuchi equation

Formulation	SAIB/EtOH (80/20, w/w)		SAIB/DMSO (80/20, w/w)		SAIB/NMP (80/20, w/w)		SAIB/PLGA/DMSO (78/2/20, w/w)	
	0–10 day	10–60 day	0–10 day	10–60 day	0–4 day	5–60 day	0–10 day	10–60 day
Slope	10.29	2.94	9.07	2.46	16.03	2.98	12.14	3.80
R^2	0.9886	0.9951	0.9915	0.9936	0.9856	0.9974	0.9829	0.9918

using 20% (w/w) DMSO as a solvent. The initial release of risperidone was reduced with increasing PLGA concentration. When 10% (w/w) PLGA was added to the system, the burst release of risperidone was reduced from 4.95 to 1.05%, and the overall drug release behavior was altered (Fig. 6b). On adding 2% (w/w) PLGA to the system, the drug release profile followed the Higuchi equation (Table 3), which was similar to that of 80% (w/w) SAIB/DMSO. When the content of PLGA increased to 5%, superficial solidification happened in a short period. Drug-diffusion through the formed shell around the system became the rate-limiting step for drug release. The drug release rate is close to zero-order at the initial state (0–34 day). A slope of 1.21 with an R^2 of 0.9944 was obtained by plotting the cumulative release against the time. On day 36, the drug-diffusion was zero-order ($R^2 = 0.9976$) with a relatively lower release rate. On adding 10% (w/w) PLGA, markedly altered release profiles were observed. The release of risperidone followed a classical two-phase release profile, which was an indication of early diffusion control, followed by erosion control. The profile was similar to that of the PLGA depot reported in earlier studies [21–24]. During the first diffusion phase (0–34 day), the release kinetics of risperidone most closely fitted zero-order release kinetics ($R^2 = 0.9849$) with a slope of 0.72. After 34 days, a fast drug release was induced by the erosion process.

3.8 Influence of PLGA on the pharmacokinetics of risperidone-loaded SAIB depots

Due to the lower in vitro burst release in the DMSO system, the effects of PLGA on the in vivo pharmacokinetics were further evaluated using 20% (w/w) DMSO as solvent. As reported in the literatures [25, 26], risperidone is metabolized fairly quickly to 9-OH-risperidone, which is pharmacologically active metabolite and has equipotent pharmacodynamics with risperidone. Therefore, the total active moiety plasma concentration (the sum of risperidone and 9-OH-risperidone) was employed to evaluate the pharmacokinetics of risperidone-loaded SAIB depots in rats. The plasma concentration–time profiles of the total active moiety (risperidone plus 9-OH-risperidone) after IM administration (at a dose of 12.5 mg kg^{-1}) are shown in Fig. 7. During the first day, significantly lower and more steady active moiety concentrations were obtained by adding 10% w/w PLGA. The active moiety concentration of the depot without PLGA decreased rapidly from 2 h to 6 days, and fell below 10 ng ml^{-1}. However, the active moiety concentrations from 4 to 20 days were in the range

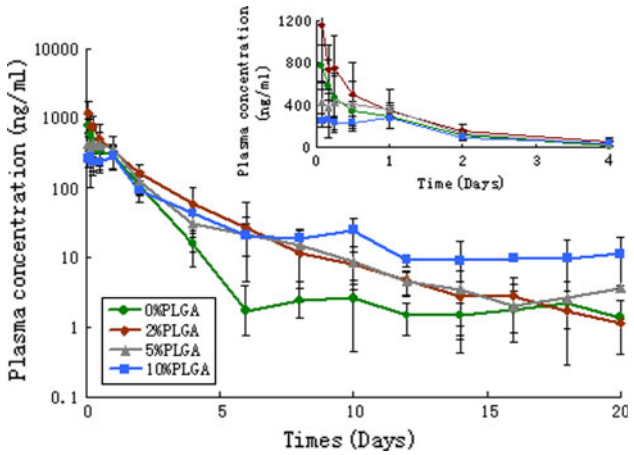

Fig. 7 Mean plasma concentration–time profile of the active moiety (risperidone plus 9-OH-risperidone) after intramuscular administration of 25 mg g^{-1} risperidone-loaded SAIB–PLGA mixture matrix depots with different contents of PLGA to rats at dose of 12.5 mg kg^{-1} ($n = 6$)

of 11.45–42.70 ng ml^{-1} by adding 10% (w/w) PLGA. The C_{max} was significantly reduced from 1161.7 ± 550.2 to 280.3 ± 98.5 ng ml^{-1}, the initial release ($AUC_{0–4\ days}$) decreased from 1028.7 ± 208.4 to 554.6 ± 106.8 ng d ml^{-1} when the PLGA content was increased from 2.0 to 10% w/w (Table 4). By inclusion of 10% (w/w) PLGA, the persistence ($AUC_{4–20\ days}$) over the 20 days was markedly increased to 343.8 ± 88.9 ng d ml^{-1} compared with that of the formulation without PLGA ($AUC_{4–20\ days}$ =79.9 ± 22.1 ng d ml^{-1}). The presence of a higher PLGA level leaded to a steadier and sustained drug release in vivo, which was consistent with the drug release behavior in vitro.

4 Discussion

In this study, a risperidone-loaded SAIB–PLGA mixture matrix depot with a reduced burst release was developed. And the physical characterizations of different SAIB systems were assessed systematically to illustrate the effects of solvents and PLGA on drug release behaviors in vitro/in vivo.

As reported previous [7, 10], the drug release from SAIB depot can be influenced by the type of solvent, the type of additive and the amount of additive. A higher drug solubility in solvent leaded to a greater burst release. Thus, the risperidone solubility in different solvents was determined to select proper solvents. The solvents with relatively low drug solubility, including EtOH, NMP and DMSO, were used to investigate the effects of solvents on the drug release behaviors of SAIB systems. In addition, the compatibility of additives and SAIB was evaluated to obtain a stable and homogeneous SAIB depot system. PLGA showed a good compatibility with SAIB compared with PLA. The polymer tended to associate with SAIB through the hydrogen bonds formed between the carboxyl end groups in the polymer and the carbonyl groups in SAIB. The glycolide section of PLGA might form a stronger hydrogen bond with SAIB than the lactide section due to the lack of an extra methyl group. Thus, PLGA exhibited a higher intermolecular force with SAIB than PLA. Consequently, PLGA with a molecular weight of 10 kDa was added into SAIB systems to modulate the drug release behavior.

The in vitro release of risperidone from 80% (w/w) SAIB in different solvents was investigated to evaluate the effects of solvents on drug release. As shown in Fig. 6a, the burst release of 80% (w/w) SAIB in different solvents was in a rank order of EtOH > NMP > DMSO. The cumulative release of drug after 1 day was only 4.95% by using 20% (w/w) DMSO as solvent. The initial release kinetics was affected by several factors that changed the drug diffusion rate. The primary factor was the solvent release rate [16]. Figure 4a indicated that the solvent release rates decreased in the rank order of EtOH > NMP > DMSO. This was in complete agreement with the rank order of the burst release. Systems with fast solvent diffusion showed an increased initial drug release. After injection of SAIB solutions into buffer, the solvent diffused rapidly out of the system into the water phase. A lower solvent release rate resulted in a slower mass transfer rate (drug diffusion rate) for SAIB/DMSO solution. The reduced burst release can also be caused by other factors. On the one hand, the mass transfer rate can be affected by the viscosity of the depot solution during phase inversion [15]. Figure 2a showed that the dynamic viscosity decreased in the order of DMSO > NMP > EtOH. In addition, the SAIB/DMSO (80/20, w/w) solution exhibited a higher intermolecular force (Ea) than SAIB/EtOH (80/20, w/w) and SAIB/NMP (80/20, w/w) solutions. The enhanced viscosity and

Table 4 The non-compartmental model pharmacokinetic parameters of the active moiety (risperidone plus 9-OH-risperidone) after intramuscular administration of 25 mg/g risperidone-loaded SAIB-PLGA mixture matrix depots with different contents of PLGA to rats at dose of 12.5 mg kg^{-1} ($n = 6$)

PLGA content (%, w/w)	0	2	5	10
$AUC_{0–4\ days}$ (ng d/ml)	728.8 ± 121.8	1028.7 ± 208.4	779.5 ± 284.7	554.6 ± 106.8
$AUC_{4–20\ days}$ (ng d/ml)	79.9 ± 22.1	294.4 ± 204.1	193.1 ± 97.2	343.8 ± 88.9
C_{max} (ng ml^{-1})	766.9 ± 211.7	1161.7 ± 550.2	431.8 ± 190.9	280.3 ± 98.5

intermolecular force of SAIB/DMSO solutions reduced the solvent diffusion rate and the burst drug release. On the other hand, the phase inversion dynamics also contributed to the drug release behavior. As displayed in Fig. 3, only 1.07% water was required to initiate precipitation for 80% (w/w) SAIB/DMSO system, while 2.89% water for 80% (w/w) SAIB/EtOH system. The less water was required for the precipitation of the SAIB system, the more rapid was the solidification rate. Thus, drug dissolved in the system is more likely to be locked in the system efficiently, resulting in a lower initial release.

Although the 80% (w/w) SAIB/EtOH system exhibited a higher burst release, the 80% (w/w) SAIB/NMP system displayed a higher initial release rate (a steeper slope, shown in Table 3). This could be interpreted by the results of the morphology and porosity study. As shown in Fig. 4, the SAIB/NMP depot exhibited a higher overall porosity ($32.58 \pm 2.57\%$). The presence of massive water-channels undoubtedly provided an avenue for rapid diffusive release of the drug, which increased the drug release rate of the SAIB/NMP system at an early stage. Moreover, the high solubility (95.27 mg ml^{-1}) of risperidone in NMP also significantly increased the initial release rate.

From the above results, SAIB/DMSO system showed a low burst release and decelerated solvent release kinetics. Thus, PLGA was added to SAIB/DMSO system to slow the drug release kinetics further. Figure 6b indicated that the burst release of risperidone was decreased with increasing PLGA content. For the SAIB/PLGA/DMSO (70/10/20, w/w/w), the burst release of risperidone was 1.05%. This result was in agreement with the solvent release of the SAIB/PLGA/DMSO solutions. The solvent release rate of decreased with increasing PLGA concentration. A slower solvent release rate leaded to a lower burst release. The reduced mass-transfer rate can be explained in two ways. On the one hand, the addition of PLGA increased the viscosity and intermolecular force of SAIB systems, which decreased the diffusion rate of DMSO. On the other hand, the ternary phase diagrams (Fig. 3) indicate that less water was required to initiate the precipitation by inclusion of 10% PLGA. Therefore, superficial solidification happened in a short period, which facilitated the successful encapsulation of the solvent and drug. This can also be supported by the fact that visible precipitation surrounding the depot occurred immediately when SAIB/PLGA/DMSO (70:10:20, w/w/w) was injected into the water phase. The fast precipitated systems developed a skin (shell) at the interface. The shell around the system became an obstacle for free drug to be "released" in a burst. The diffusion coefficient of the drug will fall on increasing the thickness of the shell and the diffusion rate of the drug will accordingly be reduced.

After the formation of SAIB–PLGA mixture matrix depot, the overall drug release behavior was significantly influenced by the porosity. At the early stage, the porosity of SAIB–PLGA mixture matrix depot was decreased with increasing the content of PLGA (Table 2). The drug dispersed in the matrix-rich phase diffused into the release buffer through the water-filled pore structure. Thus, a lower porosity of the SAIB/PLGA/DMSO (70:10:20, w/w/w) system caused a decreased drug release rate. At a later stage, PLGA was hydrolytically degraded to water-soluble fragments, the presence of which undoubtedly increased the water influx. Owing to the excellent compatibility of SAIB and PLGA, PLGA was distributed uniformly in the depot. The water diffused into the hydrophobic region of the depot along with the hydrolysis of PLGA. The visible collapsing depot structure of the SAIB/PLGA (70/10, w/w) mixture matrix depot took place along with the erosion of PLGA. However, for the depots containing 5% (w/w) PLGA, the PLGA ratio was too low to destroy the matrix depot structure. Consequently, a fast drug release was induced at a later stage by the inclusion of 10% PLGA. Because of the lack of a system to degrade SAIB in vitro, further in vivo investigation of the drug delivery was carried out.

The in vivo investigation indicated that the presence of a higher PLGA level leaded to a steadier and sustained drug release in vivo, which was consistent with the drug release behavior in vitro. For the in situ forming SAIB–PLGA mixture matrix depot, the drug release process in vivo can be illustrated by the Fig. 8. The drug release kinetics of risperidone from the SAIB–PLGA mixture matrix depot can be described using the diffusion-based model established by Raman [27]. Upon injection into tissue, the depot developed a shell at the interface of the matrix-rich phase and the water-rich phase, leaving the solvent and drug locked in the matrix-rich phase. The drug and solvent were released steadily by diffusion from the matrix-rich phase into the water-rich phase and released out through the shell. On increasing the content of PLGA, a thicker shell formed at the interface and a lower volume fraction of the water-rich phase (Fig. 5) was obtained. These factors both reduced the drug diffusion rate. As a result, the C_{max} was significantly reduced by adding 10% (w/w) PLGA to the depot. At a later stage, the PLGA shell at the depot surface slowed the SAIB erosion rate and the drug diffusion rate. Thus, the in situ

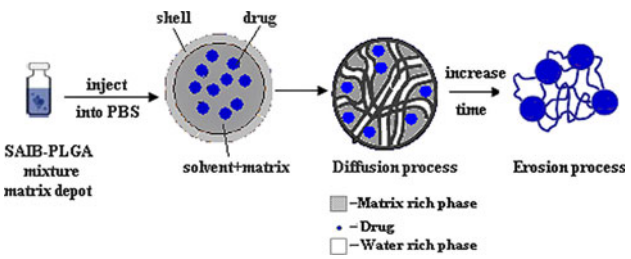

Fig. 8 The illustration of drug release process of SAIB–PLGA mixture matrix depots

forming SAIB–PLGA mixture matrix depots displayed more sustained release profiles compared with the SAIB depot without PLGA, especially for the SAIB/PLGA/DMSO (70/10/20, w/w/w) depot. Based on the above results, an ideal drug release can be obtained by modulating the content of PLGA in SAIB–PLGA mixture matrix depot. And the drug release behavior of SAIB depot can be well predicted by its physical characterizations, such as viscous properties, phase inversion, solvent diffusion kinetics, porosity and morphology. However, other factors influencing the drug release in vivo, such as drug loading and injection volume, should be studied in future investigation. The in vivo investigations on other animals, such as Beagle, need to be carried out to provide more data for the final application of depot. Moreover, therapeutic effect on the schizophrenic animal and the relationship between drug plasma concentration and its biological effect after the administration of SAIB/PLGA/DMSO system will be the topic of future research.

5 Conclusions

In summary, a novel in situ forming SAIB–PLGA mixture matrix depot was developed as a sustained release depot preparation for risperidone. The addition of PLGA reduced the mass transfer rate mainly by a thicker shell formed at the interface after contact with water. Consequently, the initial drug release rate in vitro and the initial release in vivo ($AUC_{0-4\ days}$) was reduced compared with the SAIB depot without PLGA. On increasing the PLGA content, the drug release rates at early stage in vitro and in vivo were both reduced due to the formation of a shell surrounding the depot after injection into release buffer or intramuscular environment. When the PLGA content increased to 10%, the C_{max} was reduced to 280.3 ± 98.5 ng ml^{-1}, and a more stable plasma concentration (11.45–42.70 ng ml^{-1}) was obtained from 4 to 20 days. Based on these results, it can be concluded that the SAIB–PLGA mixture matrix depot is a promising carrier for the sustained release of risperidone with an ideal release behavior.

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