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Design of a long-term antipsychotic in situ forming implant and its release control method and mechanism

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ABSTRACT

Two kinds of in situ forming implants (ISFIs) of atypical antipsychotics, risperidone and its 9-hydroxy active metabolite, paliperidone, using poly(lactide-co-glycolide)(PLGA) as carrier, were investigated. Significant difference was observed in the solution–gel transition mechanism of the two systems: homogeneous system of N-methyl-2-pyrrolidone (NMP) ISFI, in which drug was dissolved, and heterogeneous system of dimethyl sulfoxide (DMSO) ISFI, in which drug was dispersed. Fast solvent extractions were found in both systems, but in comparison with the high drug release rate from homogeneous system of drug/polymer/NMP, a fast solvent extraction from the heterogeneous system of drug/polymer/DMSO was not accompanied by a high drug release rate but a rapid solidification of the implant, which resulted in a high drug retention, well-controlled initial burst and slow release of the drug. In vivo study on beagle dogs showed a more than 3-week sustained release with limited initial burst. Pharmacologic evaluation on optimized paliperidone ISFIs presented a sustained-suppressing effect from 1 day to 38 day on the MK-801 induced schizophrenic behavior mice model. A long sustained-release antipsychotic ISFI of 50% drug loading and controlled burst release was achieved, which indicated a good potential in clinic application.

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1. Introduction

Among various kinds of polymeric delivery systems, the in situ forming implant (ISFI) provides a cost-effective alternative to conventional sustained release systems for both local and systematic drug delivery, which possesses advantages such as abilities to carry a wide category of drugs including proteins and hydrophobic molecules, less invasive placement, and relatively easier preparation methods (Graham et al., 1999; Lambert and Peck, 1995; Patel et al., 2010).

The solvent removal ISFI is an injectable liquid comprised of a water insoluble biodegradable polymer and a water miscible biocompatible solvent. Upon subcutaneous administration into the in vivo environment, the solvent is extracted from the polymer matrix while the water penetrates in. A semi-solid drug depot is formed in situ through the polymer precipitation (Dunn, 1990). Compared to thermoplastic paste and crosslinked system, the solvent removal implant avoids the uncontrolled interference of temperature and potent incomplete reaction of pre-polymers (Hatefi and Amsden, 2002). In case of a need, the implant can be easily retreated. Besides, the fluid polymer solution performs a very smooth injection while the administration of microspheres suspension may be hampered by needle clogging of particulates (Deadman et al., 2007).

A pain-free subcutaneous ISFI system named Atrigel™ has already been marketed. Products approved by FDA using this Atrigel technology including: Eligard® for advanced prostate cancer treatment, Atridox® for periodontal treatment, Atrisorb® used in guided tissue regeneration barrier membranes, Atrisorb® D product and Doxyrobe® product (Solanki et al., 2010). Besides, biocompatible solvents ranged from hydrophilic N-methyl-2-pyrrolidone (NMP), dimethyl sulfoxide (DMSO), tetraglycol, and glycol furol to the more hydrophobic solvents such as propylene carbonate, triacetin, ethyl acetate, and benzyl benzoate, have been screened to improve the medication compliance, among which NMP and DMSO are preferred because of their excellent solubilities and more acceptable safety/toxicology profiles. According to ICH Guidance for Industry Q3C, NMP belongs to Class 2 solvents in pharmaceutical product, the maximum daily intake of which is 5.3 mg, the marketed one-month dosage of Eligard® includes 82.5 mg PLGA and 160 mg NMP per injection, one-week dosage of Atridox® includes 165.15 mg PLGA and 284.85 mg NMP per injection. DMSO belongs to solvents in class 3, which may be regarded as less toxic and of lower risk to human health.

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Nevertheless, a challenge still exists in minimization of the solvent consumption, considering the polymer elimination burden in vivo and the injectable fluidity, there is an increase limit of the polymer/solvent ratio. Another solution is to improve the drug loading, which often causes a new tough issue: burst release, which meant a high initial drug release within the first 24 h post administration. Though some modified systems which integrate the microparticles into ISFIs have been researched to deal with the burst release (Rungseevijitprapa et al., 2008), an extra external phase may complicate the manufacturing process and add to the stability risk of the system.

The cause of burst release reported so far related to the rate of gelation (Graham et al., 1999; Kostrzewa, 1998; Luan and Bodmeier, 2006a), hydrophobic characteristic of formulation (Chhabra et al., 2007), viscosity of polymer solutions (Liu et al., 2010; Luan and Bodmeier, 2006a), type of polymers (Luan and Bodmeier, 2006b) and solvent nature (Graham et al., 1999; Lambert and Peck, 1995). However, no rule applies successfully to all, the gelation rate may positively or negatively influence the drug release depending on influential factors like: the drug's homogeneous or heterogeneous state, the solvent extraction rate, and their effect on the phase inversion dynamics. The type of PLGA could influence the drug release behavior in varied ways: a higher molecular weight usually increases the viscosity of the polymer solution, thus lead to a slower drug release rate. However, in the case of in situ forming microparticle (ISM) systems, a lower molecular weight PLGA system results in a slow solvent diffusion and lower initial drug release (Luan and Bodmeier, 2006b). PLGAs with different end groups exert their influences by means of presenting different solubilities in the solvent, introducing molecular interactions and changing the hydrophobic characteristics of the system (Chhabra et al., 2007). Hence, PLGA of 50:50 monomer ratio seems like to provide more of those influential end groups resulted from its a much shorter half-life than that of other monomer ratios. Besides, the hydrophobic/hydrophilic characteristics of involved elements also play an important role on the drug release behavior, all of which inspired our study and have been taken advantage of in manipulating the drug release behavior and burst release control.

Current long-term drug delivery systems applied to antipsychotics include: RISPERDAL® CONSTA®, a long-acting injection of microspheres, for which an oral medication should be given for the first three weeks to maintain adequate therapeutic plasma concentration, and INVEGA® SUSTENNATM, an extended-release injectable suspension of paliperidone palmitate, which dissolves slowly in vivo and then hydrolyzes to its active moiety, paliperidone, the pharmacologic effect largely depend on the metabolizing ability of patients. A sucrose acetate isobutyrate (SAIB) in situ system of risperidone has been reported before, the homogeneous system of which results in two active moieties in vivo, risperidone and paliperidone, and with a relatively high C_{max}/C_{min} value (Lu et al., 2007, 2008) Here a novel PLGA/DMSO or NMP heterogeneous ISFIs of antipsychotics have been researched, the sustained drug release from which only depends on the dissolving of a single active moiety. Risperidone and paliperidone are used widely for the treatments of schizophrenia (Su et al., 2011), they have different physical properties of which may exhibit different interactions in the ISFIs. The long sustained-release antipsychotic ISFI system is promised to provide a potent alternative in clinic application.

2. Materials and methods

2.1. Materials

Poly(DL-lactide-co-glycolide) (PLGA 50:50: 4A, MW 58,000 Da, inherent viscosity 0.36 dl/g; 5E, MW 65,000 Da, inherent viscosity 0.49 dl/g; 7A, MW 105,000 Da, inherent viscosity 0.70 dl/g, 75:25: 7A, MW 111,000 Da, inherent viscosity 0.73 dl/g; 7E, MW 113,000 Da, inherent viscosity 0.74 dl/g) was obtained from Lakeshore Biomaterials. Risperidone was purchased from Jiangsu Nhwa-group Pharma Corporation (Jiangsu, China). Paliperidone and MK-801 were purchased from Sigma Aldrich, St. Louis, MO, USA. Dimethyl sulfoxide (DMSO) was purchased from Amresco Inc., USA. NMP was purchased from Tianjing Guangfu Fine Chemical Research Institute. CMC-Na was purchased from Anhui Shanhe Pharmaceutical Excipients Co. Ltd.

Male mice (Kunming strain, body weight 20 ± 2 g) were purchased from Experimental Animal Center of Jilin University, China. Beagle dogs (body weight 12 ± 1 kg) were provided by Tianyao Pharmaceutical Co. Ltd. All experiments were performed according to the Guidelines for Animal Experiments, Jilin University, China.

Table 1
ISFI formulations of different drug/polymer/solvent ratio.

	Polymers	Solvents	Polymer/solvent ratio (w/v)	Drug/polymer ratio (w/w)	Drug loading (%)
1	5050 5E	NMP	1/5	1/4	20
2	7525 7E	NMP	1/5	1/4	20
3	7525 7A	NMP	1/5	1/4	20
4	5050 4A	DMSO	1/5	1/4	20
5	5050 5E	DMSO	1/5	1/4	20
6[a]	7525 7A	DMSO	1/5	1/4	20
7	5050 7A	DMSO	1/5	1/4	20
8[b]	5050 7A	DMSO	1/4	1/4	20
9	5050 7A	DMSO	1/4	1/1	50

[a] Formulation used in pharmacokinetic study in beagle dogs.
[b] Formulation used in pharmacologic effect evaluation in mice.

2.2. Solubility determination of drugs in the release medium and organic solvents

The solubility of drugs in the PBS release medium and organic solvents of NMP and DMSO were investigated. Exceeded amounts of drugs were dissolved in 1 ml solvents, the drug/solvent mixtures were treated for 30-min intermittent ultrasound exposure, then centrifugated at 10,000 rpm for 5 min, and both of the drugs were stable when applied to this short-term ultrasound exposure in condition of controlled water bath temperature. 10 μl of the supernatant was collected and diluted for further assay. Drug concentration was determined by reverse phase HPLC system of zorbax extend-C18 (4.6 mm × 250 mm, pore size 5 μm, Agilent) analytical column. The mobile phase consisted of HPLC grade methanol, water and triethylamine (80/19.5/0.5, v/v/v).

2.3. Preparation of polymer solutions

The polymer solutions were prepared by dissolving PLGA in appropriate amounts of organic solvents in a glass vial under intermittent vortexing at room temperature. Designed portion of drugs were added to be dissolved (risperidone in NMP) or uniformly dispersed just before the in vitro and in vivo experiment, all formulations are listed in Table 1. Each of the preparations was tested to be injectable through a 22G needle. Drug loading (DL%) was determined by the formula below:

$$\text{Drug loading}\% = \frac{\text{weight of drug}}{\text{weight of (drug + polymer)}} \times 100.$$

2.4. In vitro release evaluation method

2.4.1. Determination of in vitro drug release rate

The in vitro release method of formulations 1–8 were performed as follows: polymer solutions containing 1 mg of drug

were precisely weighed into the conical bottom of a 50 ml screw-cap centrifuge tube using a transfer pipette, in which 35 ml of 37.5 °C phosphate buffer (pH7.4 PBS, 50 mM, 0.02% Tween 80, 0.05% sodium azide) was immediately added to form a tablet-like implant, those tubes were then incubated in a 37.5 °C, 100 rpm shaker. Considering that there could be more than one dosage in potential clinic use, we designed the 1 mg and its quadruple dosage of formulation 9 to study the release behavior come from the same formulation but different surface/volume ratios: (1) 1 mg drug containing ISFIs loaded by 1 mg PLGA were calculated and precisely weighed into the 50 ml screw-cap centrifuge tubes, (2) 4 mg PLGA loaded of 4 mg drug were calculated and precisely weighed under the same method. At specified time intervals, samples were centrifugated by 3000 rpm for 5 min, 20 ml of supernatant was replaced and assayed. All experiments were performed in triplicate and proved by in vitro–in vivo correlation.

2.4.2. Determination of organic solvent release rate

ISFIs of 0%, 50% drug loading of paliperidone were designed to investigate the solvent release rate of NMP and DMSO based on the same polymer/solvent ratio of 1/4. Four parts of 50507A polymers were dissolved in NMP and DMSO respectively, designed portion of paliperidone were added to be uniformly dispersed. Each ISFI containing 16 μl of organic solvent was calculated and weighed into the conical bottom of a 50 ml screw-cap centrifuge tube, in which 35 ml of 37.5 °C phosphate buffer was immediately added, the whole experiment was performed under the same way as in Section 2.4.1. At predetermined time points, samples were centrifugated, supernatants were replaced and assayed. Solvent concentration in the release medium was determined by Gas–solid Chromatography performed on Shimadzu GC-14C of Rxi-17 quartz capillary column and FID detector with the acetone as internal standard. GC conditions: the injection temperature was 250 °C, initial oven temperature 80 °C, initial hold time 5 min, rate of temperature program 20 °C/min, final oven temperature 200 °C, final hold time 3 min, FID detector temperature 300 °C, the carrier gas flow rate was 30 ml N_2/min.

2.5. Morphology study on the ISFI implants

2.5.1. Real-time optical microscopic observation on initial solution–gel transition

To investigate the solvent diffusion and the solution–gel transition process during the initial implant forming period, a real-time optical microscopic observation was performed on the drug-free polymer solutions to avoid interruption of dispersed drug particles. Experiments were carried out in a PBS saturated environment to mimic the in vivo condition. 1 μl of drug-free NMP/DMSO ISFIs (50507A/solvent = 1/4) were dropped on two glass slides with a transfer pipette, drops of fresh PBS were added closely on one side of the polymer solution and absorbed on the other side by a piece of filter paper, optical pictures were recorded in 5 min, 15 min, and 30 min.

2.5.2. Morphology study by scanning electron microscope

The influence of solvent nature on the phase inversion dynamics of ISFI system was investigated by morphology study on drug-free NMP/DMSO ISFIs (50507A/solvent = 1/4). SEM samples were prepared under the same way as the in vitro evaluation method in Section 2.4, each of 50 μl volume of the ISFIs was formed at the bottom of the centrifuge tube and retrieved at pre-designed time points and freeze drying for 96 h. The freeze-dried implants were then cooled in liquid nitrogen and cut for cross-section observation. All samples were sputter-coated with platinum, and examined by Field Emission Scanning Electron Microscopy (FE-SME, JEOL JSM-6700F).

2.6. In vivo study on beagle dogs

Pharmacokinetics evaluation was performed on beagle dogs (body weight of 12 ± 1 kg, n = 3). Food and water were of free access during the whole experiment period. A single dose of 0.37 mg/kg paliperidone of formulation 6 (injection volume 0.1 ml) was administered subcutaneously on the forelimb of beagle dogs. Blood samples of 1 ml were collected pre-dose and at post-dose time points, plasma concentrations were determined by HPLC-MS/MS (Sciex API 4000 triple–quadrupole mass spectrometer).

2.7. Development of in vitro–in vivo evaluation model

The development of in vitro–in vivo evaluation model was based on the in vivo cumulative release profile calculated by trapezoid method. In vitro–in vivo correlation (IVIVC) coefficient was obtained between in vitro/in vivo release profiles. Perfect sink conditions were provided in all experiments to avoid artificial drug saturation effects (Klose et al., 2010; Martinez et al., 2008).

2.8. Pharmacologic effect evaluation of paliperidone ISFI

The animal's schizophrenic behavior model was built on mice by MK-801 induced stereotypy activities. A dose of 0.6 mg/kg MK-801 was adopted to maintain a sustained 100-min stereotypy activity effect (Sun et al., 2010). A total of 30 male mice (body weight 20 ± 2 g, n = 30) were assigned randomly into 3 groups: saline group (blank control), MK-801 group (positive control) and experiment group (MK-801 + paliperidone ISFI). A single dose of 90.82 mg/kg paliperidone of formulation 8 (injection volume 0.05 ml) was administered subcutaneously on the back of mice (n = 10), this high dose was based on pre-experiment to facilitate observation of significant effect. 15 min pre-experiment, MK-801 of 0.06 mg/ml was administered intraperitoneal to the positive control group and experiment group, 0.9% saline was administered intraperitoneal to the blank group (injection volume 0.2 ml).

MK-801 induced stereotypy activities were characterized by increased locomotion, head weaving, body rolling and sniffing progressing in mice (Kostrzewa, 1998; Li and Wolf, 1999). Evaluation of suppressing effects of paliperidone ISFI was scored according to 5 levels of inhibition degree: 0 – absent of stereotypy activities, 1 – equivocal activities, 2 – present activities, 3 – intense activities, 4 – intense and continuous stereotypy activities. Behavioral recordings began 20 min post-dose, the mice were placed immediately into a 28 cm × 18 cm × 12 cm cage, and allowed for at least 5 min adaptation. Evaluations were made every 10 min for 2-min period during a 90-min session by two trained observers who were blind to the experimental group (Li and Wolf, 1999; Taylor et al., 2002). The experiment continued for 42 days and performed only in the daytime on pre-designed date. The 90-min mean stereotypy score was averaged for daily data. Student's t-test was applied as the statistical measurement.

3. Results and discussion

3.1. Solubility determination of drugs in organic solvents

The results of drug solubility in the release medium and organic solvents are shown in Table 2, where "AUFS" stands for "absorbance units, full scale" here. Both of risperidone and paliperidone possessed lower solubility in the aqueous medium and DMSO, according to which different drug loading amount would lead to a

Table 2
Solubilities of drugs in PBS and solvents and their testing method.

Drug in solvents	Solvents	Detection wavelength (nm)	AUFS	Drug solubility (mg/ml)
Risperidone	PBS	278.00	2.00	0.18
Paliperidone	PBS	280.00	1.00	0.12
Risperidone	NMP	278.00	2.00	69.08
Risperidone	DMSO	278.00	2.00	9.84
Paliperidone	NMP	280.00	1.00	38.98
Paliperidone	DMSO	280.00	1.00	8.25

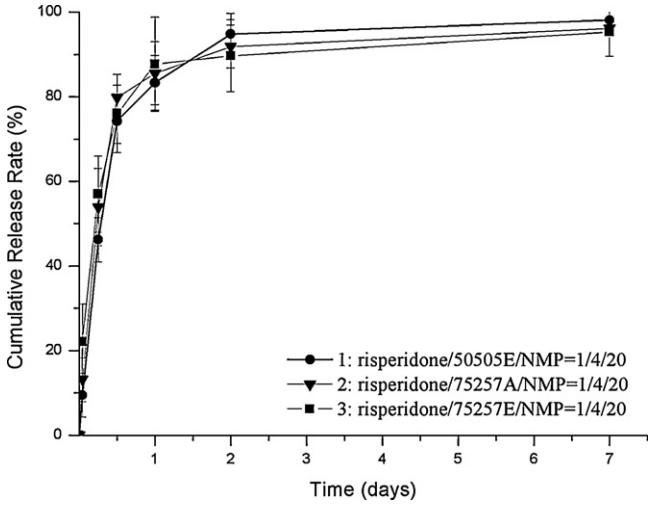

Fig. 1. In vitro releasing profile of risperidone from PLGA/NMP ISFIs.

homogenous or heterogeneous ISFI system. In our study, a dissolving system of the formulations 1–3 in the case of risperidone, and mixing systems in which the drugs were partially dissolved in other formulations were rationally designed.

3.2. Determination of in vitro drug release rate

As shown in Figs. 1 and 2, the drug release from formulations 1–3 were extremely fast, most of the drugs were released within one day, and no obvious influence of polymer monomer ratio or molecular weight were shown on the release profiles. However, those influences were noticed in the case of DMSO ISFIs (Figs. 3 and 4), from which the drug release lasted for more than one week. The

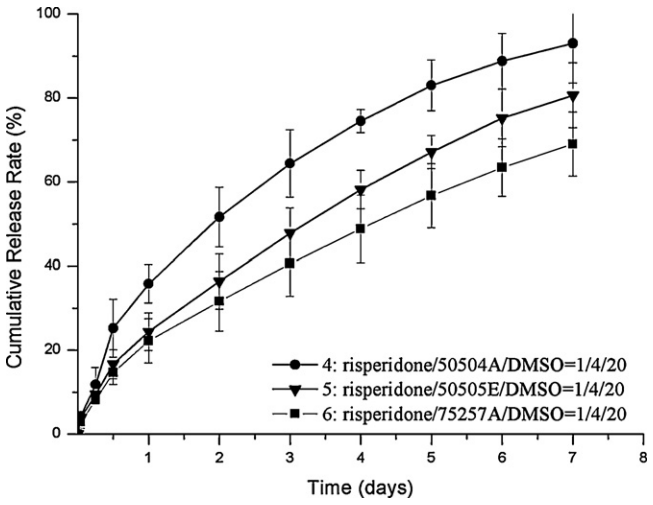

Fig. 3. In vitro releasing profile of risperidone from PLGA/DMSO ISFIs.

solvents release rate as well as the drug homogenous or heterogeneous states were supposed to contribute together to the significant difference between the two kinds of ISFIs. According to the drug solubility results of Section 3.1 and solvent release study in Section 3.3, all or most of the drugs were dissolved in NMP ISFIs and dispersed in DMSO ISFIs, the fast extraction of the NMP to the surrounding aqueous medium led to a rapid release of the dissolved drugs. In DMSO ISFIs, most of the dispersed drug particles were more tend to be encapsulated in the matrix during the solution–gel transition which enabled a more sustained release.

In the DMSO ISFIs (Figs. 3 and 4), 75257A exerted the lowest drug release, its high MW, which led to a high viscosity and a fast solidification (Graham et al., 1999; McHugh and Miller, 1995), could hamper the drug particles from diffusion. A slow degradation of the high MW PLGA further prolonged the drug release.

One exception was noticed in the case of paliperidone ISFIs (Fig. 4), the drug release rate from higher molecular weight system of 50505E exceeded that of 50504A. A-type PLGA terminated with carboxylic moiety may be more compatible with paliperidone, 9-hydroxyl derivative of risperidone, the effect of which would like to help in the drug retention.

Paliperidone and A-type PLGAs were selected for further formulation research, based on the low initial burst and sustained drug release. Considering the 50:50 monomer ratio would exhibit

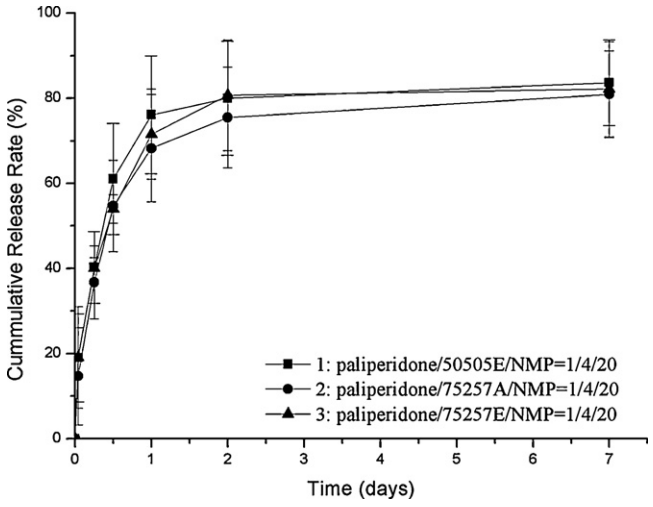

Fig. 2. In vitro releasing profile of paliperidone from PLGA/NMP ISFIs.

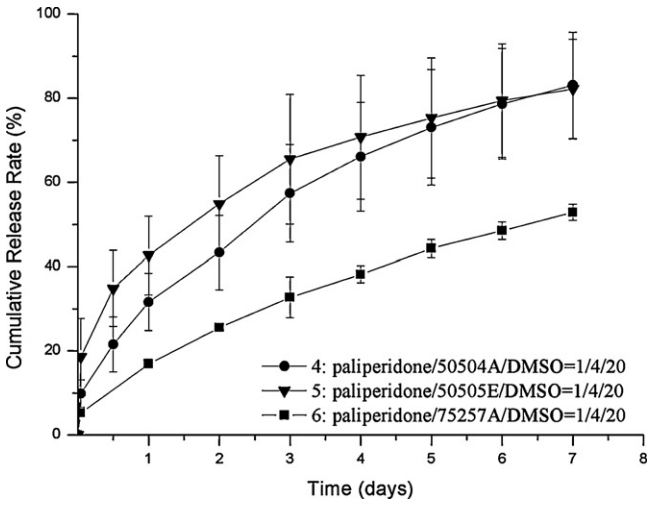

Fig. 4. In vitro releasing profile of paliperidone from PLGA/DMSO ISFIs.

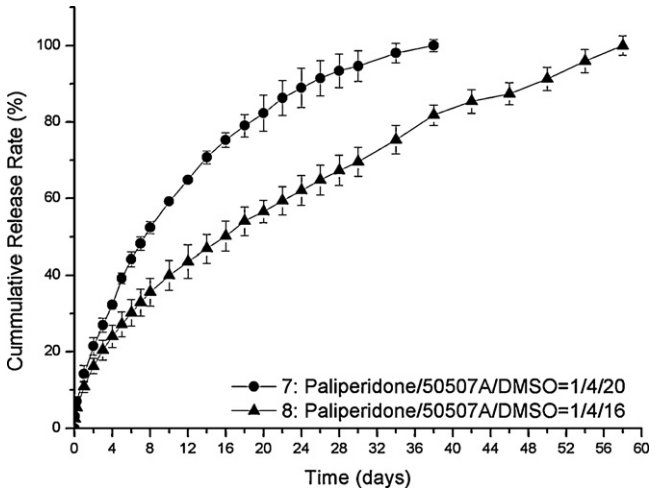

Fig. 5. In vitro releasing profile of paliperidone of formulations 7 and 8.

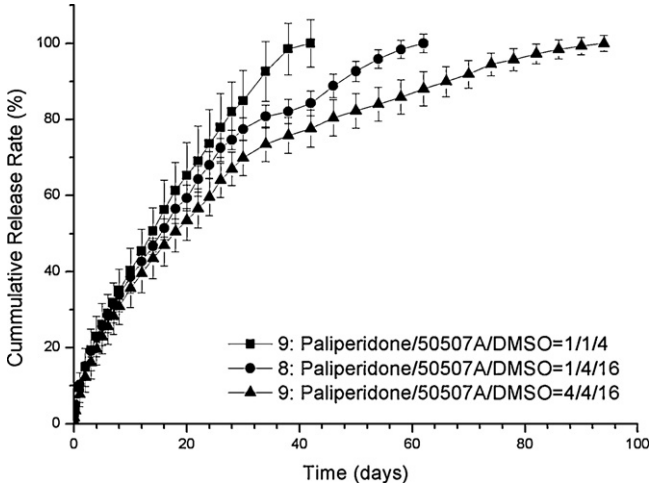

Fig. 6. Cumulative drug release profile of paliperidone ISFIs of formulations 8 and 9.

a faster degradation rate which may relieve the elimination burden in vivo, 75257A was replaced by 50507A. The polymer/solvent ratio was adjusted from 1/5 to 1/4 without sacrificed injectability. In vitro results showed that the increase of viscosity sped up the phase inversion, a lower initial burst and longer sustained release profile was observed in Fig. 5.

The in vitro release experiment of 50% drug loading of paliperidone ISFIs was performed in two methods according to Section 2.4.1. A two-phase release profile was observed in Fig. 6. Within the first week, the cumulative drug release profile of the three curves nearly overlapped on each other, a low burst release was found even for the 50% DL formulation 9 of paliperidone/50507A/DMSO = 1/1/4 and its quadruple volume depot of 4/4/16. This interesting phenomena indicated for a similar drug release mechanism within the first week, it acted independent of drug content or depot volume, which exerted their influence in the second release phase.

A semi-emprical Korsmeyer ± Peppas model (Costa and Sousa Lobo, 2001) was employed to characterize the drug release mechanism of the different phase. The Peppas mathematical equation is presented as below:

$$\frac{M_t}{M_\infty} = at^n$$

where a is the release constant indicative of characteristics of the drug depot form, n is the release exponent, characterizing the drug release mechanism, M_t/M_∞ is the fractional release of drug as the function of t. The diffusion mechanism can be characterized as Fickian diffusion when $n = 0.5$, or non-Fickian model with an n value between 0.5 and 1.0.

As shown in Table 3, the drug diffusion rate of the first week well fitted to the Korsmeyer ± Peppas model with the n value around 0.5, which indicated a quasi-Fickian, dissolution controlled drug release mechanism, during which, an saturated drug concentration reached an equilibrium in the semi-solid gel due to the poor solubility of paliperidone in the remaining DMSO, and resulted in an approximately constant concentration gradient in the system, thus all formulations possessed a similar constant diffusivity in the first phase.

After one week, the more solid matrix entered into a non-Fickian, anomalous drug transport phase. A higher drug loading of the formulation 9 led to a faster release than that of the formulation 8 which has half lower drug loading. But when increasing the volume of the matrix, a low surface/volume ratio reduced the release.

3.3. Determination of organic solvent release rate

As shown in Fig. 7, the solvents diffused mostly into the medium within one day after the system was immersed in the aqueous medium, and completed extraction one week later. Under the same condition, the release rate of DMSO far exceeded that of NMP, almost 20% of DMSO was already released into the medium within 5 min in comparison with 5% of NMP, this disparity lasted for 6 h until the 50% DL NMP ISFI catched up, in which a high DL% or less PLGA content may result in a low viscosity of the system which helped the extraction of the solvent from the matrix.

The solvents release kinetics was critical to phase inversion dynamics of viscous solution to gel or semi-solid to solid transition. In the case of drug dissolved ISFI, a fast solvents extraction often accompanied by a high drug release. However, it is a different story for dispersed drug system, in which the drug particles tend to be encapsulated in the polymeric matrix. It is expected that for homogeneous ISFI system, a low solvent release rate of NMP was preferred, and a high DL% could increase the burst release. On the other hand, for the drugs of poor solubility, a fast diffusion of DMSO would lead to a fast solidification of the implant, resulting in a high drug retention rate, which was reflected by the drug release results of Figs. 1–4.

3.4. Real-time optical microscopic observation on solution–gel transition morphology

The initial solution–gel transition morphologies within 30 min are shown in Fig. 8. Different phase inversion dynamics of the two

Table 3
Modeling of drug release mechanism using Korsmeyer–Peppas equation.

Formulation: release methods	Time period	Korsmeyer–Peppas model	Time period	Korsmeyer–Peppas model
8:1/4/16	0–7 d	$Q = 0.1094t^{0.5195}$ $R^2 = 0.9978$	7–28 days	$Q = 0.0866t^{0.6467}$ $R^2 = 0.9974$
9:1/1/4	0–7 d	$Q = 0.1031t^{0.5640}$ $R^2 = 0.9973$	7–28 days	$Q = 0.0836t^{0.6850}$ $R^2 = 0.9993$
9:4/4/16	0–7 d	$Q = 0.0867t^{0.5763}$ $R^2 = 0.9937$	7–28 days	$Q = 0.0871t^{0.6086}$ $R^2 = 0.9989$

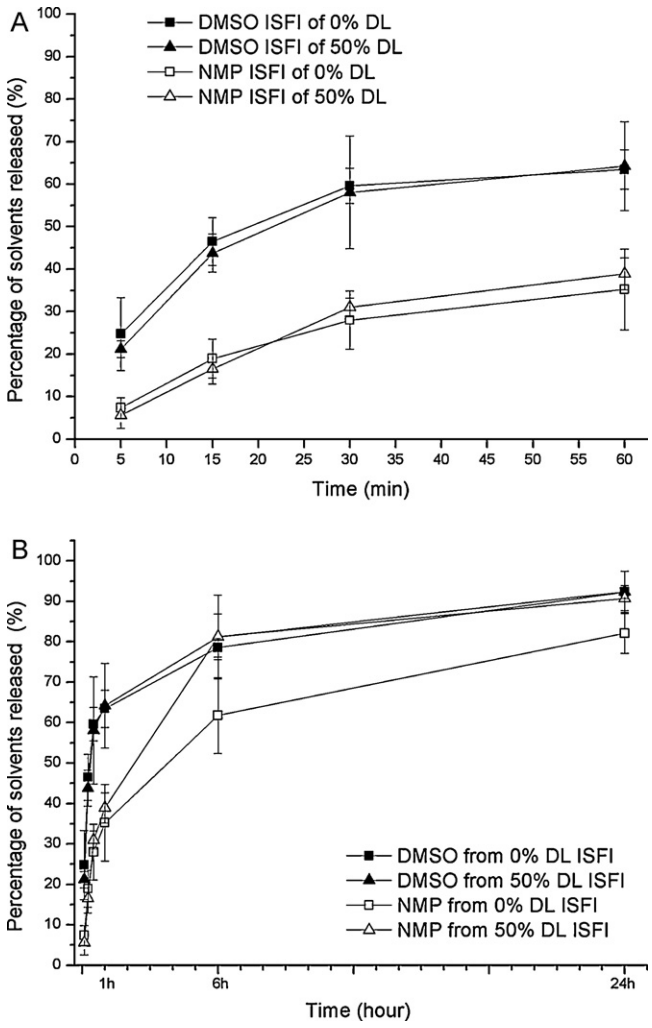

Fig. 7. Percentage of solvents released into the PBS medium within 1 h (A) and 1 day (B).

kinds of ISFIs were clearly presented in the real-time recorded pictures, in which a more transparent district indicated unsolidification solution, and the dark points indicated the solidified polymer matrix.

In the 50507A/NMP system, a front layer was formed immediately in contact with the PBS medium, as a result, more of the organic solvent remained in the matrix, thus slowed down the inner polymer solidification, more transparent districts in the 30-min NMP ISFIs was observed. In the case of 50507A/DMSO system, an obvious solvent dissipating route was observed, and the polymer precipitation occurred soon after contacting with PBS medium, this noticeable solvent extraction phenomenon lasted for a 15-min period and a more solidified matrix was formed in situ, with a few scattered small pores in the core area.

3.5. Morphologies examined by scanning electron microscope

The influence of solvent extraction rate on the morphology of ISFIs during the solution–gel transition phase within one day was investigated (Fig. 9). The 6-h cross-section pictures of NMP ISFIs revealed a semi-solid matrix of internal macro-cavities and a thin, dense external layer of about 100 μm. In the case of DMSO, an alveolate internal structure and an external layer of more than 200 μm were observed, numerous micro-paths which might be due to DMSO extraction were presented, which was agreed to the results in Fig. 8. At 6 h and 1 day, almost 40% and 20% of NMP still remained in the implant, respectively, the higher solvent/polymer ratio of NMP ISFI resulted in a macrovoided matrix after freeze drying (Fig. 9A). Meanwhile, as 80% of the DMSO had already been extracted at 6 h, a much dense alveolate internal structure was presented (Fig. 9B).

The SEM morphology examination showed consistent results with the real-time optical microscopic observation and solvent release rate study. The solvent extraction rate influenced the phase inversion of the polymer solution, resulting in different morphologies of the implants, which eventually exerted an impact on the drug release behavior.

The dense external layer of NMP ISFIs was expected to slow down the continuous extraction of the solvent and water up-taken during the initial transition phase, which would delay the solidification of the polymeric gel, and resulted in a fast diffusion of the dissolved drugs. However, for poor soluble drugs in DMSO,

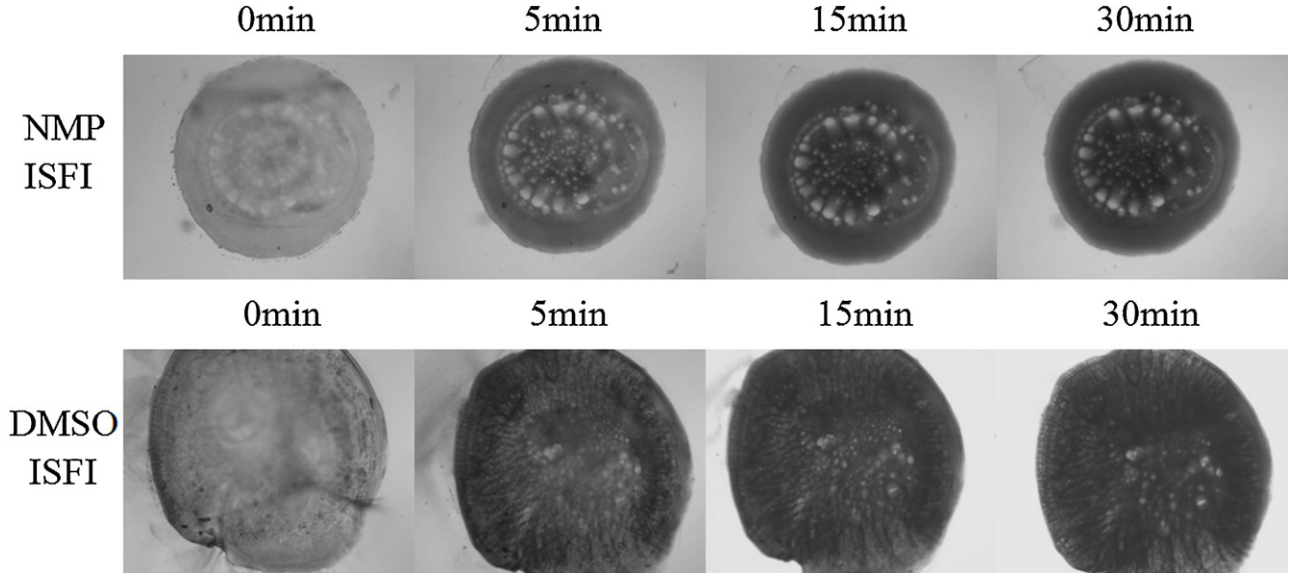

Fig. 8. Optical microscopic observation on solution–gel transition phase of NMP and DMSO ISFIs.

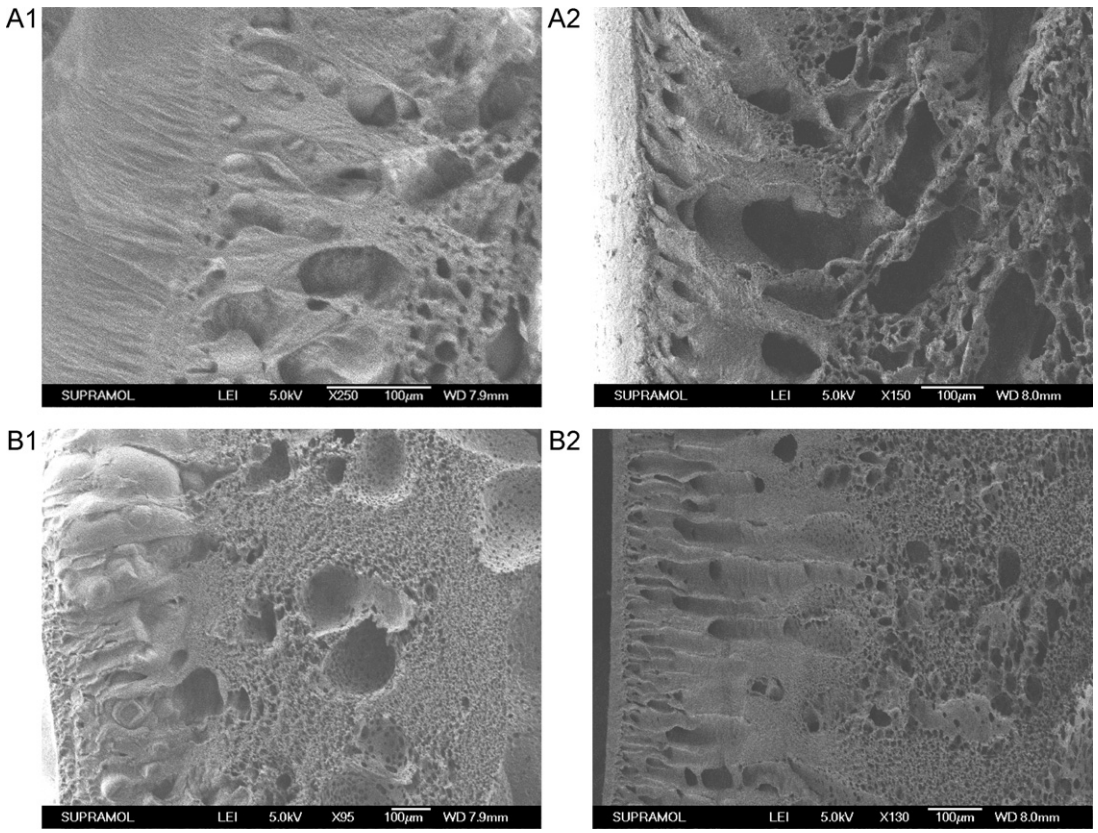

Fig. 9. SEM morphologies of NMP and DMSO ISFIs: A1, A2 indicate for cross-section structure of blank NMP ISFIs at 6 h, 1 day, respectively. B1, B2 indicate for cross-section structure of blank DMSO ISFIs at 6 h, 1 day, respectively. The left to right side of the cross-section picture indicates for external–internal part of the implant.

a quick solidification of PLGA/DMSO gel favored the encapsulation of dispersed drug particles, and resulted in lower initial burst and sustained-release. The thick front layer and profoundly formed dense matrix limited further water penetration thus slowed down the drug dissolution and diffusion.

3.6. In vivo study on beagle dogs

Formulation 6 of paliperidone/75257A/DMSO = 1/4/20 was selected for preliminary pharmacokinetic study on beagle dogs ($n = 3$). As shown in Fig. 10, C_{max} of 34 ng/ml of the drug was achieved after 8 h and reduced slowly. The total releasing period lasted for more than 3 weeks. The in situ forming implant had made a tiny visible implant of 5–6 mm diameter on the dog's forelimb. During the whole experiment, the animals were healthy and active, however, irritate reaction upon injection was noticed on one dog, but no inflammation or ulceration was observed on site. It took more than 5 weeks for the implants to be completely eliminated in vivo and disappeared.

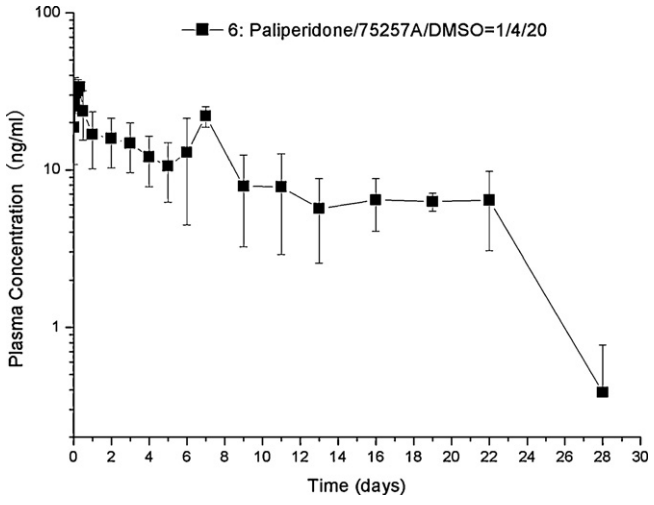

Fig. 10. In vivo pharmacokinetic evaluation of formulation 6 on beagle dogs.

3.7. Development of in vitro–in vivo evaluation model

The in vitro–in vivo evaluation model was developed based on the in vivo pharmacokinetic results, from which, the in vivo cumulative release rate profile was obtained using trapezoid method. In vitro–in vivo correlation (IVIVC) coefficient was calculated to assess the model. A PBS formulation of 50 mM, pH 7.4 phosphate buffer containing 0.02% Tween 80 and 0.05% sodium azide was determined to be the IVIVC release medium.

As shown in Fig. 11, the in vitro cumulative release profile gained through our protocol present a close resemblance to the in vivo release result. As the designated time interval of in vitro experiment did not uniformly according to that of in vivo, the IVIVC correlation were calculated based on the consistent time-point data from 0.04 days to 28 day, and resulted in a correlation coefficient of 0.994. Since all the in vitro drug release evaluation was performed according to this IVIVC protocol, all the in vitro results presented in our research were well supported.

3.8. Pharmacologic evaluation of paliperidone ISFI

As shown in Fig. 12, a dose of 0.60 mg/kg of MK-801 had successfully built the schizophrenic model on mice with enhanced

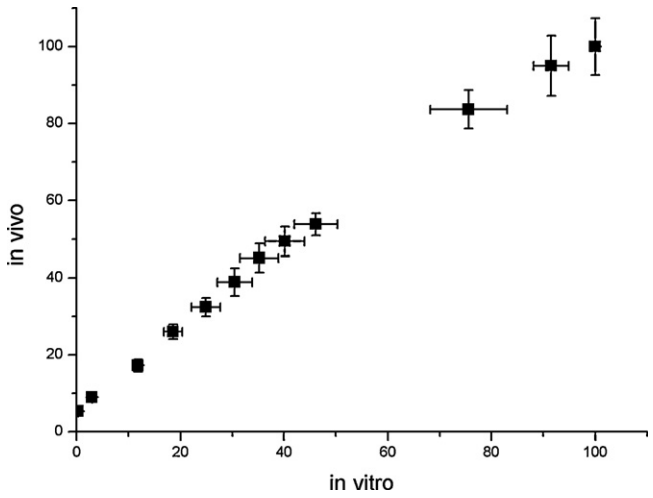

Fig. 11. In vivo–in vitro correlation of paliperidone ISFI of formulation 6.

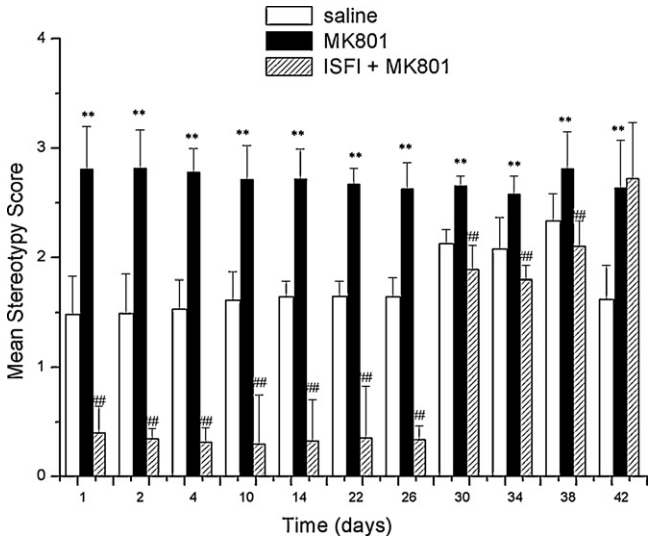

Fig. 12. Pharmacological study of paliperidone ISFI on MK-801 induced schizophrenic model. Statistical analysis was based on Student's *t*-test: $^{*}p < 0.05$, $^{**}p < 0.01$ (comparison of MK-801group with saline group); $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ (comparison of experiment group with MK-801group).

stereotypy activities, the effect of which had lasted for more than 90 min. A sustained-suppressing effect of the paliperidone ISFIs in experiment group lasted for more than one month from 1 day to 38 day, as the release of paliperidone was reduced after 26 days according to the in vitro release profile of formulation 8 in Fig. 5, the effective response in schizophrenia mice model weakened accordingly. Paliperidone is an atypical-antipsychotic of D2 and 5-HT2A receptor antagonism, and exerted a therapeutic effect on positive symptoms by suppressing D2 receptor, which may also present effect of sedation on mice. In the paliperidone ISFIs treatment group, the score-level was lower than that of blank control, extreme manifestations like body trembling, sedation and anorexia was observed on the first day post dose due to high dose strength, however, 93% of the mice survived after the whole experiment of 42 days.

4. Conclusion

Novel solvent removal ISFI systems of paliperidone and risperidone were investigated in this paper. Heterogeneous ISFIs were rationally designed to achieve high drug retention rate and low burst release by matching with a fast solvent extraction system of PLGA/DMSO.

A two-phase release profile was observed for both 20% and 50% drug loading paliperidone ISFIs. Model fitting results indicated a quasi-Fickian, dissolution controlled drug release mechanism within the first week, which acted independent of drug content or depot volume. After one week, the drug diffusion was influenced by the combined effects of dissolution inhibition and polymer degradation.

Through a real-time optical microscopic observation and SEM morphology study, distinct phase inversion dynamics of the NMP and DMSO ISFIs were observed in consistent with the drug and solvent release behavior. A slower solvent extraction rate of NMP ISFIs formed a dense external layer in the initial transition phase, which hampered further water up-taken, and slowed down the inner solidification. In the case of DMSO ISFIs, numerous micro-paths forming through the implant were observed at the very beginning of transition, which might due to the fast solvent extraction, and a alveolate matrix with thick external layer was found after freeze drying.

In vivo studies of paliperidone ISFI on beagle dogs achieved a more than 3-week sustained release after a single dose, the plasma concentration of drug reached a C_{max} of 34 ng/ml at T_{max} of 8 h, and then reduced slowly. An in vitro–in vivo evaluation model was also developed with the IVIVC correlation coefficient of 0.994.

Pharmacologic evaluation of optimized formulation was performed on animal's schizophrenic behavior model, and resulted in a sustained-suppressing effect from 1 day to 38 day on the MK-801 induced stereotypy activities in mice.

Paliperidone ISFIs of 50% drug loading, with well controlled initial burst, was achieved to last for more than one month sustained release. The one-month injection of formulation 9 in this paper included 25.2 mg PLGA and 100.8 mg DMSO, which reduced the intake of both solvent and polymer, thus decreased the potential risk. This long sustained-release ISFI system of the antipsychotics could provide a valuable alternative in potential clinic application.

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