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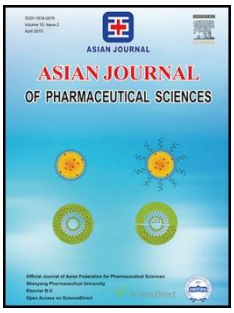

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Author: Li Liu, Jia Li, Mei-hui Zhao, Hui Xu, Lin-sen Li, Shao-ning Wang

PII: S1818-0876(16)30055-1
DOI: http://dx.doi.org/doi: 10.1016/j.ajps.2016.07.005
Reference: AJPS 388

To appear in: *Asian Journal of Pharmaceutical Sciences*

Received date: 15-4-2016
Revised date: 22-6-2016
Accepted date: 20-7-2016

Please cite this article as: Li Liu, Jia Li, Mei-hui Zhao, Hui Xu, Lin-sen Li, Shao-ning Wang, Loading of tacrolimus containing lipid based drug delivery systems into mesoporous silica for extended release, *Asian Journal of Pharmaceutical Sciences* (2016), http://dx.doi.org/doi: 10.1016/j.ajps.2016.07.005.

Title page

Loading of Tacrolimus Containing Lipid Based Drug Delivery Systems into Mesoporous Silica for Extended Release

Li Liu[a], Jia Li[a], Mei-hui Zhao[a], Hui Xu[a,] *, Lin-sen Li[b], Shao-ning Wang[c]

[a] School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, P. R. China;

[b] Department of Biochemistry, Shenyang Medical College, Shenyang 110034, P. R. China;

[c] School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, P. R. China

*Corresponding authors: Hui Xu,

School of Pharmacy, Shenyang Pharmaceutical University,

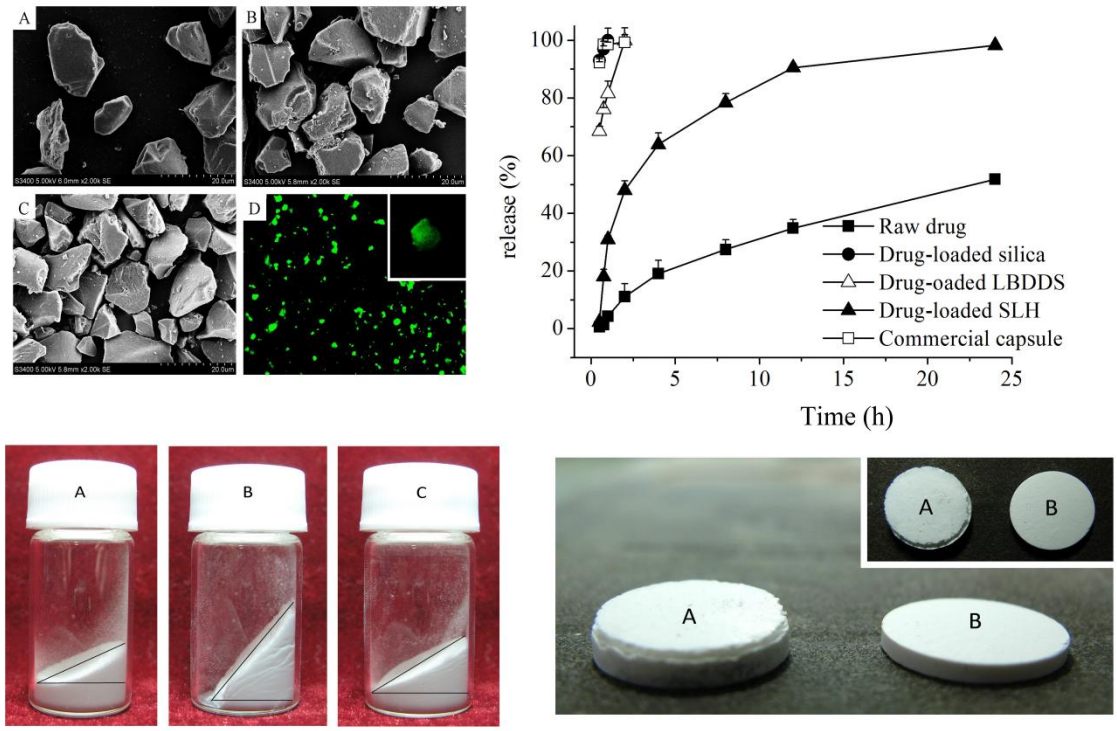
Shenyang 110016, P. R. China.

Tel: 024-23986356.

Email: xuhui_lab@163.com

Graphical Abstract

Tacrolimus loaded silica-lipid hybrid (SLH) powder prepared by mesoporous silica and lipid had good sustained release behavior, flowability and compressibility. The drug was distributed into the pores of the silica.



Abstract:

Many studies had been focused on designing tacrolimus sustained release preparations based on solid dispersion technique, but no one had tried to employ mesoporous silica as the carrier material to realize this goal. The purpose of this study **was** to develop a novel, simple and **environmental** friendly drug loading method with mesoporous silica to obtain tacrolimus sustained-release preparation. Tacrolimus was firstly dissolved in the molten mixed lipid composed of Compritol 888 ATO and Gelucire 50/13 to prepare a drug loaded lipid-based drug delivery systems (LBDDS), **then the liquid LBDDS was adsorbed by mesoporous silica to transfer the liquid into solid powder, ie. the tacrolimus sustained release silica-lipid hybrid (SLH). The SLH was characterized by**

SEM, CLSM, XRPD and DSC, and the *in vitro* drug release was tested using a paddle method. SEM and CLSM observation showed that the LBDDS was efficiently distributed throughout the pores of the silica. The results of DSC and XRPD illustrated that the lipid existed inside the silica at amorphous state. **The** drug-loaded SLH **showed** good flowability, compressibility, compactibilty **and two-phase *in vitro* drug release process within 24 hours, which did not** change obviously even after storage at 40 ºC for 10 d. **The** present study provided a novel and **simple method to prepare tacrolimus sustained release powder, which provided a feasible solution to solidify the liquid LBDDS of not only extended drug release behavior, but also improved stability and micromeritic properties.**

Keywords: Tacrolimus; Lipid-based drug delivery system; Mesoporous silica; Silica-lipid hybrid; Sustained-release

1. Introduction

Tacrolimus, previously known as FK506, is a macrolide immunosuppressant produced by *Streptomyces tsukubaensis*. **Due to its** potent immunosuppressive activity, low dosage, high organ survival rate and low incidence of acute rejection rate, tacrolimus is clinically used in the prophylaxis of organ rejection after hepatic and renal transplantation procedures [1]. As a BCS class **II** drug, the poor water solubility of tacrolimus greatly limited its oral bioavailability. Thus, increasing the solubility **or dissolution** of tacrolimus was one of the key problems in improving oral bioavailability, and many studies have been devoted to the modification of various drug delivery systems, including solid dispersion [2,3], nanoparticles [4-6], liposome [7,8], and self-emulsification [9]. **Tacrolimus extended release**

preparations attracted great attention in both clinical practice and drug development due to its superior therapeutic profits, such as decreased ratio of peak/trough blood drug level, improved oral bioavailability and eliminated need for frequent dosing. There **were** already two modified release products of tacrolimus approved and marketed worldwide. A once-daily tacrolimus sustained release capsule supplied by *Astellas Pharma Inc* **was approved in Europe (Advagraf®) and Japan (Graceptor®) in 2007 [10-11].** After that, tacrolimus sustained-release tablet produced by *Veloxis Pharmaceuticals* was approved both in Europe and USA with the brand name of Envarsus® and Envarsus®XR, respectively. In addition, there was an explosion of interest in developing tacrolimus extended release preparations in recent years. *Cho et al.* had focused on the development of novel fast-dissolving tacrolimus solid dispersion-loaded prolonged release tablet. *Wang et al.* **reported** a novel gastro-retentive sustained-release tablet of tacrolimus based on self-microemulsifying mixture [12].

Lipid excipients aroused great interest to drug formulation researchers owing to their ability of providing solutions to drug delivery challenges, such as low drug solubility and dissolution rate, poor oral absorption of water-insoluble drugs, as well as the simplified and safety manufacturing processes, and so on [13-15]. **For poorly water-soluble drugs, lipid-based drug delivery systems (LBDDS) unveiled distinguish prospects as it can mimic the food effect via creating a lipophilic microenvironment within the gastrointestinal tract,** thus enhancing the solubilization of poorly water-soluble drug molecules and providing a concentration gradient that drive the absorption of drug molecules in the intestinal tract [16-20]. One of the major disadvantages of LBDDS lies in its liquid or low melting-point semi-solid state (eg. lipid solutions, suspensions, and emulsions) at ambient environment, which may result **in** physicochemical instability during storage, **thus greatly limited applications of LBDDS [21-22].**

Mesoporous materials with tunable pore size of 2 nm to 50 nm, large surface area and porosity, and **high adsorbing capacity have been selected as ideal carriers for both fast and extend drug release. Mesoporous silica was widely investigated as drug delivery systems since *Vallet-Regi* firstly used it for loading of drug in 2001 [23-27]**. The use of

mesoporous silica in drug delivery systems is mainly due to its excellent adsorptive properties. In previous reports, organic solvent solution soaking method was often used to load poorly water-soluble drugs for fast drug release. ***Wang et al.*** **achieved sustained release of drug by employing supercritical fluid technique to load poorly water-soluble drug deep into the pore channels of mesoporous silica** [25]. It is the physical interaction between silica and adsorbed guest molecules that **delays drug release** [28]. Mesoporous silica can also be used as a carrier of LBDDS via physical adsorption **to convert the** liquid or semi-solid state LBDDS into solid powder and formed silica-lipid hybrid (SLH) with improved the flowability, compressibility and compactibility. The powdered SLH combined the well-known advantages of LBDDS with those of solid dosage forms, **and what's more**, **it bore the ability to greatly improve the physicochemical stability of LBDDS** [29].

Till now, there is no report on the using of lipid excipients and mesoporous silica as the organic/inorganic hybrid carrier to achieve solidified LBDDS for sustained tacrolimus release. In this study, tacrolimus was dissolved in a mixture of molten lipids composed of Glyceryl Behenate and Stearoyl polyoxyl-32 glycerides to obtain drug loaded LBDDS, and then mesoporous silica was used to adsorb the liquid LBDDS to obtain SLH powder. The physicochemical properties of SLH powder and their *in vitro* drug release were studied. It is desirable to achieve a novel, **simple and solvent-free technique** to fabricate tacrolimus sustained release preparations with this method.

2. Materials and methods

2.1 Materials

Tacrolimus (Chinese Pharmacopeia) was a gift from Zhejiang Hisun Pharmaceutical Co. Ltd (Jiangsu, China); **Glyceryl Behenate (**Compritol 888 ATO) and **Stearoyl polyoxyl-32 glycerides (**Gelucire 50/13) were kindly donated by Gattefossé (France); Mesoporous silica **(Parteck SLC)** was provided by Merck & Co. Inc (Germany). **Tacrolimus capsules: brand name Prograf; standard 1mg/capsule; batch number 1E2201A.** All other chemicals used were of chemical or analytical grades and used as received.

2.2 Preparation of tacrolimus loaded LBDDS and SLH

Tacrolimus loaded SLH was prepared in a process of two-steps, ie., preparation of drug-loaded LBDDS and the solidification of LBDDS to form the drug-loaded SLH as described below. (1) Tacrolimus, and/or Compritol 888 ATO and Gelucire 50/13 were accurately weighed according to the formulations listed in Table 1, and placed into a glass round bottom flask and heated at 80°C with constant stirring until all the excipients were melted; then tacrolimus was added into the molten mixture at 70°C with stirring to form a homogenous mixture. (2) The mesoporous silica was mixed with the above molten mixture, and the molten mixture was adsorbed into the pores inside the silica under decreased pressure. The mixture was stirred for another half an hour at 70°C and then cooled down to room temperature to form the final solid hybrid mixture. The lipid free, tacrolimus loaded mesoporous silica was prepared with similar method using ethanol as the solvent and fabricated at room temperature.

2.3 Scanning electron microscopy (SEM) observation

The mesoporous silica, drug-loaded SLH and drug-loaded SLH after *in vitro* drug release were spattered with gold, their morphologies were observed using a S-3400 SEM (Hitachi, Japan) at 5.0 KV electron acceleration voltage.

2.4 Confocal laser scanning microscopy microphotographs (CLSM)

Coumarin, a fluorescent dye, was loaded **into SLH** as the model molecule instead of tacrolimus to observe the distribution of drug inside the mesoporous carrier. Briefly, about 1 µg of coumarin was dissolved in 100 mg of lipid mixture molten at 70°C, the mixture was then adsorbed into 300 mg of mesoporous silica. Confocal laser scanning microscopic images of the coumarin-loaded mesoporous silica were taken with LSM 710 and Axio Inverted Microscope (ZEISS, Germany), and laser of 464 nm wavelength was used to excite

the fluorescence coumarin.

2.5 X-ray powder diffraction (XRPD)

The crystalline characteristics of **tacrolimus, drug-loaded** LBDDS, mesoporous silica and **drug-loaded** SLH were determined by XRD-6000 X-ray Powder Diffractometry (Shimadzu, Japan) at 40kV and 40mA using Cu Kα radiation. The samples were measured in the 2θ range between 5° and 60° at a scan rate of 2.8° per second with step of 0.0167°.

2.6 Differential scanning calorimetry (DSC)

The physical state of **tacrolimus, drug-loaded** LBDDS, mesoporous silica and drug-loaded SLH were measured with DSC-6 (Mettler-Toledo, Switzerland). Samples (about 5 mg) were weighed accurately and sealed in an aluminum pan and the DSC curves were determined at a heating rate of 10°C/min from 25°C to 180°C under N_2 gas purge of 40 ml/min, and an empty pan was used as reference.

2.7 In vitro drug release

The *in vitro* release test was performed using the paddle method described in Chinese *Pharmacopoeia **(2015)*** with small beakers using a ZRS-8G Dissolution Apparatus (Tianda Tianfa Technology Co. Ltd., China). 100 ml of distilled water containing 0.005% hydroxypropyl cellulose (adjust pH to 4.5 with phosphoric acid) was used as the dissolution media according to ***USP 35***, and the stirring speed was set at 50 rpm/min. Samples equivalented to 1 mg of tacrolimus was placed in the dissolution medium, 3.0 ml of the medium was withdraw from the beakers and replaced with the equal volume of fresh dissolution medium at pre-determined time intervals. The medium was filtered through a

membrane filter of 0.45 μm pore size (Millipore, USA), the content of tacrolimus was then assayed by L-2000 High Performance Liquid Chromatography (HPLC) (Hitachi, Tokyo, Japan), and the UV-vis detector was set at 210nm. Tacrolimus was analyzed using Dikma ODS C18 chromatography column (200mm×4.6mm, 5μm). The mobile phase consisted of acetonitrile and distilled water (75:25, v/v) and was pumped at a flow rate of 1.0 ml/min at the temperature of 50ºC. **Validation of assay method showed good linearity in the concentration range of 0.5μg/ml to 12.0μg/ml (A=14669C+13.799, R^2=0.999) and precision (RSD<2%).**

2.8 Properties of the powder

2.8.1 Density

Bulk, tapped, and true densities of the mesoporous silica powder, drug-loaded LBDDS **of formulation No.1** and drug-loaded SLH **of formulation No.3** were measured with methods in literatures [30,31]. About 0.5~1.0 g mesoporous silica, LBDDS or SLH were placed in a graduated cylinder to measure the bulk and tapped density. Bulk density was calculated by the equation mass/volume before tapping while the tapped density was obtained after 100 taps to allow the powder volume to plateau. The true density was measured as follows: about 0.5~1.0 g mesoporous silica, drug-loaded LBDDS or drug-loaded SLH were compressed into tablet at maximum pressure, and the true density was calculated by mass/volume of the tablet.

2.8.2 Flowability

Two methods were used to evaluate the flowability of the powders, ie. Carr's index and Hausner ratio, and the tilt method.

The Carr's index and Hausner ratio could be calculated by Eq.1 and Eq.2, respectively [32]

Carr's index = (tapped density - bulk density)/tapped density (Eq. 1)

Hausner ratio = tapped density/bulk density (Eq. 2)

And to measure the angle of repose, the cylindrical container with powder samples which took up about 1/3 of the container volume was fluctuated, after the powder remained repose, the angle formed by the surface of the powder was defined as the angle of repose.

2.8.3 Compressibility

About 300mg of pure silica or drug-loaded SLH powder was accurately weighed, and the volume of the powder (V_0) was determined. Then, the powders were compressed into flat tablet with a diameter of 13 mm using a single punch press at pressure *P* and the maximum pressure, respectively, and the corresponded volumes of the samples (V and V_∞) were determined. The slope (c_{15}) calculated according to Heckel equation (Eq.3) was used to evaluate the powder compressibility.

$$\ln V/(V - V_\infty) = c_{15}P + \ln V_0/(V_0 - V_\infty) \quad \text{(Eq.3)}$$

3.Results and discussion

3.1 Morphology and physicochemical properties

The SEM images of mesoporous silica, drug-loaded SLH, and drug-loaded SLH after *in vitro* drug release test were shown in Fig.1, A, B, and C. **The mesoporous silica was small particles with irregular shape and smooth surface, with the particle size ranging from 5 μm to 25 μm. The surface morphology, size and size distribution of the drug-loaded SLH were similar with those of the mesoporous silica except for a small amount of residual lipid material adhered on the surface.** The CLSM image provided further insight into the drug distribution inside the mesoporous silica particles (Fig.1,D). Green fluorescence (owing to coumarin) was clearly visualized to be uniformly concentrated inside the silica, which meant the active drug can be efficiently adsorbed into the mesoporous silica together with the hot-melt LBDDS.

Fig.2 showed the DSC curves and the XRPD patterns of tacrolimus, drug-loaded

LBDDS, mesoporous silica and drug-loaded SLH, respectively. Tacrolimus showed an endothermic peak at 130°C that corresponded to its melting point. The DSC curve of drug-loaded LBDDS didn't showed the endothermic peak of tacrolimus, and it exhibited two endothermal peaks, which corresponded to the melting point of Compritol 888 ATO (~70°C) and Gelucire 50/13 (~50°C), respectively. However, there were no peaks of tacrolimus, Compritol 888 ATO or Gelucire 50/13 in the DSC curve of drug-loaded SLH. As the drug content in both drug-loaded LBDDS and drug-loaded SLH was very low (9.1% and 2.3% respectively), the results of DSC analysis could only indicate that LBDDS existed in the mesoporous silica at amorphous state.

XRPD analysis could clearly describe the crystalline degree of materials. As seen in Fig.2, B, the XRPD pattern of tacrolimus displayed multi-peaks in the range between 2θ=5° and 2θ=40°, while that of drug-loaded LBDDS had 3 peaks at 2θ=19°, 2θ=21°, and 2θ=23°, respectively, which could be attributed to the presence of crystalline lipid. The mesoporous silica and drug-loaded SLH showed no typical crystal peak, which indicated that the drug-loaded LBDDS (mainly the lipids) existed inside the mesoporous silica at amorphous state. Due to the extremely low content of tacrolimus, the XRPD results could only give information that the mixed lipids in mesoporous silica was presented at amorphous state, **which was different** to the crystalline state in LBDDS.

3.2 In vitro release of tacrolimus

The *in vitro* release test were conducted with the formulations obtained, and the results were compared with that of commercial capsule (Table 1, Fig.3.). As shown in Fig.3, A, for the raw tacrolimus, only about 50% of the drug could release within 24 h, while the drug of commercial capsule released completely within 1 h. The **drug-loaded** LBDDS and drug-loaded mesoporous silica free of lipids demonstrated fast release of 100% **within 2 h and 0.5 h, respectively.** Drug-loaded SLH **(formulation No.3)** that was obtained by co-loading of the lipid matrix and tacrolimus into the pores of mesoporous silica showed extended and complete drug release at 24 h, which may be attributed to the retardation effect

of the semisolid lipids matrix and the mesopores of the silica, as well as the solid dispersion nature of the lipid carrier.

The mixed lipids composed of high melting point and weak hydrophilicity Compritol 888 ATO and low melting point and better hydrophilicity Gelucire 50/13 were used as the carrier material of tacrolimus loaded LBBDS to improve the solubility/dissolution of tacrolimus significantly. The mixed lipid at hot-melt state (about 70°C dependent on the ratio of the two lipids) can readily dissolve water-insoluble tacrolimus, and after cooling down to room temperature forms drug loaded solid dispersion. In our art, mesoporous silica was directly introduced into the hot-melt lipid, the lipid and the **tacrolimus dissolved in** were then adsorbed inside the mesopores under vacuum. After totally sucked in, the finally obtained SLH was solid powder of good flowability, instead of wax-like, semi-solid state of the mixed lipid. Different to the volatile organic solvents commonly used for loading water-insoluble active drugs into mesoporous materials **in previous studies,** the hot-melt lipid was not only used as the solvent to tacrolimus, but also the retardant material to sustain drug release. The advantages of such organic solvent-free technique also include simple, low cost/profit ratio, and environmental friendly, and by varying the ratio of the two lipids, it will be easy to adjust the melting points and drug **release** rate.

One of the commonly faced difficulties in developing a lipid based modified release formulation may lie in the instability of the lipid crystalline, and consequent changes on the melting points of the carrier material and even the drug release behavior after storage under ambient or challenged conditions.

Many researches had been devoted to investigate the impact of time and storage condition on drug release of lipid-based modified release preparations. **Normally, the lipid excipients were glyceride mixtures and they naturally bore polymorphism that was the main reason of changed drug release behavior over time due to the crystalline transformation. Fig.3, B indicated significant change on *in vitro* drug release behavior of tacrolimus loaded LBDDS (formulation No.1) after being stored at 40°C, a high temperature, challenge condition. Ironically, no apparent differences on drug release behavior of drug-loaded SLH (formulation No.3) were observed again after being**

311 **stored for 10 d** (**Fig.3, C**). The similarity factor f_2 of the two release curves was calculated
312 by Eq.4,

313 $$f_2 = 50 \times \log\{[\ 1 + 1/\mathrm{n} \sum_{j=1}^{n} (R_j - T_j)^2\ ]^{-0.5} \times 100\ \} \quad \text{(Eq.4)}$$

314 The value of f_2 was 59.6, higher than 50, which demonstrated similar of the two release
315 curves. This result also indicated improved stability by loading the lipid carrier inside
316 mesoporous carrier. As the LBDDS was dispersed in the channels of mesoporous silica at
317 amorphous state, **it** was separated and restricted inside huge amount of fine pores in the
318 silica, **thus the crystal growth of LBDDS or re-crystallization was greatly inhibited.** In
319 addition, the LBDDS adsorbed in the pore was avoided the contact with atmospheric oxygen,
320 which **would** be helpful to improve the durability against oxidative degradation [**33,34**].
321 The ratios of drug to mixed lipids and Compritol 888 ATO to Gelucire 50/13 had a
322 marked influence on drug release behaviors (**Fig.4, A and B**). With the decrease of the ratios
323 of drug to lipids, the release of tacrolimus from SLH remarkably decreased. When the ratio
324 of drug to lipids was 1:10, tacrolimus released slowly and completely at 24 h from the SLHs,
325 and higher or lower ratios resulted **in** fast release or uncompleted release even after 24 h
326 (**Fig.4, A**). The more content of Compritol 888 ATO in the mixed lipids, the slower the drug
327 release rate was observed (**Fig.4, B**).
328 Since the lipids used also played the role of retarding material to sustain drug release,
329 too high ratio of mixed lipids to tacrolimus used would lead to stronger inhibition effect
330 which limited the diffusion of tacrolimus outward and resulted in an uncompleted release,
331 while too low ratio was not enough to retard **drug diffusion into medium and thus resulted**
332 **in fast release.** As it is well known, the melting point and the HLB values of the lipids are
333 the most important properties when considering lipid excipients for extended drug release
334 formulation. The lipid with high melting point and low **HLB value**, such as Compritol 888
335 ATO, is more effective in retarding drug release [**35-37**]. As a hydrophobic lipid with a
336 melting point of 74ºC and a HLB value of 2, Compritol 888 ATO **may obviously** inhibit the
337 diffusion of release medium into LBDDS, and subsequently retard the drug release; On the
338 contrary, Gelucire 50/13 is a more hydrophilic lipid **with a melting point of 50ºC and a**

HLB value of 13 [**38,39**], which can dissolve rapidly as soon as they contact with the release medium and then enhances the dissolution of loaded drug.

The ratio of lipids to mesoporous silica was another important factor that greatly influenced drug release behavior from the SLHs as presented in Fig.4, C. As the results showed the co-loading of the lipid matrix and tacrolimus into mesoporous silica was effective in retarding drug release, and the lower ratio of lipids to mesoporous silica corresponded to slower drug release. When the ratio of lipids to silica was 1:1, the formulation showed a relatively fast drug release of about 90% **within** 2 h, while with the increase of silica, for example the ratios lower than 1:2, the drug release began to display the sustained release behavior. What's more, there was no obvious difference when the ratios were 1:3 and 1:4. Thus, the significance of the ratio of lipids to silica in designing optimal sustained-release composition was highlighted beyond doubt. This was because i**f the amount of the silica was not enough to adsorb all the lipid completely (eg. the ratio of lipid to silica higher than 1:2), the lipids together with the dissolved tacrolimus were adsorbed on the surface of the mesoporous silica particles which resulted in a fast release. According to the above results, formulation No.3 was selected as the optimum one for further studies.**

The *in vitro* drug release data of SLH **(formulation No.3)** was fitted very well to first-order kinetic model (see Table 2), which indicated a diffusion-controlled mechanism of finite dosed drug delivery device. The release of tacrolimus from SLH **was** a two-phases process, ie, (1) **diffusion** of tacrolimus through the mesoporous channels to the surface of silica carrier, and (2) **release** of tacrolimus to the medium. Phase 1 **was** obviously the rate-control step of complete drug release process. The possible drug release process was depicted and illustrated in Fig.5. For drug-loaded mesoporous silica, the organic solvent used was completely removed after drug loading, tacrolimus was adsorbed on the inner surface of the channels and presented at a highly **dispersing** state. The release medium **penetrated** into the mesoporous channels just after its contacting with the medium, and drug molecules **diffused** through the medium-filled channels, which led to a fast drug release. **While for the SLH, the situation was obviously different due to that the mesopores were filled with**

the mixed lipid that inhibited both the penetration of medium inward and the release/diffusion of tacrolimus outward. Although the water-soluble lipid (Gelucire 50/13) was readily dissolved into the release medium, the diffusion of tacrolimus through the mesoporous channels filled with the residual water-insoluble lipid matrix was still relatively slower (low diffusion coefficient) compared to that through the medium-filled channels like in the case of drug-loaded mesoporous silica.

3.3 Properties of the powder

3.3.1 Density

Powder densities, including true density, bulk density and tap density, of mesoporous silica, SLH, and LBDDS were listed in Table 3. **It could be seen that mesoporous silica had the most significant difference between the bulk density and the tap density to the true density,** followed by SLH and LBDDS powders.

3.3.2 Flowability

To the best of our knowledge, flow behavior of the powders is a major concern in handling and processing operation such as flow from hoppers and silos, transportation, mixing compression and packaging [40], and it is also important to decide the dose uniformity of drug preparations. The compressibility index and the closely related Hausner ratio have become the simple and popular methods to predict the flow properties of powder according to ***USP 35.*** The values of the Carr's index and Hausner ratio of mesoporous silica, SLH, and LBDDS were also determined and presented in Table 3. According to the specification of flowability in ***USP 35***, the LBBDS with a Hausner ratio of 1.09 and Carr's Index of 8.45, and the pure silica with a Hausner ratio of 1.25 and Carr's Index of 20.0 were classified as good-flowing and fair-flowing, respectively. While the SLH had an intermediate flowability with a Hausner ratio lower than 1.18 (1.16) and Carr's Index lower than 15 (13.75), which was also classified as good-flowing. The angle of repose measured by tilt

method gave a direct indication to the flowability of the powders tested (Fig. 6). We could draw the conclusion that the order of the flowability was LBDDS > SLH > pure mesoporous silica, which was in accordance to the results of Hausner ratio and Carr's Index.

3.3.3 Compressibility

The slope of compress curve of SLH calculated **by** Heckel equation was 0.16, which was bigger than that of mesoporous silica (0.10). According to the theory of Heckel equation, the slope reflects the degree of plastic deformation. The bigger the slope is, the better the compressibility is. **As shown in Fig.7 the** tablet obtained from SLH showed smooth surface and intact appearance, while the tablet obtained from mesoporous silica had relatively rough surface and capped just after withdrawal from the die. It could be directly perceived that SLH **had** better compactibility than mesoporous silica.

As a low density and rigid inorganic powder, mesoporous silica has poor flowability and compressibility, which could **hardly** be compressed into tablet at all. LBDDS was wax-like solid at room temperature, which could be **readily** crushed and grinded into fine powder. What's more, the LBDDS powder has a good flowability and apt to aggregate during storage. **In fact, Compritol 888 ATO has various properties, such as the lower shear stress, appropriate melting point, high specific surface area, amphiphilic and film forming tendency, and the use of lipid as lubricant initially in pharmaceutics could be traced back to the 1980s** [41,42]. As a low melting-point and wax-like powder, Compritol 888 ATO also **played** the role of binder in this study. The SLH powder was almost the same as mesoporous silica in appearance. However, the key differences between mesoporous silica and SLH powder were that SLH had a good flowability and compressibility which could be compressed into tablet easily. No wonder the good flowability and compressibility of SLH powder are mainly due to the good lubricity and plasticity of Compritol 888 ATO absorbed on the surface of the silica.

4. Conclusion

In this study, we provided a novel method to prepare a sustained release powder containing tacrolimus by employing mesoporous silica as the carrier to adsorb drug-loaded LBDDS. Using this method, the low melting-point, wax-like LBDDS could be transformed to a rigid solid powder with good flowability, compressibility and compactibility. **By the combination of lipids and mesoporous silica, we successfully obtained a kind of sustained-release powder, and** the stability of LBDDS under challenge condition could also be obviously improved. Such organic solvent-free technique supplied a novel, simple, low cost, ecologically friendly and easy to obtain at industrial scale method to prepare tacrolimus solid sustained-release **powder,** the **powder** we obtained had a better stability and **micromeritic properties**, which could be made into dry suspension, granules, capsules, and tablets according to the clinical requirements.

Declaration of interest

The authors report no declarations of interest.

Reference

[1] Cho JH, Kim Y, Kim D et al. Development of novel fast-dissolving tacrolimus solid dispersion-loaded prolonged release tablet. Eur J Pharm Sci.2013; 54: 1-7.

[2] Park YJ, Ryu DS, Li DX, et al. Physicochemical characterization of tacrolimus-loaded solid dispersion with sodium carboxylmethyl cellulose and sodium lauryl sulfate. Arch Pharm Res. 2009; 32(6): 893-898.

[3] Joe JH, Lee WM, Park YJ, et al. Effect of the solid-dispersion method on the solubility and crystalline property of tacrolimus. Int J Pharm, 2010; 395(1-2): 161-166.

[4] Watts AB, Cline AM, Saad AR et al. Characterization and pharmacokinetic analysis of

tacrolimus dispersion for nebulization in a lung transplanted rodent model. Int J Pharm. 2010; 384(1-2): 46-52.

[5] Shin SB, Cho HY, Kim DD et al. Preparation and evaluation of tacrolimus-loaded nanoparticles for lymphatic delivery. Eur J Pharm Biopharm. 2010; 74(2): 164-171.

[6] Lamprecht A, Yamamoto H, Takeuchi H, et al. A pH-sensitive microsphere system for the colon delivery of tacrolimus containing nanoparticles. J Control Release. 2005; 104(2): 337-346.

[7] Dutta S, Mezei M, Lee TD, et al. Liposomal tacrolimus and intestinal drug concentration. Transplant Proc. 1998; 30(6): 2651-2652.

[8] McAlister VC. Liposomal tacrolimus: drug migration within blood compartments. Transplant Proc.1998; 30(4): 1000-1001.

[9] Borhade V, Nair H, Hegde D. Design and evaluation of self-microemulsifying drug delivery system(SMEDDS) of tacrolimus. AAPS PharmSciTech. 2008; 9(1): 13-21.

[10] Christine E, Staatz, Susan E.T. Clinical Pharmacokinetics of Once-Daily Tacrolimus in Solid-Organ Transplant Patients. Clin Pharmacokinet. 2015; 54(10): 993-1025.

[11] Nakamura Y, Hama K, Katayama H, et al. Safety and Efficacy of Conversion from Twice-Daily Tacrolimus (Prograf) to Once-Daily Prolonged-Release Tacrolimus (Graceptor) in Stable Kidney Transplant Recipients. Transplantation Proceedings. 2012; 44: 124-127.

[12] Wang Y, Gan Y, Zhang X, et al. Novel gastroretentive sustained-release tablet of tacrolimus based on self-microemulsifying mixture: *in vitro* evaluation and *in vivo* bioavailability test. Acta Pharmacologica Sinica. 2011; 32: 1294-1302.

[13] Yuan T, Qin L, Wang Z, et al. Solid lipid dispersion of calcitriol with enhanced dissolution and stability. **Asian J Pharm Sci. 2013; 8: 39-47.**

[14] Rosiaux Y, Jannin V, Hughes S, et al. Solid lipid excipients-Matrix agents for sustained drug delivery. **J Control Release. 2014; 188: 18-30.**

[15] Strickley R.G. An Overview of Lipid Excipients currently available: Strenghts, Weaknesses and Opportunity Gaps: The Options for the Formulators. Bull.tech.Gattefossé. 2007; 100: 31-37.

[16] Humberstone AJ, Charman WN. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. Adv Drug Delivery Rev. 1997; 25(1): 103-128.

[17] Kossena GA, Boyd BJ, Porter CJH, et al. Separation and characterization of the colloidal phases produced on digestion of common formulation lipids and assessment of their impact on the apparent solubility of selected poorly water-soluble drugs. **J Pharm Sci. 2003; 92(3): 634-648.**

[18] Porter CJH, Trevaskis NL, Charman WN. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nat Rev Drug Discov. 2007; 6(3): 231-248.

[19] Tan A, Rao S, Prestidge CA. Transforming Lipid-Based Oral Drug Delivery System into Solid Dosage Forms: An Overview of Solid Carriers, Physicochemical Properties, and Biopharmaceutical Performance. Pharm Res. 2013; 30: 2993-3017.

[20] Dening TJ, Rao S, Thomas N, et al. Novel Nanostructured Solid Materials for Modulating Oral Drug Delivery from Solid-State Lipid-Based Drug Delivery System. The AAPS Journal. 2016; 18(1): 23-40.

[21] Chakraborty S, Shukla D, Mishra B, et al. Lipid-an emerging platform for oral delivery of drugs with poor bioavailability. Eur J Pharm Biopharm. 2009; 73(1): 1–15.

[22] Pouton CW. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. Eur J Pharm Sci. 2000; 11(S2): S93-S98.

[23] Vallet-Regi M, Balas F, Arcos D. Mesoporous materials for drug delivery. Angew Chem. Int. Ed. 2007; 46: 7548-7558.

[24] Vallet-Regi M., Ramila A., Real R.P., et al. A new property of MCM-41:drug delivery system. Chem. Mater.2001; 13: 308-311.

[25] Wang L, Che X, Xu H, et al. A novel strategy to design sustained-release poorly water-soluble drug mesoporous silica microparticles based on supercritical fluidtechnique. Int. J Pharm. 2013; 454:135-142.

[26] **Zhang H , Shahbazi MA, Mäkilä ME, et al.** Diatom silica microparticles for sustained release and permeation enhancement following oral delivery of prednisone and

mesalamine. Biomaterials. 2013; 34: 9210-9219.

[27]Kohno Y, Kato Y, Shibata M, et al. Fixation and stability enhancement of beta-carotene by organo-modified mesoporous silica.Microporous Mesoporous Mater. 2016; 220: 1-6.

[28]Kim KS, Park SJ. Effect of porous silica on sustained release behaviors of pH sensitive Pluronic F127/poly(acrylic acid) hydrogels containing tulobuterol. **Colloids Surf. B Biointerfaces. 2010; 80: 240-246.**

[29]Chakkumkal A, Arun K, Devinder S, et al. Influences of process and formulation parameters on powder flow properties and immunogenicity of spray dried polymer particles entrapping recombinant pneumococcal surface protein A. Int J Pharm. 2014; 466: 198-210.

[30]Benkovic M, TuŠek AJ, Lenart A, et al. Artifical neural network modelling of changes in physical and chemical properties of cocoa powder mixtures during agglomeration. **LWT-Food Sci Technol. 2015; 64: 140-148.**

[31] Wells J. I. Pharmaceutical Preformulation: The Physicochemical Properties of Drug Substances. Chichester, U.K. 1988.

[32]Xanthakis E, Ruud van Ommen JRV, Ahrné L, et al. Flowability characterization of nanopowders. Powder Technol. 2015; 286: 156-163.

[33]Brigo L, Scomparin E, Galuppo M, et al. Mesoporous silica sub-micron spheres as drug dissolution enhancers:Influence of drug and matrix chemistry on functionality and stability. Mater. Sci. Eng. 2016; C59: 585-593.

[34]Wang J, Wen H, Desai D. Lubrication in tablet formulations. Eur J Pharm Biopharm. 2010; 75(1): 1-15.

[35]Jannin V, Rosiaux Y, Doucet J, et al. Exploring the possible relationship between the drug release of Compritol®-containing tablets and its polymorph forms using micro X-ray diffraction. J Control Release. 2015; 197: 158-164.

[36]Roberts M, Vellucci D, Monstafa S, et al. Development and evaluation of sustained-release Compritol®888 ATO matrix mini-tablets. Drug Dev. Ind. Pharm. 2012; 38(9): 1068-1076.

[37]Gupta M.K, Goldman D, Bongner RH, et al. Enhanced drug dissolution and bulk

properties of solid dispersions granulated with a surface adsorbent. Pharm.Dev.Technol. 2001; 6(4): 563-572.

[38] Jannin V, Musakhanian J, Marchaud D. Approaches for the Development of Solid and Semi-solid Lipid-Based Formulations. Adv. Drug Deliv. Rev. 2008; 60(6): 734-746.

[39] Shen SC, Ng WK, Chia LSO, et al. Preparation and application of monodispersed mesoporous submicron carbon particles as a drug carrier. Powder Technol. 2014; 261: 241-249.

[40] Rao M, Ranpise A, Borate S, et al. Mechanistic Evaluation of the Effect of Sintering on Compritol®888 ATO Matrices. AAPS Pharm Sci Tech. 2009; 10(2): 355-360.

[41] Jannin V, Berard V, N'Diaye A, et al. Comparative study of the lubricant performance of Compritol 888 ATO either used by blending or by hot melt coating. Int J Pharm. 2003; 262(1-2): 39-45.

[42] Krause J, Thommes M, Breitkreutz J. Immediate release pellets with lipid binders obtained by solvent-free cold extrusion. Eur J Pharm Biopharm. 2009; 71(1): 138-144.

Figure and Table legends

Fig. 1. SEM images of mesoporous silica (A), drug-loaded SLH (B) and drug-loaded SLH after *in vitro* drug release test (C), and CLSM image of the coumarin loaded SLH (D, The insert showed an enlarged image).

Fig. 2. DSC curves (A) and XPRD patterns (B) of drug-loaded LBDDS (a), mesoporous silica (b), drug-loaded SLH (c) and **tacrolimus**(d).

Fig. 3. The *in vitro* release of raw tacrolimus, commercial capsules, drug-loaded mesoporous silica, drug-loaded LBDDS and drug-loaded SLH (n=3) (A), the *in vitro* release of drug-loaded LBDDS (B) and drug-loaded SLH (C) before and after being stored at 40ºC for 10 d (*n*=3).

Fig. 4. The *in vitro* release of tacrolimus from various formulations of drug loaded SLH (n=3). The effect of the ratios of tacrolimus to mixed lipids (Compritol 888 ATO : Gelucire 50/13=7 : 3 and mixed lipids : silica=1 : 3) on *in vitro* drug release (A), the

effect of the ratios of Compritol 888 ATO to Gelucire 50/13 (tacrolimus : mixed lipids=1 : 10 and mixed lipids : silica=1 : 3) on *in vitro* drug release (B) and the effect of the ratios of mixed lipids to mesoporous silica (Compritol 888 ATO : Gelucire 50/13=7 : 3 and tacrolimus : mixed lipids=1 : 10) on *in vitro* drug release (C) .

Fig. 5. The processes of drug loading and drug release from the drug-loaded SLH. Phase 1: Diffusion of tacrolimus through the mesoporous channels to the surface of silica carrier; Phase 2: Release of tacrolimus to the media.

Fig. 6. Flowability of LBDDS (A), mesoporous silica (B) and SLH (C) evaluated by tilting angles.

Fig. 7. The appearance of the tablets compressed from pure silica (A) and SLH (B), respectively.

Tables:

Table 1 Formulations of tacrolimus loaded LBDDS and SLH

No.	Ingredients, mg			
	Tacrolimus	Compritol888 ATO	Gelucire 50/13	Mesoporous Silica
1	20	140	60	
2	20	70	30	360
3	20	140	60	660
4	20	210	90	960
5	20	120	80	660
6	20	160	40	660
7	20	140	60	220
8	20	140	60	440

9	20	140	60	880
10	20			800

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586 Table 2 The release patterns of tacrolimus loaded SLH

Release pattern	Fitting equation	R^2
First order	Ln(1-Q)=0.1973t-0.1094	0.9939
Higuchi	$Q= 21.955t^{1/2}+7.4484$	0.8528
Korsmeyer-Peppas	$Q=5.5596t^{0.94}$	0.8715

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588 Table 3 Density and flow properties of mesoporous silica, SLH and LBDDS

	Bulk density mg/cm^3	Tap density mg/cm^3	Ture density mg/cm^3	Carr's index	Hausner ratio
Mesoporous silica	298	373	1340	20.00	1.25
LBDDS	441	482	1080	8.45	1.09
SLH	448	519	1480	13.75	1.16

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