Optimization and characterization of primaquine-loaded solid lipid nanoparticles (SLN) for liver schinonticide targeting by freeze drying

Abstract
The aim of this study was preparation of a liver schinonticide Primaquine phosphate (PQ) directly to the hepatocytes using solid lipid nanoparticles (SLN). The PQ-loaded solid lipid nanoparticles (PQ-SLNs) were prepared by a modified solvent emulsification evaporation method based on a water-in-oil-in-water (w/o/w) double emulsion and dried freeze drying (PQ-SLNFD) to obtain the nanoparticles. The mean particle size, zeta potential, drug loading, and encapsulation efficiency of the PQ-SLNFD were 236nm, +23 mV, 14%, and 75%, respectively. A spherical morphology of PQ-SLNFD was seen by scanning electron microscope that lacked traces of drug crystals. Differential scanning calorimeter thermograms demonstrated presence of drug in drug-loaded nanoparticles along with disappearance of decomposition exotherms, suggesting increased physical stability of drug in prepared formulations. The nanoformulated PQ was 20% more effective as compared with conventional oral dose when tested in Plasmodium berghei-infected Swiss albino mice. This study established an effective process of developing a nanomedicine delivery system for PQ.

Keywords: double emulsion, solid lipid nanoparticles, freeze dried

Introduction
One of the strategies employed to improve PQ is reformulation into nanoparticles. Nanoparticles have been investigated for many applications due to the enhanced material properties that result from reduction in particle dimensions. The most active area of research is in the pharmaceutical industry, where nanoparticles have the potential to provide drug-delivery vehicles. Some advantages of nanomaterials include maintaining drug therapeutic concentrations and prolonged circulation time at target sites; protection from premature degradation in the gastrointestinal tract; improved pharmacokinetics, solubility, bioavailability, and stability; reduced toxicity; reduced in dose and dose frequency; enhancement of patient compliance; and prevention, reduction, or delay of onset of resistance.

The aim of using nanocarriers as drug-delivery systems is to promote drug protection against extracellular degradation, to improve selectivity in relation to the target, and to reduce dose frequency as well as duration of the treatment via enhancing the pharmacokinetic profile of the drug. Nanoformulation of drugs has made an impact in therapies for diseases like cancer. For example, Doxil® (Alza Corp., Mountain View, CA, USA) is a nanomedicine formulation of the anthracycline doxorubicin that is currently in use for cancer treatment. The four first-line antituberculosis drugs rifampicin, isoniazid, ethambutol, and pyrazinamide have been nanoformulated in polymeric nanoparticles. In contrast, nanotechnology has not been widely applied to transform therapies for poverty-related diseases such as malaria. Nevertheless, albumin and gelatin nanoparticles of PQ have been synthesized and shown to reduce toxicity in mice. Increased life-span index related to PQ-loaded nanoparticles was demonstrated after administration of free PQ diphosphate, free poly (diethyl methylidenemalonate) (poly [DEMM]) nanoparticles, and PQ-loaded poly (DEMM) nanoparticles as a single intraperitoneal injection to P. berghei-infected mice. In yet another study in mice, PQ-loaded liposomes were shown to protect nontarget tissues such as lung, kidneys, heart, and brain from PQ accumulation, thereby reducing the toxicity of PQ.

The current study envisaged that reformulating PQ would enhance its efficacy and half-life, which may impact on its dosing regimen by enabling lower dosing and longer frequency. These will lead to reduced toxicity and better patient compliance. The strategy employed toward this was through the synthesis of PQ-loaded SLNs (PQ-SLNs). SLNs introduced in 1991 represent an alternative carrier system to traditional colloidal carriers such as emulsions, liposomes, and polymeric micro- and nanoparticles. SLNs are submicron colloidal carriers ranging from 50nm to 1,000nm. The system consists of spherical solid lipid particles in the nanometer ranges, which are dispersed in water or in aqueous surfactant solution. The solid core contains the drug dissolved or dispersed in the solid high melting fat matrix. The general ingredients include solid lipid(s), emulsifier(s), and water. A major advantage of SLNs is the fact that the lipid matrix is made from physiological lipids, which decreases the danger of acute and chronic toxicity. The solvent emulsification-evaporation method was selected for the formation of the nanoparticles. This method has emerged as a superior technique for preparing SLNs. A key step involves dispersions by precipitation in oil-in-water (o/w) emulsions. The lipophilic material is dissolved in a water-immiscible organic solvent that is emulsified in an aqueous phase. Upon evaporation of the solvent, nanoparticle dispersion is formed by precipitation of

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the lipid in the aqueous medium. Reproducibility of this method is reported to be high. The procedure used in forming the PQ-SLNs is outlined below.

The generation of SLN, the dispersion and control of the nanoparticles is a key stride. The attributes of the suspension determine the morphology of the particles and their properties. A few investigations have revealed the scattering and security of suspensions of nanosized SLN, for example, RAPAMUNE®, EMEND®, TriCor® and MEGACE® among others as further explained in Table 1.

### Materials and methods

#### Materials

All materials, reagents, chemicals, and PQ base utilized as a part of the study were provided by our partners at the Novartis, Basel, Switzerland. The stearic acid (SA), chitosan low-viscous, polyvinyl alcohol (PVA) of molecular weight 13,000-23,000 and partially hydrolyzed (87%-89%), D-lactose monohydrate, sulfanoyl, and ethyl acetate (EtOAc) were obtained from Sigma-Aldrich (Basel, Switzerland), and Pluronic® F127 Prill from BASF Corporation (Mount Olive, NJ, USA). All other synthetic items were commercially accessible and of analytical grade. In this test outline, stearic acid was the lattice; PVA and pluronic were surfactants stabilised the emulsion, and chitosan was a mucoadhesive expanding circulation time in the digestive system to enable the majority of the nanoparticles to be assimilated. Lactose improved particle size reduction, since it is a binder, while sulfanoyl was used as an antifoaming agent.

#### Preparation of SLNs via freeze drying

The nanoparticles loaded with PQ were prepared using a modified multiple emulsion solvent evaporation technique followed by freeze drying. A solution of 100mg PQ was dissolved in 2m of aqueous 2% PVA to form the aqueous phase containing the drug. The organic phase was made by dissolving 50mg of stearic acid in 10ml of EtOAC. The aqueous phase was frequently dispersed in the organic phase by means of a high speed homogenizer (silverson L4R; Silverson Machines limited, Buckinghamshire, UK), with a speed varying between 3,000 and 6,000rpm for 3 minutes. This water-in-oil (w/o) emulsion was transferred to a specific volume of water-in-oil-in-water (w/o/w) double emulsion obtained by a high speed homogenizer (silverson L4R; Silverson Machines limited, Buckinghamshire, UK), with a speed varying between 3,000 and 6,000rpm for 3 minutes. This water-in-oil (w/o) emulsion was transferred to a specific volume of aqueous 2% PVA (m=13000-23000), 5% of 0.2% % (w/v) chitosan low viscous, and 5 ml of 5% (w/v) D-lactose monohydrate. A drop of sulfanoyl was added as a stabiliser to the resultant water-in-oil-in-water (w/o/w) emulsion and the mixture was further emulsified by homogenisation at 8,000rpm. The water-in-oil-in-water double emulsion obtained was gently stirred overnight at room temperature to remove the organic solvent. Thereafter, drug-loaded SLNs were harvested by ultracentrifugation (37000g for 15 mins) followed by a series of wash steps with deionized water. The recovered pellets were dispersed in liquid Nitrogen before freeze drying (Heto DRYWINNER, Germany) at 0.05mBar for 24 hours.

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### Table 1 Current marketed pharmaceutical products utilizing nanocrystalline API

| Product       | Drug compound | Indication          | Company            | Product                                      |
|---------------|---------------|---------------------|--------------------|----------------------------------------------|
| RAPAMUNE®     | Sirolimus     | Immunosuppressant   | Wyeth              | Elan Drug Delivery Nanocrystals®             |
| EMEND®        | Aprepitant    | Antiemetic          | Merck              | Elan Drug Delivery Nanocrystals®             |
| TriCor®       | Fenofibrate   | Treatment of hypercholesterolemia | Abbott          | Elan Drug Delivery Nanocrystals®             |
| MEGACE®       | Megestrol acetate | Appetite stimulant | PAR Pharmaceutical | Elan Drug Delivery Nanocrystals®             |
| ES TriglideTM | Fenofibrate   | Treatment of hypercholesterolemia | First Horizon     | Pharmaceutical                              |
|               |               |                     | Skye Pharma IDD®-P technology |                                             |

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an aqueous 2% w/v PVA (mw=13000-23000), 5% of 0.2% (w/v) chitosan low-thick, and 5ml of 5% (w/v) D-lactose monohydrate. A drop of sulfanyl was added as a stabilizer to the resultant water-in-oil-in-water (w/o/w) emulsion and the blend was additionally emulsified by homogenisation at 8,000 rpm. The water-in-oil-in-water twofold emulsion got was delicately blended overnight at room temperature to expel the organic solvents. Drug loaded SLNs were collected by ultracentrifugation (37000g for 15 mins) followed by a progression of wash steps with deionized water. The recovered pellets were scattered in fluid Nitrogen before freeze drying (Heto DRYWINNER, Germany) at 0.05mBar for 24 hours.

**Particle characterisation**

**Determination of size and ζ-potential:** A Zetasizer Nanoseries (Malvern Instruments, Malvern, UK) was utilized to estimate particle size, poly dispersity index(PDI) and ζ-potential of all the two sorts of particles by dynamic light scattering (DLS) or photon correlation rule. All scattering-samples were diluted 1:500 in ultra-pure water, vortex and sonicated for determination of the, PDI and ζ-potential. Three replicates of each were measured at room temperature. Results are presented as mean ± standard deviation.

**Stability study and pH determination:** To guarantee stability of the formulations over some stretch of time, scatterings in water (0.5mg/ml) were checked for up to 2 months after processing of SLNwith respect to particle size, PDI, ζ-potential, and pH. For the pH deduction acalibrated potentiometer (Model AZ-8306: AZ Instruments Corp., Taichung City, Taiwan) was utilized, pH values were measured by the direct immersion of the electrode in the undiluted dispersion at 10% (w/v). The samples were stored in amber glass flasks at 25°C.

**Drug loading and encapsulation efficiency**

Drug content was broke analyzed after a modified form of the technique outlined by Fontana et al., EE% was resolved utilizing both the direct and indirect strategy. In the indirect strategy, 20mg of the synthesised nanoparticles were scattered in 10mL of water and vortexed in falcon tubes until completely dispersed. The resultant solution was then ultra-centrifuged at 15,000rpm for 20 minutes at 4°C. The supernatant was then taken for ultraviolet-visible (UV-VIS) investigation at wavelengths between 400 nm and 200 nm. For the direct technique, the precipitate was taken and disintegrated in a predetermined amount of EtOAc. Water was then added to the solution and left overnight. The aqueous stage was then isolated by means of aseparating funnel and examined for PQ concentration. The concentration of the drug was calculated by methods for a standard curve as separated from UV-VIS spectrometry examination by utilizing diverse known concentrations of the drug. The EE% and DL% were ascertained utilizing the equations underneath.11-24

\[
\text{EE\%} = \frac{\text{drug in precipitate}}{\text{total added drug}} \times 100 \quad (1)
\]

\[
\text{DL\%} = \frac{\text{drug in precipitate}}{\text{drug in precipitate} + \text{added excipients}} \times 100 \quad (2)
\]

Where “drug in precipitate”=total drug added -free drug after ultra-centrifugation (indirect method) and “added excipients”=lipids+surfactant mixtures+other ingredients used.

**Surface morphology**

Scanning electron microscopy was utilized to give an approach to specifically observe the morphological appearance of the nanoparticles.

The particles were first covered in gold to limit the impact of heat amid high-power amplification and were then run through a scanning electron microscope instrument (SU1510 model; Hitachi Ltd., Tokyo, Japan) where photos of the nanoparticles were taken. Fourier change infrared spectroscopy (FTIR) was likewise utilized to decide the useful gatherings exhibit on the surface of the nanoparticle.

**In vitro release experiment**

_In vitro_ release studies were performed utilizing the strategy outlined by Mühlén and Mehnert, with slight modification. To decide if the encapsulation procedure brings about a sustained release of drug was performed in phosphate buffered saline (0.1M PBS, pH 7.4). In this strategy, three replicates of each sample containing nanoparticles of a known concentration of PQ-stacked Freeze Drying (FD) nanoparticles strategies were set in a dialysis film and immersing dialysis film into in 20ml of PBS arrangement in a shaker at 37°C, at a predetermined interim 100µL of sample were analysed by HPLC utilizing the technique adapted from Mohan, with minor alterations. For positive controls, 10mg of free PQ drug was dialysed into 20ml of PBS sample. The investigation gave the amount of drug discharged from the nanoparticles with time.21-24

**In vitro antimalarial assays**

_In vitro_ cultivation of malaria parasite: The asexual intra-erythrocytic stage of _P. falciparum_ laboratory strain (3D7) was obtained from the Centre of Traditional Medicine and Drug Research (CTMDR), Kenya Medical Research Institute (KEMRI) The parasites were continuously cultured _in vitro_ according to the method of Hout (2006) with slight modifications.

Frozen parasite vials stored in liquid nitrogen were thawed in water bath at 37°C for 15 min, spun at 2000 rpm for 10 min and the supernatant discarded. The pellets were transferred into a sterile 15mL falcon tube and equal volume of thawing mix (3.5% NaCl in distilled water) was added, thoroughly mixed and spun at 2000rpm for 10 min. The supernatant was removed and 1mL of CPM (complete parasite medium) was added and spun again for 2000 rpm for 10 min. The washing step was repeated and supernatant discarded. The complete parasite medium (pH 7.3) used consisted of filter-sterilized RPMI 1640 solution supplemented with 0.5% AlbuMAX II, hypoxanthe (0.04%), buffered with 0.4% sodium bicarbonate (NaHCO3), 0.72% N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) and 0.005 mg/mL gentamicin. The pellets were suspended in 25mL culture flask (BD falcon) containing 5 mL of CPM and 200µL freshly prepared packed RBC (sickling negative, _O_ rehus positive) to have a haematocrit of 4%.

The culture was flushed with a mixture of gases (2% Oxygen, 5.5% Carbon dioxide and 92.5% Nitrogen) for 30s. The flask was closed and placed in an incubator (RS Biotech, Livingston, UK) set at 37°C. The culture medium was changed daily using fresh CPM. The parasitaemia was checked by preparing a thin smear on a microscope slide under sterile conditions in the laminar flow safety cabinet (Hitachi Clean Bench, Japan). The slides after drying were fixed in absolute methanol, stained with 10% Giemsa in phosphate buffer for 10 min. The slides were examined under the light microscope using×100 (oil immersion) objective lens for level of parasitaemia, growth stages and viability. The culture flask was re-incubated after gassing and adding appropriate volume of CPM. The number of infected RBCs and total number of RBCs in a field were counted and
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Prior to the study, one of the infected mice was kept and observed (Adzu and Haruna 2007). These infected mice were used for the study.

Animals:

In vivo antimalarial assay

In vitro drug sensitivity assay

In vitro antimalarial interaction assay

In vivo antimalarial interaction assay

Statistical analysis of data
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Particle size, size distribution and zeta potential: Particle characterisation

Results and discussion

Particle size, size distribution and zeta potential: The measure of nanoparticles was optimized against a benchmark of 250nm as it is accounted for smaller particles (normal size <250nm) undergo passive diffusion through hepatocytes. Different parameters were optimized to acquire an average particle size ranging between 230 and 250nm and an average polydispersity index of 0.1to 0.2. The expansion of shear rate by expanding the speed of homogenizer caused a lessening in particle size.

All particles demonstrated a positive zeta potential. The added drops of chitosan to give positive surface charge brought about microparticles. The sizes measured for the two unique sorts of particles went between ~250-260 nm (Table 1). FD gave rise to a generally larger size when contrasted to SD. This can be explained by the presence of the oil phase (U.M.A., 2009) prompting a core-and-shell structure for FD rather than a matrix system in the spray dried (SD). All nanoparticles had a PDI in the range of 23.48 and 15.50 affirming a homogeneous size appropriation. The ζ-Potential of one of the two sorts of NPs was positive, extending from 17.0 to 19.5mV. This positive potential is in all likelihood caused by the polymeric divider made by the lipids. The strength and stability of the particles is because of steric obstruction of the surfactant between the two phases counteracting mixture preventing coalescence (Table 3).

Stability study

Drug loading and encapsulation efficiency

Scanning electron microscopy (SEM) analysis was feasible for all two types of particles (Figure 1). FD the imaging was more difficult, most likely due to the presence of the organogel. Both methods confirmed the expected morphology. All particles were spherical in shape, revealed a smooth surface, and a homogeneous size distribution (Figure 2).

The FTIR chromatogram in Figure 2 shows peaks of the drug formulations. The level of drug enclosure in the nanoparticles or absence in the case of the drug-free nanoparticles could be deduced from the chromatograms. Of particular interest was the NH bending at 1,614cm⁻¹ and aromatic C=C stretching at around 1,532cm⁻¹ and 1,467cm⁻¹. As seen in Figure 4, free drug had more pronounced peaks in those particular absorbance regions, but in the case of the nanoparticles with 5mL of lactose, the peaks appeared reduced. When 15mL of lactose was used as a structural binder, the peaks reduced much further, indicating successful encapsulation in the solid lipid
matrix as has been similarly observed in other studies.

**DTA**

DTA analysis gave the degradation points of the tested samples shown in Figure 3. The first depression in the curves was a measure of moisture content. From TGA analysis, sample moisture content was found to be 4%-5%. A moisture content of less than 5% is acceptable in the pharmaceutical.

**In vitro release studies**

To determine whether the encapsulation process results in a sustained release of drug, the dialysis dialysis membrane method was applied. The release profiles of the SLNs in suspension versus free drug in a PBS solution as a control is depicted in Figure 4. A noteworthy issue amid the work with lipid nano pellets was the burst discharge seen with these systems. At the point when not washed properly, PQ-SLNs could demonstrate a burst discharge due to unencapsulated drug. An extended drug discharge has been acquired when contemplating the consolidation of prednisolone. As can obviously be found in Figure 4, no significant differences were observed between the release profiles of the two nano carriers (FD(p>0.05)). However, as was anticipated, all the two PQ-loaded particles showed an sustained discharge of the drug, as following 24 hours just around half of the typified drug was discharged for each of the three carriers. Indeed, even toward the finish of a 72 h period, the mean concentration of discharged drug still stayed beneath 80%. Free drug, conversely, showed a quick discharge profile with ~80% of PQ as of now being distinguished after just 5h.

**In vivo antimalarial assay**

Determination of ED$_{50}$ of Nanoformulated primaquine in $P$. berghei infected mice. Free Primaquine, Nanoformulated primaquine and standard antimalarial chloroquine produced a dose dependent reduction in parasitaemia levels with similar reduction as in the standard antimalarial chloroquine -treated group (positive control). The ED$_{50}$ of Nanoformulated primaquine on the 5th and 6th days of treatment was calculated to be 10.45±0.5mg/kg and 45mg/kg, respectively.

Suppressive efficacy against the malaria parasite was evaluated by comparing the percentage reduction of parasitemia (chemosuppression) between the treated and untreated groups. Results are shown in Table 5. Average parasitemia in both nanoformulated and free PQ drug were significantly lower ($P<0.05$) than that of the untreated group for both test concentrations. There was no significant difference in parasitemia levels and survival time between the unloaded nanoparticles and the untreated control group, indicating that the empty nanoparticles and the excipients therein did not exert any antimalarial activity. When mice were treated with PQ-SLNs at a dose of 2mg/kg/day, chemosuppression of 93.5% was observed. In comparison, only 71.9% chemosuppression was observed when mice were treated with free PQ at a similar dose. This indicated that nanoformulation of PQ increased its efficacy by more than 20%. The mean survival time of mice treated with the nanoformulated PQ was similarly enhanced when compared with the group of mice that received the conventional dose of PQ. In a previous study, it was observed that formulating PQ into the nanoemulsion shows effective antimalarial activity against $P$. berghei infection in Swiss albino mice at 25% lower dose level compared with the conventional oral dose.

**Combination antimalarial assay**

Table 5 shows the antimalarial activity, thus the mean percentage reduction in parasitaemia produced by the drugs alone or in nanoformulated form, compared to the control, on days 1 to 6. The Nanoformulated primaquine (ED$_{50}$=40mg/kg) and Nanoformulated primaquine (ED$_{50}$=6mg/kg) produced a significant reduction in parasitaemia from days 1 and 6. The Nanoformulated primaquine at all dose levels produced high percentage suppression in the first three days compared to free primaquine only. The lowest dose ratio combination (1/8:1/8) showed high parasite levels on days 5 and 6 compared to the negative control group.

**In vivo synergistic interaction of nanoformulated primaquine and free primaquine**

The theoretical ED$_{50}$ of Free Primaquine and Nanoformulated primaquine combination was 8.3±0.35mg/kg. The experimental ED$_{50}$ (Zexp) of the mixture was 1.03±0.03mg/kg. The degree of interaction calculated as the interaction index was 0.14 (Table 5).

Table 2 Combination ratio of nanoformulated primaquine to standard antimalarial drugs (PQ)

| Combination solution | Ratio of nanoformulated primaquine to PQ | Volume (μL) |
|----------------------|-----------------------------------------|-------------|
|                      | Nanoformulated PQ Free PQ Nanoformulated PQ Free PQ |
| 1                    | 5            | 0           | 10          | 0          |
| 2                    | 4            | 1           | 8           | 2          |
| 3                    | 3            | 2           | 6           | 4          |
| 4                    | 2            | 3           | 4           | 6          |
| 5                    | 1            | 4           | 2           | 8          |
| 6                    | 0            | 5           | 0           | 10         |
Table 3 Size distribution, PDI values, and ζ-potential of nanoparticles (NP) using dynamic light scattering (DLS) analysis; mean ± standard deviation

| Type of particle | Size (nm)     | PDI          | ζ-potential (mV) | PQ DL(%)  | Encapsulation efficiency (%) |
|------------------|---------------|--------------|------------------|-----------|-----------------------------|
| FD               | 267.6±3.7     | 0.13±0.02    | 17.17±1.11       | 23.48±0.02| 78.46±1.0                   |
| FD(placebo)      | 228±2         | 0.17±0.02    | 13.59±0.73       | -         | -                           |

Table 4 Physiochemical characteristics of Freeze Dried (FD and Spray Dried (SD) tracked for 2 months; mean±SD (size measured using DLS analysis)

| Type of particle | Month | Size (nm)     | PDI          | ζ-Pot (mV) | pH          |
|------------------|-------|---------------|--------------|------------|-------------|
| FD               | 0     | 295±4         | 0.08±0.02    | 17.10±1.93 | 5.99±0.23   |
|                  | 1     | 218±3         | 0.16±0.02    | 16.30±1.71 | 5.92±0.19   |
|                  | 2     | 208±4         | 0.13±0.03    | 15.63±0.65 | 5.42±0.12   |

Table 5 Percentage suppression of P. berghei infected mice given combination treatment

| Drugs             | Day 1    | Day 2    | Day 3    | Day 4    | Day 5    | Day 6    |
|-------------------|----------|----------|----------|----------|----------|----------|
| Nanoformulated PQ | 40.74±10.4 | 88.72±3.3 | 97.09±2.1 | 88.60±5.7 | 89.30±6.6 | 99.44±3.3 |
| Free primaquine   | 40.54±11.1 | 68.62±2.2 | 86.91±2.4 | 87.16±5.3 | 89.30±6.6 | 90.31±7.7 |
| ED50(1 :1)        | 73.14±9.4 | 95.72±9.3 | 93.09±1.1 | 68.60±5.7 | 79.34±6.3 | 89.54±3.1 |
| ED50/2(1/2 :1/2)  | 80.56±3.1 | 78.62±5.2 | 87.91±2.4 | 84.06±2.3 | 89.90±6.6 | 91.34±5.7 |
| ED50/8(1/8 :1/8)  | 86.74±1.46| 85.72±3.3 | 77.09±2.1 | 0.60±15.7 | -29.30±9.6 | -9.04±10.3 |
| Zadd(mg/kg)       | 8.3±0.35            |            |           |           |           |           |
| Zexp(mg/kg)       | 1.03±0.03           |            |           |           |           |           |
| Interaction       | 0.14                |            |           |           |           |           |

Figure 1 Scanning electron microscopy images of drug-loaded solid lipid nanoparticles at ×20,000.

Figure 2 Fourier transform infrared spectroscopy graphs for primaquine nanoparticle formulations. First from top (A) is formulation with 5 ml of lactose, second (B) has no drug, third (C) has 15ml of lactose, and the bottom one (D) is free drug.

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Conflicts of interest

The authors report no conflicts of interest in this work.

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Figure 3  DTA cooling scans and heating scans. Notes: empty nanoparticles refer to solid lipid nano-particles that do not contain drug.

Figure 4  In-vitro release study of PQ loaded SLN in PBS pH 7.4, in 37 C water baths. PQ Drug Crystals were used as con-trol. Data are expressed as mean±SD (n=3). Lines drawn represent non-linear regression fit of the data.
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