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ORIGINAL ARTICLE

In vitro and in vivo evaluation of self-microemulsifying drug delivery system of buparvaquone

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Abstract

Aim: The aim of this study was to prepare a lipid-based self-microemulsifying drug delivery system (SMEDDS) to increase the solubility and oral bioavailability of a poorly water-soluble compound, buparvaquone (BPQ). *Methods*: The solubility of BPQ was determined in various vehicles, and pseudo-ternary phase diagrams were constructed to determine the microemulsion region. A series of formulations with different compositions were selected in the microemulsion region for assessment of self-emulsification time and droplet size. The optimized SMEDDS formulation was used for in vitro dissolution and pharmacokinetic studies in rabbits. *Results*: The optimum formulation of SMEDDS consisted of Capryol 90 (9.82%), Cremophor EL (70.72%), Labrasol (17.68%), and BPQ (1.78%). Emulsification time and the mean droplet size were found to be 1 minute and 18.0 ± 0.25 nm, respectively, for the optimum formulation. The cumulative percentage of drug released in 90 minutes was 100% in both SGF and SIF. The calculated absolute oral bioavailability for BPQ was found to be 40.10%. Conclusions: The optimum SMEDDS formulation was increased the rate and extent of absorption of BPQ. The formulation is suitable for oral administration of BPQ. It would be useful to conduct efficacy studies of BPQ in diseased animal models and subsequently for toxicokinetics studies.

Key words: *Bioavailability; buparvaquone; HPLC; pharmacokinetics; self-microemulsifying drug delivery system*

Introduction

As part of a program to find new drugs for the treatment of leishmaniasis, a series of hydroxynaphthoquinones were tested against *Leishmania donovani* in vitro and in vivo. In vitro experiments showed buparvaquone (BPQ) to be 100-fold more active against *L. donovani* amastigotes than other hydroxynaphthoquinones with ED_{50} value of 0.005 μM[1]. BPQ, which is chemically known as 4-hydroxy-3-[(4-tert-butylcyclohexyl) methyl] naphthalene-1,2-dione (Figure 1), has very poor aqueous solubility (<300 ng/mL) with high lipophilicity (Log D–7.02 at pH 3.0). Because of these properties, low in vivo activity was observed for BPQ against leishmaniasis[2]. These findings led to the synthesis of a number of water-soluble phosphate prodrugs such as BPQ-3-phosphate and 3-phosphonooxymethyl-BPQ whose aqueous solubility is more than 3.5 mg/mL over the pH range of 3.0–7.4. These prodrugs are sufficiently stable in the gastrointestinal tract before their absorption and are rapidly hydrolyzed ($t_{1/2}$ = 1.2 and 3.8 minutes) to the parent compound in the presence of alkaline phosphatases[3].

A few formulation approaches for BPQ have been reported but none of them gave information on the oral pharmacokinetics and bioavailability of BPQ. Nebulized nanosuspension has been used for the pulmonary drug delivery of BPQ for the treatment of *Pneumocystis carinii pneumonia* lung infection[4]. A formulation of BPQ nanosuspension in mucoadhesive hydrogels was found to be suitable for oral administration in the treatment of *Cryptosporidium parvum*, a protozoan parasite that persists in the entire gastrointestinal tract[5].

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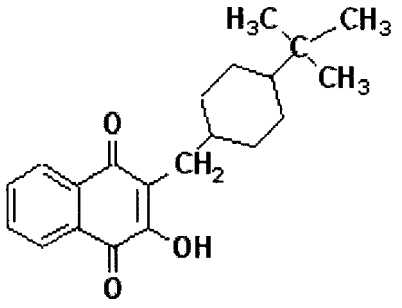

Figure 1. Chemical structure of buparvaquone (BPQ).

The in vivo antileishmanial activity of BPQ was determined with oral suspensions and cyclodextrin complexation. This study indicated that BPQ significantly decreased the liver parasite burden and confirmed its potent activity against leishmaniasis[6].

The choice of formulation is of critical importance for obtaining a successful oral formulation for BPQ. Several formulation approaches have been developed and reported in the literature to improve the bioavailability of poorly water-soluble drugs. The approaches include reduction of drug droplet size, cyclodextrin complexation, nanoparticles, solid dispersions, and lipid-based formulations[7,8].

In recent years, much attention has been focused on lipid-based formulation approaches, particularly on self-emulsifying drug delivery systems/self-microemulsifying drug delivery systems (SEDDS/SMEDDS), which are well known for their potential as an alternative strategy for delivery of hydrophobic drugs. SEDDS/SMEDDS promote drug solubilization and drug release at the absorption sites, which improves the oral bioavailability of hydrophobic drugs. Therefore, this method has been used for increasing the bioavailability of poorly water-soluble drugs such as fenofibrate, itracanazole, griseofulvin, and danazol[9,10]. The basic principle in SEDDS/SMEDDS is its ability to form an oil-in-water (O/W) emulsion or microemulsion under gentle agitation provided by the digestive motility of the stomach and intestine. The droplet size of SEDDS and SMEDDS is 100–300 nm and below 50 nm, respectively[7]. The spontaneous formation of an emulsion upon reaching the gastrointestinal tract advantageously presents the drug in a dissolved form and the small droplet size provides a large surface area for drug absorption, thereby enhancing the bioavailability[11]. The clinical usefulness of SMEDDS is evident from the commercially available formulations such as cyclopsorin A (Neoral Sandimmun) and three HIV protease inhibitors, ritonavir (Norvir®), saquinavir (Fortovase®), and amprenavir (Agenerase®)[12,10]. In view of this, SMEDDS formulation approach was selected for the preparation of an oral formulation of BPQ.

The aim of this study was to develop an optimum SMEDDS oral formulation for BPQ and to assess its absolute bioavailability in rabbits.

Materials and methods

Materials

BPQ was obtained as a gift sample from GlaxoSmithKline (Middlesex, UK). Acetonitrile and methanol of high performance liquid chromatography (HPLC) grade were obtained from J.T. Baker (Phillipsburg, KS, USA). Glacial acetic acid was purchased from Merck (Darmstadt, Germany). Sodium deoxy cholate, Brij 96 (polyethylene glycol monooleyl ether), vitamin E-acetate (tocopheryl acetate), oleic acid (monounsaturated omega-9 fatty acid), and linoleic acid were purchased from Sigma Aldrich (Steinheim, Germany). Capryol 90 (propylene glycol monocaprylate 90%), Labrasol (caprylocaproyl polyoxylglycerides), Peceol (glyceryl monooleate 40 EP), Labrafil M 1944 CS (oleoyl polyoxylglycerides), Labrafac PG (propylene glycol dicaprylocaprate), Masine 35-1 (glyceryl monolinoleate), and Trascutol P (monoethyl ether of diethylene glycol) were kindly provided as gift samples by Gattefosse (Gennevilliers, France). Accnon MC8 (PEG-8 caprylic/capric glycerides) was a gift sample from Abitec Corporation (Columbus, OH, USA). Pluronic P85 and Cremophor EL (polyoxyl 35 castor oil) were obtained from BASF (Ludwigshafen, Germany). Tween 20 (polysorbate 20), Tween 40 (polyoxyethylene-sorbitan monopalmitate), and Tween 80 (sorbitan monooleate) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium lauryl sulfate was purchased from Parchem trading Ltd. (New York, NY, USA). Deionized water was used throughout the experiment (Pure Lab UHQ ELGA, Buckinghamshire, UK).

Determination of BPQ solubility

The solubility of BPQ in various oils, surfactants, and cosurfactants was determined by adding excess amount of BPQ to 2.0 mL of each selected vehicle in a glass vial wrapped with aluminum foil. The mixture was vortexed and kept in a water bath at 37°C for 48 hours to reach equilibrium. The samples were centrifuged at 704 × *g* for 5 minutes and excess insoluble BPQ was discarded by filtration using a syringe filter (0.45 μm PTFE). The concentration of BPQ was quantified by a validated RP–HPLC–UV method.

Pseudo-ternary phase diagram study

The pseudo-ternary phase diagrams were constructed with oil, surfactant/cosurfactant, and water using water titration method at room temperature. The procedure

consisted of preparing four solutions containing surfactant to cosurfactant in the following ratios by weight: 1:1, 2:1, 3:1, and 4:1. Each of these solutions was then used for preparing a mixture containing oil and combined surfactant and cosurfactant solution in the following ratios by weight: 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. Water (5%, w/w) was added to each of the mixtures and vortexed for 5 minutes. They were then placed in a water bath at 37°C for 24 hours with gentle shaking. The mixtures were then observed against a dark background after illuminating the samples with white light. Turbidity of the sample would indicate formation of a coarse emulsion, whereas a clear isotropic solution would indicate the formation of a microemulsion. To such isotropic solutions a further 5% (w/w) water was added and vortexed for 5 minutes followed by equilibration at 37°C for 24 hours with gentle shaking. This procedure was continued until turbidity was observed in the sample. The resulting phase diagram permits identifying the coarse emulsion and microemulsion regions.

Preparation and determination of maximum drug loading of BPQ in selected SMEDDS formulations

A series of six SMEDDS were prepared from the microemulsion region in the pseudo-ternary phase diagram constructed using surfactant to cosurfactant ratio by weight of 4:1. The compositions of the SMEDDS formulations selected from the microemulsifying region covered the different areas of the region and are represented in Table 2. To determine maximum drug loading of BPQ, excess amount of BPQ was added to the glass vial containing the weighed amount of surfactant in the selected formulation. Oil and cosurfactant were then accurately weighed and added to the surfactant solution of BPQ. The glass vials are protected from light by wrapping with aluminum foil, and mixtures were then equilibrated in a water bath at 37°C for 48 hours with gentle shaking. The formulations were analyzed to determine the maximum drug load for each formulation using a validated RP–HPLC–UV method.

Droplet size analysis

About 100 µL of each formulation (F1 to F6) was diluted with 10 mL of deionized water and gently mixed by vortexing. The droplet size of the resultant solution was determined after 1 hour using a laser diffraction sizer at 90° (Zetasizer 3000; Malvern Instruments Ltd., Malvern, UK).

Emulsification time

The emulsification time is used for the determination of the efficacy of emulsification and is expressed as the time required for a SMEDDS formulation to form a complete homogeneous mixture when subjected to aqueous dilution under gentle agitation. The experiment was carried out with a dissolution apparatus (Disteck Premier 5100, North Brunswick, NJ, USA) consisting of 500 mL of simulated gastric fluid (SGF) without enzyme (pH 1.2) at 37°C and paddle speed of 50 rpm. Nine milligrams of the formulation was delivered via a syringe pump (Green stream® sy-P ARGUS 600; Argus Codan, Heimberg, Switzerland) 1 cm below the surface of the SGF medium at a flow rate of 10 mL/min. Samples were withdrawn at 1, 1.5, 2, 3, 5, and 10 minutes, respectively. The samples were filtered through 0.45-µm PTFE syringe filters and analyzed in triplicate by a validated RP–HPLC–UV method.

In vitro dissolution test

The United States Pharmacopoeia paddle method was used for the in vitro dissolution studies. SGF (pH 1.2) and simulated intestinal fluid (pH 6.8) without enzymes were used as the dissolution media. SMEDDS containing 9.0 mg of BPQ was filled into hard gelatin capsules and introduced into 500 mL of dissolution medium. SMEDDS was agitated at 50 rpm at 37°C. One-milliliter sample was withdrawn at predetermined time intervals of 15, 20, 25, 30, 40, 50, 60, 75, and 90 minutes. The samples were filtered through 0.45-µm PTFE syringe filters and the drug concentration was determined using a validated RP–HPLC–UV method.

HPLC analysis of BPQ

The RP–HPLC–UV method was developed and validated for the determination of BPQ in solubility, emulsification time, and dissolution study samples. Chromatographic separations were obtained using a C-4 stainless steel column, 250 mm × 4.6 mm ID, 5 µm particle size (Thermo Hypersil-Keystone, Bellefonte, PA, USA), which was maintained at 35°C. The analytical wavelength was set at 251 nm and samples of 50 µL were injected into the HPLC system. The mobile phase consisted of 1% glacial acetic acid in water, acetonitrile, and methanol in the ratio of 30:60:10 (v/v/v). The mobile phase was filtered through a 0.45-µm filter (Sartorius, Göttingen, Germany) and degassed for 10 minutes by sonication. The flow rate was set at 1.0 mL/min. The BPQ calibration curves were linear (correlation coefficient ≥0.9997) in the selected range (0.1–2.0 µg/mL). The intraday accuracy and precision were ≤4.75% and ≤0.97%, whereas the interday accuracy and precision were ≤4.52% and ≤0.92% for BPQ, respectively.

Bioavailability study

In our previous study, intravenous pharmacokinetic study of BPQ was conducted in three healthy white New Zealand male rabbits[13]. BPQ was prepared in Lipofundin® emulsion and 1.0 mg/kg dose was given by intravenous route. The samples were collected through marginal ear veins at 0 (before dosing), 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 24, 36, and 48 hours after dosing, respectively.

The oral pharmacokinetic study was carried out using the optimized SMEDDS formulation of BPQ (F2). Three healthy white New Zealand male rabbits weighing between 2.9 and 3.2 kg were used in the study. The study protocol was approved by the Animal Ethics Committee, Universiti Sains Malaysia, Penang. The rabbits were fasted for 24 hours (but had free access to water) prior to the administration of the oral dose of BPQ. Three rabbits received the optimized SMEDDS oral formulation F2 containing 3 mg/kg BPQ. The rabbit was held in an upright position; the SMEDDS formulation was administered using a 5-mL syringe with the help of mouth holder and an oral gavage tube. Food was supplied to the rabbits after 4 hours of the dose administration. Blood samples (1.0 mL) were collected through marginal ear veins into Vacutainer™ tubes (Becton Dickinson, Franklin Lakes, NJ, USA) containing sodium heparin as anticoagulant. Blood was collected at predetermined time intervals of 0 (before dosing), 0.5, 1, 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, and 60 hours after dosing. The blood samples were immediately centrifuged at 1,252 × *g* for 15 minutes and the supernatant was then transferred into Vacutainer™ tubes without anticoagulant and kept at −70°C until further analysis.

Analysis of BPQ in rabbit plasma

The pharmacokinetic study samples were analyzed with our previously reported RP–HPLC–UV method[13]. Chromatographic separations were obtained using a Gemini C18 stainless steel column, 150 mm × 4.6 mm, ID, 5 μm particle size (Phenomenex Gemini, Torrance, CA, USA), which was maintained at 45°C. The analytical wavelength was set at 251 nm and a sample of 40 μL was injected into the RP–HPLC–UV system. The mobile phase was ammonium acetate (0.02 M; pH 3.0 adjusted with glacial acetic acid) and acetonitrile in the ratio of 18:82 (v/v) at a flow rate of 1.1 mL/min. About 50 μL of 12 μg/mL internal standard (lovastatin) was added into 250 μL of study sample and vortexed for 20 seconds. Acetonitrile (500 μL) was added to denature the plasma proteins and the solution was centrifuged at 11,269 × *g* for 15 minutes. The liquid phase was pipetted out into microcentrifuge tubes and 0.5 mL of 50 mM KH_2PO_4 was added. The SPE extraction was carried out using Oasis® HLB cartridges 1 cc/30 mg (Waters, Milford, MA, USA). The cartridge was conditioned with 2 mL of methanol followed by 2 mL of 1% acetic acid in deionized water prior to sample loading. The extraction cartridge was then washed with 2 mL of 10% methanol in 1% acetic acid in deionized water followed by 5% acetonitrile in 1% acetic acid in deionized water. BPQ and lovastatin were eluted with two consecutive aliquots of 0.5 mL of 2% acetic acid in acetonitrile. The eluent was evaporated to dryness at 40°C under a gentle stream of nitrogen gas and reconstituted in 150 μL of acetonitrile/20 mM ammonium acetate pH 3.0 (90:10, v/v).

Results and discussion

Solubility studies

Drug loading per formulation is an important factor in the development of a successful SEDDS/SMEDDS. The drug loading into a SEDDS/SMEDDS mainly depends on the solubility of the drug in the excipients used in the formulation. In vivo the SMEDDS formulation should avoid precipitation of the drug on dilution in the gut lumen. If excipients with high drug solubility are chosen to prepare a SMEDDS, then the amount of formulation to be administered will be small. It is easy to administer a small volume in an encapsulated form for accuracy of dosing.

Self-emulsifying formulations generally consist of the drug, oil, surfactant, and cosurfactant. The solubility of BPQ in various vehicles is shown in Table 1. It is clear from the table that the highest solubility of BPQ is in the oil, Capryol 90, and in the surfactants—Cremophor EL and Labrasol. Generally hydrophilic surfactants with an HLB value of greater than 10 are much better in providing fine, uniform emulsion droplets when SEDDS/SMEDDS come in contact with gastric fluids. The

Table 1. BPQ solubility at 37°C in various vehicles. Mean ± SD, $n = 3$.

Vehicle	Solubility of BPQ (mg/mL)	Vehicle	Solubility of BPQ (mg/mL)
Accnon MC8	14.11 ± 0.61	Palm oil	2.97 ± 0.34
Brij 96	12.40 ± 0.59	Peceol	9.02 ± 0.29
Capryol 90	15.58 ± 0.57	Pluronic P85	0.05 ± 0.01
Cremophor EL	15.70 ± 0.52	Propylene glycol	0.58 ± 0.12
Labrasol	17.00 ± 0.28	Sodium deoxy cholate	0.07 ± 0.01
Linoleic acid	7.03 ± 0.37	Sodium lauryl sulfate	0.02 ± 0.09
Labrafil	12.39 ± 0.35	Trascutol P	1.77 ± 0.30
Labrafac PG	13.61 ± 0.66	Tween 20	12.92 ± 0.59
Masine 35	8.48 ± 0.48	Tween 40	8.10 ± 0.27
Oleic acid	6.45 ± 0.71	Tween 80	13.86 ± 0.45
Olive oil	3.47 ± 0.35	Vitamin E-acetate	1.74 ± 0.18

surfactants, Cremophor EL, and Labrasol both have an HLB value of 14[14,15]. So, the inclusion of these surfactants will ensure a uniform and smaller droplet size of the dispersed phase (oil) containing the drug moiety. Furthermore these surfactants are nonionic, which are more compatible with biological systems than ionic surfactants. These surfactants are less affected by pH and changes in ionic strength in the gastrointestinal tract. Previous investigators have also reported that a combination of surfactants yielded a smaller droplet size[16].

In this study, Cremophor EL and Labrasol were selected as surfactant and cosurfactant, respectively. The choice was based on the observation that a coarse emulsion is formed easily on addition of small amounts of water when the ratio by weight of Labrasol to Cremophor EL was 4:1 whereas a ratio by weight of Cremophor EL to Labrasol of 4:1 yielded a microemulsion on addition of small amounts of water.

Pseudo-ternary phase diagram

The selection of oil and surfactant/cosurfactant and mixing ratio of oil to surfactant to cosurfactant plays an important role in the formation of the microemulsion. Pseudo-ternary phase diagrams were constructed to identify the microemulsion regions for optimizing the concentrations of oil, surfactant, and cosurfactant. Figure 2 shows the pseudo-ternary phase diagrams of the combination of Cremophor EL and Labrasol in the ratios by weight of 1:1, 2:1, 3:1, and 4:1. Cremophor EL and Labrasol in the ratio 4:1 was selected for optimization as it yielded the largest self-emulsifying region (Figure 2). However, it was observed that increasing the surfactant to cosurfactant ratio to more than 4:1 resulted in loss of flowability.

When BPQ was incorporated into the preparations and titrated with water, there was no change in the area of the microemulsion region in all four ratios. When the concentration of surfactant/cosurfactant was increased, there was an increase in water uptake to form a coarse emulsion. All four pseudo-ternary phase diagrams showed that emulsion formation is only possible when the surfactant to cosurfactant concentration was greater than 10%.

Preparation of SMEDDS

From the pseudo-ternary phase diagrams, six formulations were selected from the microemulsion region of 4:1 of surfactant to cosurfactant ratio. These formulations were loaded with excess amount of BPQ and allowed to reach equilibrium in a reciprocating shaker bath for 48 hours. The formulations were analyzed by RP–HPLC–UV to determine the maximum drug load for each formulation and the results are shown in Table 2. There was no significant difference in the maximum amount of drug load in the formulation with increase in surfactant/cosurfactant concentration.

SMEDDS formulations were prepared with maximum drug loading as there is no available information in literature regarding the oral therapeutic dose for BPQ against leishmaniasis. High drug loading formulation will be useful for conducting the efficacy of BPQ in diseased animal models and subsequently for toxicokinetics studies as only a small sample volume is involved in dosing.

Droplet size analysis

The mean droplet size of the formulations selected from the microemulsion region of surfactant to cosurfactant ratio of 4:1 is given in Table 2. All the formulations selected from the microemulsion region of pseudo-ternary phase diagram of surfactant to cosurfactant ratio of 4:1 yielded a mean droplet size ranging between 13.1 and 280.6 nm. It is clear from Table 3 that there was an increase in mean droplet size in the formulations with a 4:1 ratio of surfactant to cosurfactant, when the Cremophor EL was ≤57.98%. When Cremophor EL was used alone the preparation becomes too viscous to be encapsulated; addition of Labrasol decreases the viscosity of the preparation.

Previous investigators have also observed that in various self-emulsifying systems an increase in the surfactant concentration causes a decrease in droplet size[17,18]. The decrease in size has been attributed to availability of more surfactant to stabilize the oil/water interface. The decrease in droplet size also indicates formation of a closed packed film of surfactant at the oil and water interface, thereby stabilizing the dispersed phase particle[19].

Formulations F4, F5, and F6 have a particle size more than 50 nm; hence these formulations were not further evaluated. Formulations F1, F2, and F3 were subjected to stability studies in SGF and SIF for 2 and 8 hours, respectively, so as to mimic physiological conditions as the drug stays in stomach for 2 hours and for 6–8 hours in the intestinal tract. All the formulations were dissolved in simulated gastric fluids and intestinal fluid and the samples were checked for increase in mean droplet size.

The initial mean droplet size of formulations F1, F2, and F3 when dispersed in SGF were 13.1, 18.0, and 32.2 nm and in SIF were 13.2, 18.3, and 32.5 nm, respectively. The mean droplet size for formulations F1 and F2 in SGF after 2 hours were 13.50 and 17.80 nm and in SIF after 8 hours were 13.74 and 18.63 nm, respectively. There was no significant difference in the droplet size of

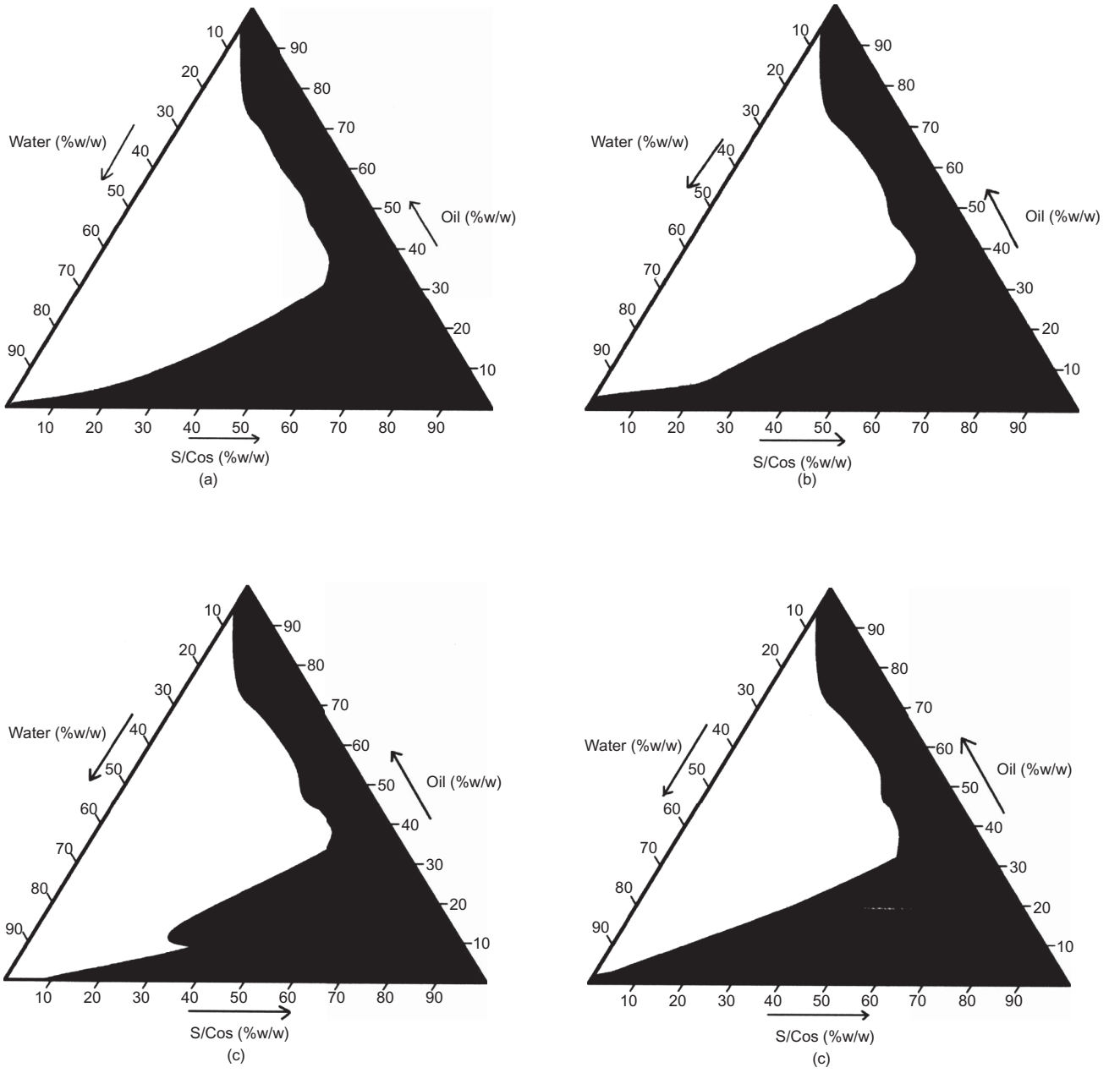

Figure 2. Pseudo-ternary phase diagram consisting of Capryol 90, Cremophor EL, and Labrasol with surfactant/cosurfactant ratios of (a) 1:1, (b) 2:1, (c) 3:1, and (d) 4:1 (%, w/w); the black area represents oil/water microemulsion region.

formulations F1 and F2 after 2 hours in SGF and 8 hours in SIF. In formulation F3, it was found that the droplet size drastically increased from 32.2 to 150.6 nm after 2 hours in SGF because of precipitation of the drug. Thus formulations F1 and F2 were found to be stable in terms of mean droplet size in both SGF and SIF with the maximum drug solubility. Formulation F2 was selected for in vitro and in vivo studies based on its higher drug solubility when compared with formulation F1.

Emulsification time

Emulsification time is a parameter to be assessed because the SEDDS or SMEDDS should disperse completely and

Table 2. Composition of formulations with incorporation of BPQ into SMEDDS formulations with surfactant to cosurfactant ratio of 4:1, solubility, and droplet size. Mean ± SD, $n = 3$.

Formulation no.	Oil (%, w/w)	Surfactant (%, w/w)	Cosurfactant (%, w/w)	BPQ (%, w/w)	Solubility (mg/g)	Droplet size (nm)
F1	7.87	72.38	18.09	1.66	16.92 ± 0.58	13.1 ± 0.23
F2	9.82	70.72	17.68	1.78	18.13 ± 0.19	18.0 ± 0.25
F3	16.41	65.64	16.41	1.54	15.64 ± 0.51	32.2 ± 3.00
F4	25.88	57.98	14.49	1.65	16.73 ± 0.74	83.4 ± 1.05
F5	46.56	41.39	10.35	1.71	17.35 ± 0.84	162.5 ± 2.62
F6	67.16	24.80	6.20	1.85	18.82 ± 0.40	280.6 ± 4.58

Table 3. Pharmacokinetic parameters of BPQ after oral administration of 3.0 mg/kg of BPQ. Mean ± SD, $n = 3$.

	Rabbit 1	Rabbit 2	Rabbit 3	Mean ± SD
T_{max} (hour)	10.00	6.00	6.00	7.33 ± 2.31
C_{max} (ng/mL)	143.56	165.01	206.64	171.74 ± 32.07
$t_{1/2}$ (hour)	33.80	28.17	19.20	27.06 ± 7.36
AUC_{0-36}(ng h/mL)	3370.95	3559.34	3438.85	3456.38 ± 95.41
$AUC_{0-\infty}$ (ng h/mL)	4787.17	4240.43	3997.48	4341.69 ± 404.47
CL (mL/min)	12.53	14.14	15.00	13.89 ± 1.25
Vd (L)	36.68	35.51	24.94	32.38 ± 6.46

quickly when subjected to aqueous media with mild agitation. A time of 2 minutes has been used as an evaluation index for emulsification process[20]. Emulsification time of the selected formulations F1 to F6 in SGF was less than 2 minutes. All the formulations emulsified very rapidly as emulsification time was not a critical parameter for selecting the optimum formulation[21].

In vitro dissolution test

Formulation F2 was used for in vitro dissolution study based on small droplet size, high drug loading ability, and stability in SGF and SIF for 2 hours and 8 hours, respectively. The amount of drug loaded was 15 mg/g (80% of maximum drug load). The cumulative percentage of drug released in 90 minutes was 100% in both SGF and SIF as shown in Figure 3. At the initial time points, the drug dissolved in simulated intestinal fluid was higher but at 90 minutes the release was similar in both media indicating that the drug was released to the same extent from SMEDDS irrespective of the media. The difference in the dissolution in SGF and SIF in initial hours may be attributed to higher solubility of BPQ at higher pH (solubility of BPQ at pH 1.2 and 7.0 were 130.46 and 210.67 ng/mL).

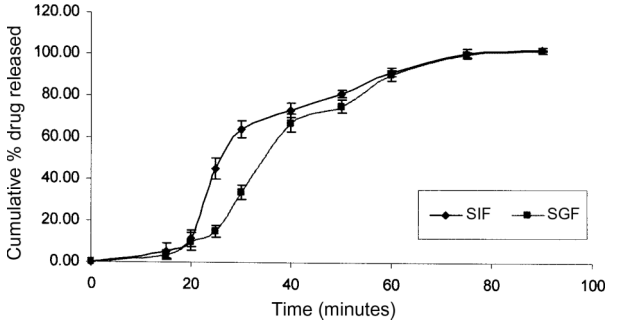

Figure 3. In vitro dissolution profile of BPQ in SGF (pH 1.2) and SIF (pH 6.8). Mean ± SD, $n = 6$.

Bioavailability study

There was no published literature regarding the pharmacokinetics of BPQ in rabbits by oral route. However, pharmacokinetic studies were reported for BPQ and parvaquone in healthy cattle for *Theileria parva* infection. BPQ was administered into the neck muscles at a dose of 2.5 mg/kg. The mean C_{max}, T_{max}, $t_{1/2}$, and area under the curve (AUC) for BPQ were 102 ng/mL, 3.17 hours, 26.44 hours, and 3.43 µg h/mL, respectively[22]. Muraguri et al. reported the clinical efficacy and pharmacokinetic parameters of two formulations of BPQ (Butalex™ and Buparvex™) in cattle infected with *T. parva* infection. BPQ was administered into the neck muscles at a dose of 2.5 mg/kg. The mean C_{max}, T_{max}, and AUC were 229 ng/mL, 2.62 hours, and 4.785 µg h/mL for Butalex™ and 253 ng/mL, 2.12 hours, and 4.156 µg.h/mL for Buparvex™, respectively[23].

The optimum formulation of SMEDDS consisting of Capryol 90 (9.82%), Cremophor EL (70.72%), Labrasol (17.68%), and BPQ (1.78%) was selected for in vivo evaluation in fasted rabbits. In SMEDDS oral pharmacokinetics, BPQ was detected for up to 60 hours for one rabbit and 48 hours for two rabbits. The mean plasma concentration versus time profile of BPQ is shown in Figure 4. The pharmacokinetic parameters of BPQ in individual rabbits following an oral dose of 3.0 mg/kg are given in Table 3. The mean C_{max}, T_{max}, $t_{1/2}$, $AUC_{0-36hour}$, and $AUC_{0\text{-}inf}$ for BPQ were 171.74 ng/mL, 7.33 hours, 27.06 hours, 3456.38 ng h/mL, and 4341.61 ng h/mL, respectively. Absolute oral bioavailability is a measure of systemic exposure of an orally administered drug relative to an intravenous administered dose. The intravenous pharmacokinetic parameters for BPQ which was

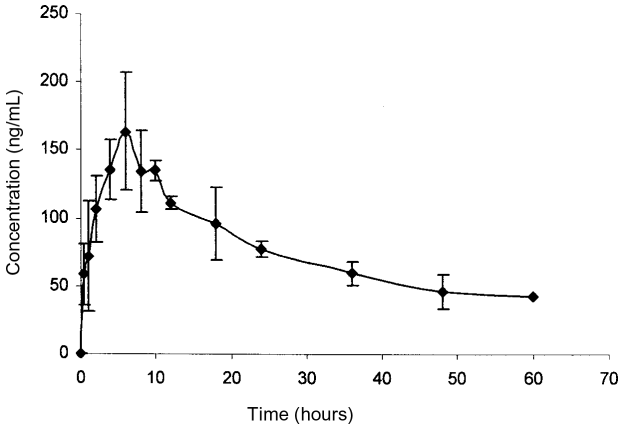

Figure 4. Plasma concentration versus time profile in rabbits following an oral dose of 3.0 mg/kg BPQ. Mean ± SD, $n = 3$.

reported earlier were 999.83 ng/mL, 0.25 hour, 2872.71 and 3233.26 ng h/mL, for mean C_{max}, T_{max}, AUC_{0-36} (ng h/mL), and $AUC_{0\text{-}inf}$ (ng h/mL), respectively. These values were used for calculating the absolute oral bioavailability of BPQ[13]. Absolute bioavailability of SMEDDS formulation was calculated using the following equation.

$$\text{Absolute oral bioavilability}(\%) = \frac{\text{IV dose} \times \text{oral AUC}_{0\text{-}36\text{hours}}}{\text{Oral dose} \times \text{IV AUC}_{0\text{-}36\text{hours}}}$$

The absolute oral bioavailability of SMEDDS formulation relative to an intravenous administration was 40.10% indicating that the SMEDDS formulation increases the rate and extent of absorption of BPQ. These results show that the developed SMEDDS formulation is suitable for oral administration of BPQ.

Conclusion

The present investigation shows the potential application of SMEDDS for oral drug delivery of the lipophilic compound BPQ. The optimized formulation was developed through solubility studies, pseudo-ternary phase diagrams, self-emulsification time, and droplet size. The optimum formulation of SMEDDS consisted of Capryol 90 (9.82%), Cremophor EL (70.72%), Labrasol (17.68%), and BPQ (1.78%). Emulsification time and the mean droplet size were evaluated for the optimum formulation and found to be 1 minute and 18.0 ± 0.25 nm, respectively. The optimized formulation was used for dosing rabbits for pharmacokinetic studies based on its small droplet size and ease of emulsification on dilution with SGF. The calculated absolute oral bioavailability for BPQ was 40.10%. The increased bioavailability of BPQ was probably because of the enhanced solubilization as well as rapid and efficient dispersion of BPQ in the gastrointestinal tract. These results show the developed SMEDDS formulation is rapidly absorbed and suitable for oral administration and would be useful to conducting efficacy studies of BPQ in diseased animal models and subsequently for toxicokinetics studies.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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