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Original research paper

Formulation development of an albendazole self-emulsifying drug delivery system (SEDDS) with enhanced systemic exposure

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The aim of the study was to develop and evaluate a self-emulsifying drug delivery system (SEDDS) formulation to improve solubility and dissolution and to enhance systemic exposure of a BCS class II anthelmetic drug, albendazole (ABZ). In the present study, solubility of ABZ was determined in various oils, surfactants and co-surfactants to identify the microemulsion components. Pseudoternary phase diagrams were plotted to identify the microemulsification existence area. SEDDS formulation of ABZ was prepared using oil (Labrafac Lipopfile WL1349) and a surfactant/co-surfactant (Tween 80/PEG 400) mixture and was characterized by appropriate studies, *viz.*, microemulsifying properties, droplet size measurement, *in vitro* dissolution, *etc.* Finally, PK of the ABZ SEDDS formulation was performed on rats in parallel with suspension formulation. It was concluded that the SEDDS formulation approach can be used to improve the dissolution and systemic exposure of poorly water-soluble drugs such as ABZ.

Keywords: albendazole, lipid based formulation, SEDDS, pharmacokinetics, rats

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Most of the new chemical entities (NCEs) are poorly water-soluble and pose a challenge to developing an optimum solid oral dosage form. Oral route has been the major route of drug delivery for the treatment of various diseases. Delivery of poorly water-soluble molecules by oral route is difficult because approximately 40 % of drug compounds are limited to low aqueous solubility, which leads to restricted oral bioavailability, high intra- and inter-subject variability and lack of dose proportionality (1).

To increase the oral bioavailability of poorly water-soluble compounds and eliminate the discussed drawbacks, various other formulation strategies have been adopted, including the use of cyclodextrins, nanoparticles, solid dispersions and permeation en-

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hancers (2, 3). In recent years, considerable attention has been focused on lipid-based formulations to improve the oral bioavailability of poorly water-soluble compounds. In fact, the most popular approach is incorporation of the drug compound into inert lipid vehicles such as oils and surfactant dispersions, self-emulsifying formulations (4–6), emulsions, liposomes, with particular emphasis on self-microemulsifying drug delivery systems (SMEDDS) (7).

SEDDS formulations are isotropic mixtures of a drug, lipid, surfactant and a co-surfactant or co-solubilizer. The basic principle of this system is its ability to form fine oil--in-water (o/w) microemulsions under gentle agitation following dilution by aqueous phases, *i.e.*, digestive motility of the stomach and intestine provide the agitation required for self-emulsification *in vivo* in the lumen of the gut. This spontaneous formation of an emulsion in the gastrointestinal tract renders the drug in solubilized form, and the small size of the formed droplet provides a large interfacial surface area for drug absorption (8). Apart from solubilization, the presence of lipids in the formulation further helps improve bioavailability by affecting the drug absorption. Selection of a suitable self-emulsifying formulation depends upon the assessment of drug solubility in various components, the area of the self-emulsifying region obtained in the phase diagram, and the droplet size distribution of the resultant emulsion following self-emulsification (9). SEDDS formulation offers the opportunity to deliver lipophilic drugs to the gastrointestinal tract in a dissolved state, avoiding the dissolution step (which can limit the absorption rate of BCS class II and IV drugs), reduction in inter- and intra-subject variability, reduction of food effect, ease of manufacturing and scale-up, ability to deliver peptides that are prone to enzymatic hydrolysis in GIT and no influence of lipid digestion process (10–12).

Albendazole (ABZ, methyl[6-(propylthio)-1*H*-benzoimidazol-2-yl] carbamate), is a benzimidazole carbamate broad spectrum oral anthelmetic used to treat a variety of worm infections caused by nematodes and cestodes (13). ABZ is poorly soluble and its aqueous solubility is reported to be 0.2 μg mL^{-1} at 25 °C. It has weak basic properties (pK_{a1} = 2.68 and pK_{a2} = 11.83) and a log *P* of 3.5 (14). ABZ falls into the BCS class II category as has high permeability and low solubility. Because of its low aqueous solubility, it is poorly and erratically absorbed following oral administration (15). Following oral administration to rats, it was found to absorb 20–30 %, whereas in humans the percent absorbed is 1−5 (16). Following oral administration, ABZ undergoes extensive metabolism in the intestine and liver (by cytochrome P450 (CYP) 3A4 and/or flavin-containing monooxygenase (FMO) into its major active metabolite, *i.e.*, albendazole sulfoxide (ABZ-SOX) or ricobendazole (RBZ). RBZ is further metabolized by CYP2C into albendazole sulfone (ABZ-SON), which is pharmacologically inactive. Due to extensive metabolism in all species, plasma concentrations of ABZ are usually low compared to the concentrations of its oxidized metabolites RBZ and ABZ-SON.

Lack of water solubility of any drug reduces its flexibility for drug formulation and administration. To overcome these drawbacks and to increase aqueous solubility of ABZ, different formulation approaches have been tried in the past to improve ABZ aqueous solubility. For instance, since ABZ is basic in nature, its solubility can be increased by ionization in an acid medium, although this increase in solubility is not sufficient for the preparation of high-ABZ concentration formulations (17). Another approach involved addition of surfactants such as Tween 80 and bile salts, or co-solvent agents such as Transcu-

tol (18–20). Use of liposomes (21) and a solid dispersion approach with polyvinylpyrrolidone (22–24) showed less promise. Recently, a formulation with a high ABZ concentration was prepared by complexation with hydroxypropyl-β-cyclodextrin (25, 26). Lately, a lipid based delivery system of ABZ, the self-microemulsifying drug delivery system (SMEDDS) for improving ABZ solubility, was published (27).

The main objectives of this study were two-fold: *i*) to develop and evaluate an optimal SEDDS formulation containing ABZ; *ii*) to evaluate the SEDDS formulation potential to improve systemic exposure of ABZ by conducting an oral pharmacokinetic study in rats. Though an earlier SMEDDS formulation of ABZ was reported and evaluated in rabbits (27), the increase in *AUC* and c_{max} was ~2-fold. Considering the precedence of erratic oral absorption and high pre-systemic metabolism of ABZ following its oral administration, we feel that ~2-fold improvement is not adequate and sometimes it will not be truly reflective because of erratic absorption of ABZ. After reviewing the earlier published SMEDDS formulation of ABZ (27), we found that the authors did not choose a suitable combination of excipients to prepare the SMEDDS formulation, which are critical for forming the droplet size when the SMEDDS formulation is dispersed.

In this paper, we made a systematic evaluation of ABZ solubility and selected the right oil (Labrafac Lipofile WL1349) and S/CoS (Tween 80/PEG 400) mixture to develop the SEDDS formulation. We characterized the optimized SEDDS formulation through the following: stability study, emulsification study and *in vitro* drug release by dissolution. We have also determined the droplet size of the emulsion and found that droplets are in the nanometer range. Having established all the *in vitro* tests, the SEDDS formulation was evaluated in rats by conducting an oral pharmacokinetic study along with a conventional suspension formulation.

EXPERIMENTAL

Chemicals and reagents

ABZ, Tween 80, soya oil, olive oil, sunflower oil, corn oil, propylene glycol were purchased from Sigma Aldrich (Milwaukee, USA). Sesame Labrasol (caprylocaproyl macrogolglycerides), Labrafac Lipofile WL1349, 1357 and 1358 (medium chain triglycerides), Capryol PGMC (propylene glycol caprylate), Lauroglycol FCC (propylene glycol laurate) and Maisine 35-1 (glyceryl monolinoleate) were gift samples from Gattefosse (Mumbai, India). Capmul PG-8 (propyleneglycol monocaprylate), Captex 300 Low CC (glyceryl tricaprylate/caprate), Captex 355 (glycerol caprylate caprate) and Capmul MCM (glyceryl mono and dicaprate) were obtained from Abitec Corporation (Columbus, OH, USA). Poloxamer 188 (ethylene oxide/propylene oxide block copolymer), Cremophor RH40 (polyoxyl 40 hydrogenated castor oil), Lutrol E300 (polyethylene glycol 300), Lutrol E400 (polyethylene glycol 400), Solutol HS15 (polyethylenglycol-660-12-hydroxystearate) and Cremophor EL (castor oil, ethoxylated) were obtained from BASF (Mumbai, India) through Signet Corporation. RBZ was procured from Jay Radhe Sales (Ahmedabad, India). Sesame oil, Span-20 and crodamol GTCC were procured from Croda Chemicals (Mumbai, India). All other chemicals used were of analytical grade.

Solubility studies

The solubility experiment was carried out in various oils, surfactants and co-surfactants, by using the 96-well plate format. The DMSO stock was spiked into each excipient at 500 μg mL^{-1} concentration. Following spiking, the 96-well plate was kept shaking for 2 h to equilibrate. After equilibration, the plate was centrifuged at 4000 rpm at 37 °C on a Sigma centrifuge for 10 min. Supernatant was used for HPLC analysis.

Construction of a pseudo-ternary phase diagram

Pseudo-ternary phase diagrams of oil, surfactant/co-surfactant (S/CoS) and water were developed using the water titration method at room temperature (25 ± 1 °C). Mixtures of oil and S/CoS at certain mass ratios were diluted with water in a dropwise manner. For each phase diagram at a specific ratio of S/CoS (2:1 and 3:1), a transparent and homogenous mixture of oil and S/CoS was formed by vortexing for 5 min. Each mixture was then titrated with water and visually observed for phase clarity and flowability. Mixtures of surfactant and co-surfactant with water were prepared at the ratios of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10. Resulting mixtures were evaluated visually for transparency and flow properties. Endpoint of titration was the point where the mixture became turbid or phase separation was observed. At that point, the amounts of water, oil, surfactant and co-surfactant added were noted. Monophasic, clear, low viscous systems were considered as micro-emulsion (ME) and shown as the ME region.

Emulsification study

Self-emulsification ability of surfactants was assessed to select the best surfactant from a large pool of surfactants. Selected oil and surfactants were mixed in 1:3, heated at 40–50 °C and vortexed to form a homogenous mixture at room temperature (25 ± 1 °C). To evaluate the effect of the co-surfactants on emulsification, the ratio of surfactant to co-surfactant was kept constant 3:1 and this mixture was mixed with the oil in the ratio of 3:1 based on the requirements stated by Pouton (2000) (28). Oil-surfactant mixture, 500 mg dispersed in 500 mL of doubly distilled water in a glass beaker, was prepared under gentle stirring. Visual test was used to assess self-emulsification of surfactants in terms of dispersability, ease of emulsification and final appearance using a grading system (29) (Table I).

Preparation of ABZ SEDDS

A series of SEDDS formulations was prepared using Tween 80, Solutol HS (surfactants), PEG 400, propylene glycol (co-surfactant) and Captex 300 Low C6, Labrafac Lipofile WL1349 and Capmul PG8 (oil). In all formulations, the level of ABZ concentration was kept constant (0.1 %, *m/m*, of total formulation weight). Drug loading was done on selected formulations, which were found satisfactory in the emulsification study. The selected oil and surfactant mixture ratio was 1:3 and surfactant and co-surfactant ratio was 3:1. Drug loading was done on the basis of *m/m* of the composite weight of the formulation.

Briefly, SEDDS formulations were prepared so that accurately weighed ABZ was placed in an Eppendorf tube, dispersed into oil phase and heated at 40–50 °C under vor-

Table I. Grading system for visual assessment of self-microemulsification efficiency

Dispersability and appearance	Time of self-emulsification (min)	Grade
Formulation spread rapidly in water, forming a clear and transparent microemulsion	< 1	A^+
Formulation formed a transparent, gel-like intermediate structure prior to dispersing completely but could form microemulsion	3–5	A
Formulation droplets spread in water to form a turbid emulsion	> 5	B
Formulation exhibited poor emulsification with coalescence of oil droplets	NE	C

A+ – rapid microemulsion; A – microemulsion; B – emulsion; C – poor emulsion; NE – no emulsion

tex. Surfactant and co-surfactant were mixed together in a separate test tube and mixed well under vortex. Surfactant and co-surfactant were heated up to 60 °C to mix properly. Drug containing oil phase was transferred into the surfactant and co-surfactant mixture under continuous mixing. They were vortex mixed and heated at 50 °C in a sonicator until ABZ was perfectly dissolved. The azotropic mixture was stored at room temperature until further use.

In vitro *dissolution studies*

A quantitative *in vitro* release test was performed in 500 mL of phosphate buffer pH 6.8 and 0.1 mol L^{-1} HCl using glass beakers maintained at 37 °C on a magnetic stirrer at 100 rpm. The SEDDS formulation (400 mg) was filled into size-3 hard gelatin capsules for *in vitro* dissolution evaluation. Samples were taken at 5, 8, 12 and 15 min time points for drug release. At each time point, the removed sample volume was replaced with 5 mL of fresh medium. Samples were centrifuged at 4000 rpm for 10 min, filtered using a 0.45 μm syringe filter and analyzed on HPLC.

Emulsion droplet size measurement

An aliquot of 500 μL of each SEDDS formulation was diluted to 250 mL with Milli-Q water in a beaker using a magnetic stirrer. The resultant emulsion was then subjected to particle size analysis using a Malvern Zetasizer (Worchestershire, UK) equipped with 2000 Hydro MU at 25 °C, with a particle size measurement range of 0.02 to 2000 μm. Particle size was calculated from the volume size distribution.

Freeze-thawing

Freeze-thawing was employed to evaluate the stability of formulations. Thermodynamic stability was evaluated at different temperatures. To check the effect of temperature, the formulation was subjected to freeze-thaw cycles (–20 °C, 1–4 °C for 1, 2 and 3 days, followed by 40 °C for 1, 2 and 3 days). Another sample was kept at 40 °C for 1, 2

and 3 days. At the end of the cycle, the formulation was diluted and centrifuged. Dilution was 1:10, 1:50, 1:100 and 1:500, followed by centrifugation at 5000 rpm for 10 min. The formulations were then observed for phase separation. Only formulations that were stable to phase separation were selected for further studies.

Animals

Male Sprague-Dawley rats were procured from Bioneeds, Bangalore, India. The animals were housed in the Jubilant Biosys animal care facility in a temperature and humidity controlled room with 12:12 h light/dark cycles, had free access to food (Lipton India) and water *ad libitum*. Animal experiments were approved by the Jubilant Biosys Ltd. Institutional Animal Ethics Committee and were in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Environment, Government of India.

Oral pharmacokinetic (PK) study

Blood samples were collected following oral administration of ABZ using SMEDDS (self emulsfying drug delivery system) and conventional suspension (0.5 % methyl cellulose + 0.1 % Tween-80) formulations at a dose of 30 mg kg^{-1} to overnight fasted (~12 h, during fasting animals had free access to water) Sprague Dawley rats (n = 4 each group, 189–193 g). Blood samples (100 µL) were collected into polypropylene tubes containing Na_2EDTA solution as an anti-coagulant at pre-dose, 0.25, 0.5, 1, 2, 4, 8 6, 10, 18, 20 and 24 h from retro-orbital plexus.

Plasma collection and processing

From the above PK study blood samples, plasma was harvested by centrifuging the blood using an Eppendorf 5430R centrifuge (Germany) at 5000 rpm for 5 min. Harvested plasma was stored frozen at –80 ± 10 °C until analysis. Plasma (50 µL) samples were processed as described below.

Plasma sample analysis

Plasma samples were analyzed for simultaneous quantification of ABZ and RBZ using a validated LC-MS/MS published by us (30). Briefly, to an aliquot of 50 µL plasma sample, 400 µL of 10 % tetrahydrofuran (THF) in acetonitrile, containing 100 ng mL^{-1} of internal standard (phenacetin), was added and samples were centrifuged at 14000 rpm in 5430R (Eppendorf, Germany) at 10 °C for 10 min. About 400 µL of clear supernatant was transferred into the sample vial and 10 µL was injected onto the analytical column for analysis. Resolution of analytes and internal standard was achieved on a Chromolith Performance, RP-18e (100 x 4.6 mm, Merck, Darmstadt, Germany) column using 5 mM ammonium acetate (pH 6.0)/acetonitrile (20:80, *V/V*) as mobile phase delivered at a flow rate of 1.0 mL min^{-1}. Tandem mass spectrometric (MS/MS) detection was performed with a triple quadrupole MDS Sciex (Foster City, CA, USA) API-4000 mass spectrometer equipped with a Turboionspray™ (ESI) source and the analytes were monitored and quantified using Analyst software (version 1.5). Detection of ions was performed in the multi-

ple reaction monitoring (MRM) mode, monitoring the transition of the *m/z* 266.1 precursor ion to the *m/z* 234.4 product ion for ABZ, *m/z* 282.2 precursor ion to the *m/z* 240.4 product ion for RBZ and *m/z* 180.1 precursor ion to the *m/z* 110.1 product ion for IS. Quadrupole Q1 and Q3 were set to unit resolution. The dwell time was 200 ms. The method had reproducible linearity over a range of 2.01–2007 and 6.02–6020 ng mL^{-1} for ABZ and RBZ, respectively. Along with PK samples, QC samples at low, medium and high concentration were assayed in duplicate and were distributed among calibrators and unknown samples in the analytical run. The criteria for acceptance of analytical runs encompassed the following: *i*) 67 % of the QC samples accuracy had to be within 85–115 % of the nominal concentration; *ii*) 50 % of each QC concentration level had to meet the acceptance criteria.

PK data analysis

Plasma concentration *vs.* time data of ABZ and RBZ was analyzed by the non-compartmental method using WinNonlin Version 5.1 (Pharsight Corporation, Mountain View, CA) to derive various pharmacokinetic parameters, *viz.*, $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, c_{max}, t_{max} and $t_{½,\beta}$.

RESULTS AND DISCUSSION

Solubility study

Various oils and surfactants were used in this study. The results of solubility studies of ABZ in various oils and surfactants are presented in Table II. It is evident from the data obtained that ABZ does not have good solubility in any of the excipients used. The highest solubility 498.90 μg g^{-1} was found in PEG 400. These results concur with those of Torrado *et al.*, who found low solubility of ABZ in similar solubility enhancers (18). Some excipients showed a relatively high solubilizing ability, for example, polyethylene glycol 300 (340.45 μg g^{-1}), Lauroglycol FCC (186.05 μg g^{-1}), Cremophor EL (179.76 μg g^{-1}), Solutol HS15 (193.44 μg g^{-1}), Labrafac Lipofile WL1349 (96.78 μg g^{-1}), Capmul PG-8 (139.88 μg g^{-1}), Span 20 (185.79 μg g^{-1}), Captex 355 (99.37 μg g^{-1}), Labrasol (188.13 μg g^{-1}), Capryol PGMC (179.48 μg g^{-1}) and Tween 80 (172 μg g^{-1}). Based on these results, Tween 80, Solutol HS15, PEG 400, Captex 300 Low C 6, Labrafac Lipofile WL1349, Propylene glycol, Lauroglycol FCC and Capmul PG8 were selected for drug loaded SEDDS formulation development. Although some other excipients show good solubility, we have considered emulsification and drug loading parameters for selection of surfactants and oils and selected the above listed excipients.

While formulating SEDDS, it is important to avoid precipitation of the drug upon dilution in the gut lumen *in vivo*. Therefore, the components used in the formulation of SEDDS should have a high solubilizing capacity to ensure drug solubilization in the resultant dispersion. From the results of solubility studies, the highest solubility for ABZ was found in PEG 400 (498.90 μg g^{-1}).

Table II. Solubility of ABZ in different vehicles

Vehicle	Total solubility ($\mu g\ g^{-1}$)
Sunflower oil	0.43
Captex 300	40.23
PEG 300	340.45
PEG 400	498.90
Soya oil	3.62
Olive oil	0.79
Crodamol oil	23.61
Corn oil	0.05
Lauroglycol FCC	186.05
Cremophore EL	179.76
Solutol HS15	193.44
Labrafac Lipofile WL1349	96.78
Capmul PG8	139.88
SPAN 20	185.79
Captex 355	99.37
Tween 80	172.19
Acrysol K150	46.18
Labrasol	188.13
Cremophore RH40	46.72
Acrysol K140	46.60
Maisine	18.43
Sesame oil	3.73
Capmul MCM	5.59
Capryol PGMC	179.48
Propylene glycol	194.47

Construction of a pseudo-ternary phase diagram

In the present study, oil – Labrafac Lipofile WL1349, surfactant – Tween 80 and co-surfactant – PEG 400 mixture were used. The surfactant to co-surfactant ratios 2:1 and 3:1 were maintained. As seen from the ternary plots (Fig. 1 and 2), Labrafac Lipofile WL1349 gave a wider microemulsion region when the 3:1 ratio of the surfactant to co--surfactant was maintained compared to 2:1.

Upon their introduction into aqueous media, self-emulsifying systems form fine oil--water emulsions with only gentle agitation. Surfactant and co-surfactant get preferentially adsorbed at the interface, reducing the interfacial energy as well as providing a mechanical barrier to coalescence. Decrease in the free energy required for emulsion formation consequently improves the thermodynamic stability of the microemulsion for-

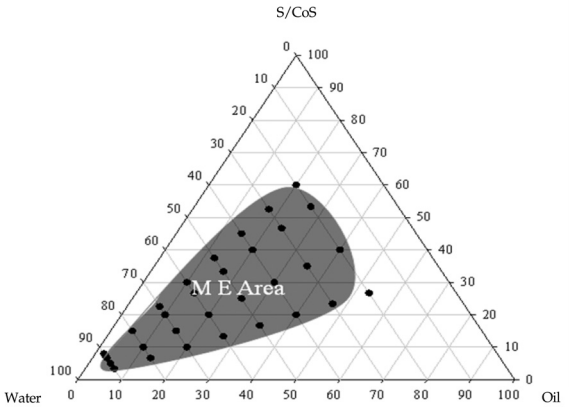

Fig. 1. Ternary phase diagram prepared with oil-Labrafac Lipofile WL1349, surfactant-Tween 80 and co-surfactant PEG400 (S/CoS ratio 3:1).

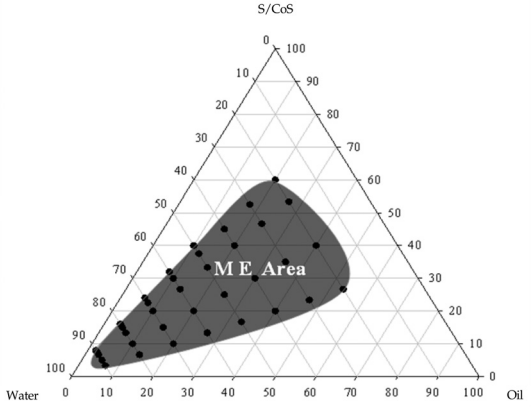

Fig. 2. Phase diagram prepared with oil-Labrafac Lipofile WL1349, surfactant-Tween 80, and co-surfactant PEG400 (S/CoS ratio 2:1).

mulation (34, 35). Therefore, the selection of oil and surfactant, and the mixing ratio of oil to S/CoS, play an important role in the microemulsion formation. The phase diagram was constructed using Labrafac Lipofile WL1349 as oil, Tween 80 as surfactant and PEG 400 as co-surfactant. From the results of the phase diagram, the selected surfactant to co-surfactant ratio is 3:1 and 1:3 for oil to S/Co-S because the microemulsion is formed in this region. These data are supported by the phase diagram (28).

Emulsification study

Emulsification grading for relative emulsification of surfactants is shown in Table I. It clearly distinguishes the ability of surfactants to emulsify selected oily phases. The results

Table III. Emulsification efficiency of various surfactants

Surfactant	Oils								
	Soya oil	Maisine 35-1	Capmul MCM	Captex 300 Low	Labrafac Lipofile WL1349	Capryol PGMC	Olive oil	Captex 355 Low	Capmul PG8
Tween	C	A^+	A^+	A	A^+	A^+	D	A	A^+
Gelucire 44/14	D	D	D	C	C	A^+	D	C	A^+
Creomophor EL	D	C	C	A	B	A^+	C	A	A^+
Labrasol	C	C	C	C	C	D	C	C	C
Cremophore RH 40	C	A^+	A^+	A^+	A	A^+	A^+	A	A^+
Acrysol K150	C	A^+	A^+	A	A	A^+	C	A	A^+
Solutol HS15	D	C	A^+	A^+	A^+	A^+	D	A^+	A^+

A^+ – rapid microemulsion; A – microemulsion; B – emulsion; C – poor emulsion; D – no emulsion

Table IV. Emulsification study of surfactant and co-surfactant combinations (surfactant/co-surfactant 3:1 and Smix/Oil 3:1)

Surfactant	Oils		
	Captex 300 Low	Labrafac Lipofile WL1349	Capmul PG8
Tween 80 + PEG400	A^+	A^+	A^+
Tween 80 + Lauroglycol FCC	C	B	C
Tween 80 + Propylene glycol	A^+	A^+	A^+
Cremophore EL+ PEG400	A	A^+	A^+
Cremophore EL+ Lauroglycol FCC	A^+	A	A^+
Cremophore EL+ Propylene glycol	A^+	A^+	A^+
Acrysol K150 + PEG400	A^+	A^+	A^+
Acrysol K150 + Lauroglycol FCC	A^+	A^+	A^+
Acrysol K150 + Propylene glycol	A^+	A^+	A^+
Solutol HS15 + PEG400	A	A^+	A^+
Solutol HS15 + Lauroglycol FCC	A	A^+	C
Solutol HS15 + Propylene glycol	A^+	A^+	A

A^+ – rapid microemulsion; A – microemulsion; B – emulsion; C – poor emulsion; D – no emulsion

of emulsification with different oils and surfactants are summarized in Table III and the effects of the co-surfactant results are summarized in Table IV using the grading system.

Although HLB values of surfactants used in study were > 10, there were considerable differences in their ability to emulsify oils. The results obtained indicated that apart from the HLB value, other factors such as structure and relative length of hydrophobic chains of surfactants influenced microemulsification. These results are in agreement with the results reported in literature (36, 37). Cremophor EL and Tween 20 rendered effective microemulsification and were selected for further study. Among the oils, soya oil and olive oil were difficult to emulsify, followed by Capmul MCM, Maisine 35-1, Captex 300 Low, Labrafac Lipofile WL1349, Capryol PGMC, Captex 355 low and Capmul PG8, which were emulsified easily. This is explained by fact that the ease of oil emulsification and the amount incorporated in microemulsion are affected by the molecular volume of oil. As the number and length of hydrophobic alkyl chains increases, molecular volume increases. These observations are in line with studies reported by Warisnoicharoen *et al.* (38) and Malcolmson *et al.* (39). Labrafac Lipofile WL1349 provides good emulsion compared to other oils; the oil HLB value was near 1.0, so it has a high lipophilic nature and can load more of the compound. We have selected them as oily phases for further study due to their relative ease of self-microemulsification. Emulsification capacities of the surfactants were also evaluated with the above oils from Table III, all surfactants showing good emulsification power except Labrasol, which has a low HLB value compared to other oils. Table IV shows relative efficacy of co-surfactants of improving the emulsification of surfactants. PEG 400 and Lauroglycol FCC were used as co-surfactants and Tween 80, Cremophore EL and Acrysol K 150 were used as surfactants. All combinations increased the spontaneity of microemulsion formation. As the ratio of surfactant to co-surfactant was constant, the study clearly distinguished the ability of co-surfactants to improve the emulsification of surfactants. Furthermore, as co-surfactants improve emulsification of surfactants by penetrating the interfacial surfactant monolayer, their performance is affected by their structure and chain length. Thus, the study gave an insight into relative emulsification properties of SEDDS components and forms the basis for their selection as selected excipients.

Preparation of ABZ SEDDS

A series of SEDDS formulations was prepared using Tween 80, Solutol HS15 as surfactant; PEG 400, Propylene glycol, as co-surfactant, and Captex 300 Low C6, Labrafac Lipofile WL1348, Capumul PG-8 as oil phase. All formulations were able to take a load of 0.1 % ABZ and were homogenous and transparent. ABZ was completely in soluble form. Combinations of all formulations are given in Table V.

In vitro *evaluation of formulations*

In vitro dissolution of ABZ SEDDS was done in 0.1 mol L^{-1} HCl and phosphate buffer pH 6.8. The release of ABZ in all different drug loaded formulations was checked. Release of ABZ in all formulations was over 90 % in 10 min both in phosphate buffer pH 6.8 and 0.1 mol L^{-1} HCl. The results are shown in Figs. 3 and 4.

ABZ has low solubility and dissolution rate in conventional formulations (14). Using the proposed formulation approach, we were able to attain more than 90 % release in 10 min in both dissolution media. These results indicate that SEDDS formulation resulted in spontaneous formation of a microemulsion with a small droplet size, which permitted a faster rate of ABZ release in dissolution media. As the drug is present in dis-

Table V. Composition of selected ABZ SEDDS formulations

Formulation	Fraction in formulation (%)	F1	F2	F3	F4	F5	F6
Drug	0.1	ABZ	ABZ	ABZ	ABZ	ABZ	ABZ
Oil	25	Captex 300 Low C6	Captex 300 Low C6	Labrafac Lipofile WL1349	Labrafac Lipofile WL1349	Labrafac Lipofile WL1349	Capmul PG8 —
Surfactant	56.25	Solutol HS15	Solutol HS15	Tween 80	Solutol HS15	Solutol HS15	Tween 80
Co-surfactant	18.75	PEG 400	Propylene glycol	Propylene glycol	PEG 400	Lauroglycol FCC	Propylene glycol

Formulation	Fraction in formulation (%)	F7
Drug	0.1	ABZ
Oil	25	Labrafac Lipofile WL1349
Surfactant	56.25	Tween 80
Co-surfactant	18.75	PEG 400

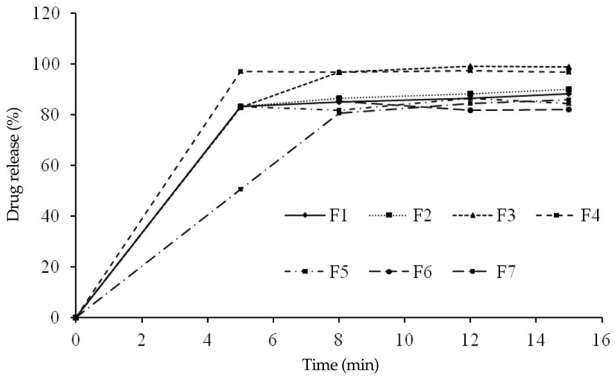

Fig. 3. Dissolution profile of albendazole formulation in pH 6.8 phosphate buffer.

solved state, the dissolution step is omitted for the GI tract, which permits faster release of the drug into aqueous phase, which could affect oral bioavailability.

The average droplet size of the selected formulation was obtained as Z-average 127.5 and PDI 0.264 nm. Three peaks in Fig. 5 indicate that three different sizes of droplets existed in the formulation, all droplets sizes being in nanometers, peak 1 (29.94 nm), peak 2 (283.2 nm) and peak 3 (75.2 nm). Their corresponding intensity was 57.1, 37.2 and 10.3 %, respectively, indicating that major droplets were 29.94 nm. Nanometer particle size of droplets after emulsification was the most important property of SEDDS.

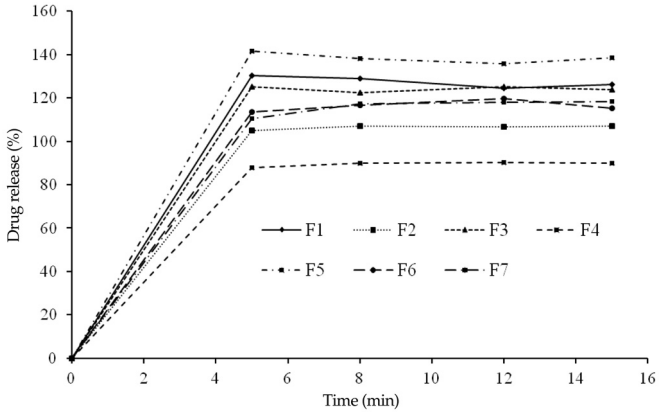

Fig. 4. Dissolution profile of albendazole formulation in 0.1 mol L^{-1} HCl.

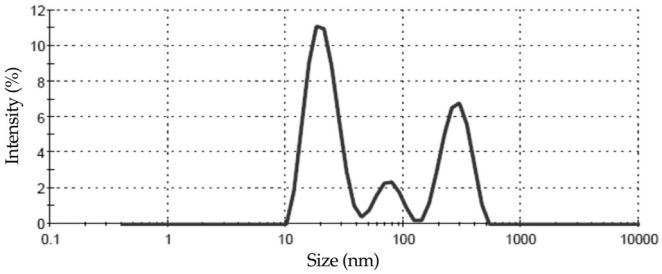

Fig. 5. Particle size distribution of the selected albendazole microemulsion by Zetasizer.

Freeze-thawing

The freeze-thaw cycling test was performed to test the robustness of the formulation. One freeze-thaw cycle consisted of microemulsion storing at –20 °C, 1–4 °C for 24, 48 and 72 h. After this, formulations were stored at 40 °C for another 24, 48 and 72 h. No phase separation of the ABZ loaded SEDDS formulation was observed upon centrifugation. However, the thermal cycling study created a thermodynamically unstable microemulsion, which had larger droplet size distribution upon dilution. Visual observation indicated that there was no phase separation in any formulation and the physical appearance of all formulations was similar.

The thermal cycling study created a thermodynamically unstable microemulsion, which had larger droplet size distribution upon dilution. Visual observation indicated that there was no phase separation in all formulation and the physical appearance of all formulations was similar. This study has revealed that a sudden change in temperature causes a change in the entropy of the system, which results in coalescence of droplets. The overall stability of the formulation under normal conditions was found to be acceptable.

Oral pharmacokinetic (PK) study

Due to extensive metabolism, plasma concentrations of ABZ are usually low and it has been already reported that RBZ is the active metabolite of ABZ; we have quantified both ABZ and RBZ simultaneously using a validated method published by us (30). In fact, it was reported that pharmacokinetics of ABZ had been studied by determining the plasma concentrations of RBZ and ABZ-SON (31–33). From the results of two studies [*i.e.*, suspension *vs.* SEDDS] it was evident that the SEDDS formulation increased both c_{max} and *AUC* of ABZ and its active metabolite, *i.e.*, RBZ. The PK parameter results for ABZ and RBZ are shown in Table VI. Time *vs.* plasma concentration for ABZ and RBZ is shown in Fig. 6. Following ABZ suspension formulation, both ABZ and RBZ (formed from ABZ) were seen only up to 10 h, whereas following SEDDS formulation administration of ABZ, ABZ and RBZ (formed from RBZ) were seen up to 18 and 20 h, respectively.

Elimination half-life ($t_{½,ß}$) observed for ABZ after dosing of suspension formulation was 2.61 h, while extended $t_{½,ß}$ of 1.78 h was observed for the SEDDS formulation profile. Peak concentration was found to be 6-fold higher for SEDDS formulation, *i.e.*, c_{max} value of 309 ng mL^{-1} for suspension formulation *vs.* 1876 ng mL^{-1} for SEDDS formulation. Exposure was found to be 11-fold higher for SEDDS formulation, *i.e.*, $AUC_{0\text{-}t}$ value of 980 ng h mL^{-1} for suspension formulation *vs.* 11167 ng h mL^{-1} for SEDDS formulation.

Elimination half-life ($t_{½,ß}$) observed for RBZ after dosing of ABZ suspension formulation was 4.71 h, while lower $t_{½,ß}$ of 1.36 h was observed for the SEDDS formulation profile. Peak concentration was found to be 3.7-fold higher for SEDDS formulation *i.e.*, c_{max} value of 2.410 μg mL^{-1} for suspension formulation *vs.* 8.976 μg mL^{-1} for SEDDS formulation. Exposure was found to be 6-fold higher for SEDDS formulation, *i.e.*, $AUC_{0\text{-}t}$ value of 16.904 μg h mL^{-1} for suspension formulation *vs.* 99.962 μg h mL^{-1} for SEDDS formulation. Lower $t_{½,ß}$ was attributed to rapid elimination and inadequate elimination phase time points (as the concentration of RBZ was 7.025 μg mL^{-1} at 10 h and decreased to 4.55 ng mL^{-1} at 24 h post dose).

Increase in exposure was evident for both ABZ and its active metabolite RBZ from the results obtained in pharmacokinetic studies. SEDDS formulation clearly proved to

Table VI. Pharmacokinetic parameters of ABZ and RBZ (released from ABZ) following oral administration of albendazole suspension and SEDDS formulations at 30 mg kg^{-1} to Sprague Dawley rats

PK parameter	Albendazole (ABZ)		Ricobendazole (RBZ)	
	Suspension	SEDDS	Suspension	SEDDS
$AUC_{0\text{-}t}$ (ng h mL^{-1})	980 ± 527	11167 ± 1666	16904 ± 7314	99962 ± 6334
$AUC_{0\text{-}8}$ (ng h mL^{-1})	1024 ± 557	11448 ± 1359	23740 ± 12440	100517 ± 6993
c_{max} (ng mL^{-1})	309 ± 96	1876 ± 281	2410 ± 906	8976 ± 752
t_{max} (h)	1.00 ± 0.71	1.75 ± 1.50	3.00 ± 1.15	6.00 ± 2.13
$t_{½,ß}$ (h)	2.61 ± 0.99	1.78 ± 0.57	4.71 ± 1.13	1.36 ± 0.21

[a] $n = 4$

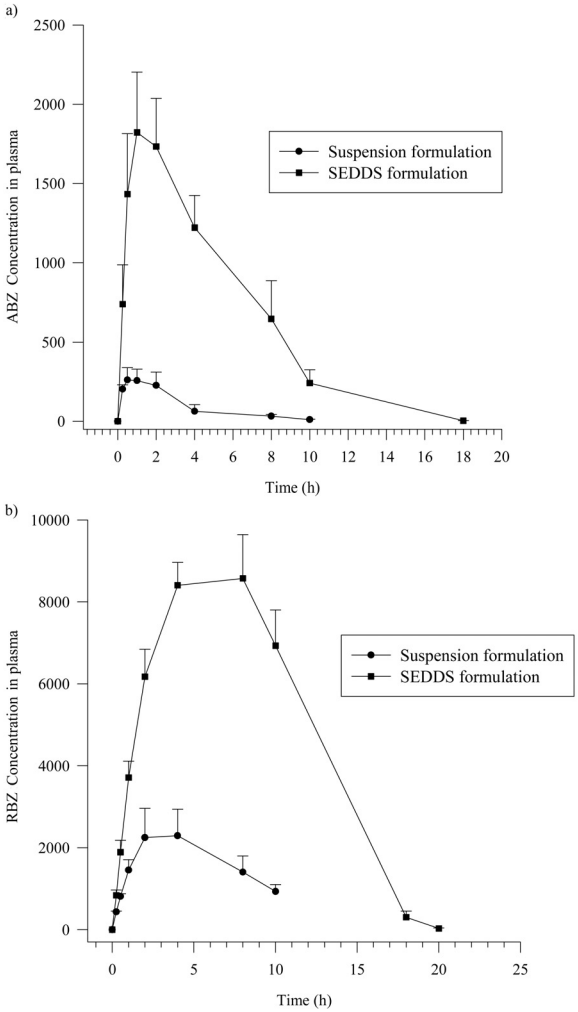

Fig. 6. Plasma concentration *vs.* time profiles for ABZ and RBZ (released from ABZ) following oral administration of ABZ suspension and SEDDS formulations at 30 mg kg^{-1} to Sprague Dawley rats (n = 4).

be superior over the conventional suspension formulation strategy and could provide a step forward for the design of PK studies of such poorly soluble and rapidly metabolizing molecules.

CONCLUSIONS

An optimized self-emulsifying drug delivery system for ABZ was successfully developed, with an increased dissolution rate and solubility compared to conventional for-

mulations, which ultimately increased the systemic exposure of ABZ and its active metabolite RBZ in rats. Our study illustrated the potential of using SEDDS to dispense poorly water-soluble drug by oral route.

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S A Ž E T A K

Razvoj samoemulzifirajućeg sustava za isporuku albendazola (SEDDS) s pojačanom sistemskom apsorpcijom

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Cilj rada bio je razvoj i evaluacija samoemulzifirajućeg sustava za isporuku lijekova (SEDDS) povećane topljivosti i oslobađanja, te povećane sistemske apsorpcije anthelmintika albendazola (ABZ), lijeka iz klase BCS II. Odabir sastojaka za pripravu mikroemulzija izvršen je na temelju topljivosti albendazola u različitim uljima, surfaktantima i kosurfaktantima. Kako bi se odredilo područje u kojem dolazi do mikroemulzifikacije izrađeni su pseudoternarni fazni dijagrami. SEDDS formulacija albendazola pripravljena je pomoću smjese ulja (Labrafac Lipopfile WL1349) i surfaktanta/kosurfaktanta (Tween 80/PEG-400). Dobivenom pripravku određena su mikroemulzifirajuća svojstva, veličina kapljica, oslobađanje *in vitro*, itd. Osim toga, određeni su farmakokinetički parametri za ABZ SEDDS u štakora i uspoređeni sa suspenzijom albendazola. Zaključeno je da se pomoću SEDDS-a može povećati topljivost i sistemska apsorpcija lijekova slabo topljivih u vodi kao što je ABZ.

Ključne riječi: albendazol, pripravak na bazi lipida, SEDDS, farmakokinetika, štakori

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