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Solubility enhancement of *Boswellia serrata* Roxb. ex Colebr. extract through a self dispersible lipidic formulation approach

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Boswellic acids (BAs) are isolated from oleo gum resin of *Boswellia serrata* Roxb. ex Colebr. and are reported to have anti-inflammatory, immunomodulatory and anti-tumor activity with better tolerance and lesser side effects compared to NSAIDs. Pharmacokinetic studies of BAs revealed its poor absorption through oral route due to poor solubility. The present study was aimed to develop and characterize a lipid based drug delivery system of *Boswellia serrata* extract (BSE) to enhance the solubility and in turn, the oral absorption of BAs. Suitable compositions for lipidic formulation were screened *via* solubility and compatibility studies. Pseudoternary phase diagrams were used to evaluate the microemulsion existence area. The self microemulsifying drug delivery system (SMEDDS) was characterized by solubility, clarity, drug precipitation, globule size, emulsification time and drug release profile. The optimal formulation of SMEDDS comprised of 37.5 % Tween-80, 12.5 % PEG-400 and 50 % oil (Caprylic/capric triglycerides). The dissolution study in hydrochloric acid buffer pH 1.2 showed significantly improved dissolution of BSE-SMEDDS (>90 %) compared to Plain BSE (practically no release) in 120 minutes. BSE-SMEDDS showed better anti-inflammatory activity than plain BSE in a carrageenan-induced rat paw edema model. The developed formulation was found to have better solubility and can be used as a possible alternative to traditional oral formulations of BSE with potential applications.

Keywords: Anti-inflammatory, Bioavailability enhancement, *Boswellia serrata* extract, Self microemulsifying drug delivery system, 11-keto-β boswellic acid, 3 acetyl-11-keto-β-boswellic acid.

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs), used for the management of pain, fever and inflammatory diseases are among the most widely prescribed of all therapeutic agents[1]. Continual treatment with NSAID effectively reduces symptoms of painful arthritis in most cases, however, chronic NSAID use may lead to gastrointestinal (GI) complications including abdominal discomfort, bleeding resulting in life threatening ulceration and GI wall perforations[2].

Numerous approaches have been carried out in order to develop NSAIDs that spare the GI complication, thus leading to the development of highly selective cyclo-oxygenase (COX)-2 inhibitors with an improved gastric tolerability profile[3]. However, severe adverse cardiovascular reactions, which have led to the withdrawal of some of these drugs, have reduced the initial enthusiasm for this new class of anti-inflammatory drugs[4]. This necessitates reinvestigation and development of well tolerated anti-inflammatory medicines of herbal origin, which may provide an alternative with possible therapeutic benefits and avoid GI and cardiovascular complications.

For centuries, *Boswellia serrata* Roxb. ex Colebr. (Family Burseraceae) has been traditionally applied in folk medicine to treat various topical and systemic inflammatory diseases[5,6] such as rheumatoid arthritis[7], osteoarthritis[8], ulcerative colitis[9]. Boswellic acids (BAs) also exhibit immunomodulatory[10] and anti-tumor activity[11]. Boswellia extracts are usually obtained from the *Boswellia carteri* Birdw. tree's resin or gum belonging to the family frankincense and

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contain at least 15 triterpene acids[6]. The highly potent anti-inflammatory components of BAs are 3-acetyl-11-keto-β-boswellic acid (AKBA) and 11-keto-β-boswellic acid (KBA) and exhibit 5-lipoxygenase inhibition[12,13], linked to anti-inflammatory activity[5]. Preliminary pharmacokinetic studies of *Boswellia serrata* extract (BSE) following oral administration revealed poor bioavailability, especially of KBA and AKBA owing to poor water solubility and high lipophilicity (1og P = 7 – 10.3)[14,15].

In studies, carried out using Caco-2 cells it was found that BAs showed moderately to poor permeation capabilities, *viz.* KBA possessed moderate permeability, whereas AKBA showed poor permeability through caco-2 cells lines. Studies in rat liver microsomes (RLMs) and human liver microsomes (HLMs) revealed that KBA is extensively first-pass metabolized and converted to hydroxylated derivatives. With RLMs and HLMs, more than 80 % of the initial AKBA concentration remained unmetabolized at the end of the incubation period[4]. BAs are poorly absorbed and extensively metabolized (especially KBA) therefore strategies to improve its bioavailability need to be worked out, so as to gain the necessary therapeutic benefit.

Much research has been carried out over the past years on lipid based formulations with particular emphasis on self emulsifying (SEDDS) or self microemulsifying drug delivery (SMEDDS) systems to improve oral bioavailability of lipophilic drugs. Self microemulsifying formulations are isotropic mixture of oil, surfactant, co-surfactant (or solubiliser) and drug, which can form an emulsion under conditions of GI fluid and motility after oral administration as evidenced by several literature reports[16-18]. These lipidic formulations owe their self emulsifying properties to a negative or low free energy requirement for emulsion formulation[19].

The oral bioavailability of various poorly water soluble herbal actives such as curcumin, co-enzyme $Q_{10,}$ and silymarin has been reported to improve by SEDDS/SMEDDS[17] but BSE formulation as self dispersible formulation (SEDDS/SMEDDS) has not been evaluated so far. Conventional dosage forms of BSE are available which are associated with the disadvantages of poor absorption through the intestine and poor bioavailability.

The objective of the present study was to formulate and evaluate SMEDDS of BSE to enhance aqueous solubility, better absorption profile, enhanced bioavailability and improved pharmacokinetics to ensure effective anti-inflammatory activity of administered BAs. The composition of SMEDDS formulation was optimized using solubility, phase diagram, particle size, drug release and pharmacokinetics studies.

Materials and Methods

Materials

BSE was purchased from M/s Herbosin Corps, Meerut, India. Caprylic/ capric triglycerides (CCTG), Ethyl oleate, Tween 80 and Polyethylene glycol 400 were obtained from M/s Croda India, Mumbai, India. Cremophor EL, Cremophor RH-40, Labrasol, Labrafil M1944CS, Lutrol E-300 and Transcutol P were obtained from M/s BASF, Mumbai. Castor oil was purchased from M/s Sd Fine Chem Ltd, Mumbai. Propylene glycol was purchased from M/s Thomas Baker Ltd, Mumbai and Ethanol was purchased from M/s Jiangsu Huaxi Int Trade Co Ltd, China. All other chemicals used were of analytical grade.

Solubility studies

The solubility of BSE in various components (oils, surfactants and co-surfactants) was determined using saturation method. An excess amount of BSE was added to 5 mL of the vehicle and each was maintained at 25 ℃ for 48 h in a water bath with intermittent stirring[20]. After 48 h, mixture was centrifuged at 3000 rpm for 10 min and the supernatant was passed through a membrane filter (0.45 µm) to remove the insoluble BSE. After suitable dilution with methanol, drug concentration in the filtrate was quantified by HPLC (Waters e2695, USA).

Construction of pseudoternary phase diagram

Pseudoternary phase diagrams were constructed in order to obtain the appropriate concentration ranges of components that can result in large existence area of stable emulsion/ microemulsion. Series of different compositions were prepared with varying concentrations of oils (CCTG and Ethyl oleate), surfactants (Tween 80 and Cremophor EL) and co-surfactants (PEG 400 and Ethanol). For each phase diagram at a specific ratio of S/CoS (i.e., 1:1, 2:1, 3:1, 4:1, 1:4, 1:3 and 1:2 w/w), a transparent and homogenous mixture of oil and S/CoS was formed on vortexing for 5 minutes. The mixtures of oil and S/CoS at certain weight ratios were then titrated with water and visually observed for appearance, spontaneity of emulsification, flowability and phase separation. Phase diagrams were constructed

identifying the transparent self-emulsifying region. Moreover, to investigate the effects of BSE on the self-microemulsifying performance of SMEDDS, phase diagrams were also constructed in the presence of drug using drug-enriched oil as the hydrophobic component.

Preparation of SMEDDS formulations

A series of SMEDDS formulations were prepared using Tween 80 and PEG 400 as the S/CoS combination and CCTG as the oil. In all the formulations, the level of BSE was kept constant (i.e., 1:1 % w/w of the oil weight). Variable proportions of oil (containing solubilised BSE), surfactant and co-surfactant were mixed by gentle stirring. SMEDDS were optimized for drug loading efficiency, stability, appearance and spontaneity in emulsification (emulsification time).

Evaluation of SMEDDS containing BSE

Optimized SEDDS formulation was evaluated for globule size, self-emulsification and precipitation assessment, emulsification time, robustness to dilution, transmittance test, refractive index, viscosity, pH, drug content, effect of pH on drug release and *in vitro* dissolution profile and morphological study.

Emulsion droplet size analysis

One gram of SMEDDS formulation I (F1A) was diluted to 250 mL in a beaker and gently mixed using a magnetic stirrer. The resultant emulsion was then subjected to particle size analysis using Malvern particle size analyser (Worchestershire, UK) equipped with 2000 Hydro MU with a particle size measurement range of 0.02 to 2000 μm. Particle size was calculated from the volume size distribution.

Morphology

The morphology of SMEDDS was studied using transmission electron microscope (TEM) (TECHNAI G 20, 200 KV HR-TEM, FEI Company, Holland). SMEDDS were diluted with distilled water (1:200) and mixed by gentle shaking to form microemulsion. One drop of diluted sample was deposited on a film-coated copper grid and then stained with one drop of 2 % aqueous solution of phosphotungstic acid (PTA), and allowed to dry before observation under the electron microscope.

Robustness to dilution

BSE loaded SMEDDS were diluted 50, 100 and 1000 times with different dilution media, viz. water, hydrochloric acid (HCl) buffer pH 1.2 and phosphate buffer pH 6.8. The diluted emulsions were stored for 24 h and observed for signs of phase separation or drug precipitation, if any.

Self emulsification and precipitation assessment

Different batches of SMEDDS formulation were characterized on the basis of spontaneity of emulsification, appearance and apparent stability of the resultant emulsion. Visual assessment was performed by drop wise addition of 1g of the pre-concentrate (SMEDDS) into 250 mL of distilled water at room temperature. The contents were gently stirred on a magnetic stirrer at ~100 rpm. Precipitation was evaluated by visual inspection of the resultant emulsion after 24 hours. The formulations were then characterized as clear (transparent), non-clear (turbid), stable (no precipitation at the end of 24 h), or unstable (showing precipitation within 24 h).

Determination of emulsification time

The emulsification time of SMEDDS formulation I (F1 A) was determined according to a previously reported method[21]. Isotropic mixture (SMEDDS, 500 mg) was added drop wise to 500 mL of purified water at 37 ^{o}C with gentle stirring provided by dissolution paddle rotating at 50 rpm and visually observed for emulsification.

Transmittance test

Effect of pH on emulsification ability and stability of optimized SMEDDS formulation with respect to dilution was checked by measuring transmittance through UV spectrophotometer (UV-1700, Shimadzu). Isotropic mixture (SMEDDS, 1 g) was diluted to 200 mL with different dilution media, viz. water, HCl buffer pH 1.2 and phosphate buffer pH 6.8 to yield fine emulsion by gentle stirring on a magnetic stirrer. The resulting emulsions were allowed to stand for 2 h and their transmittance was measured at 650 nm.

Determination of refractive index

Refractive index of SMEDDS formulation I (F1 A), before and after dilution with distilled water (1:200) was measured with a thermostated Abbes refractometer (Shijiazhuang Optical Instrument Factory, China).

Viscosity determination

The viscosity of SMEDDS formulation I (F1 A) was determined without dilution using Brookfield viscometer, spindles RV-3 at 30 rpm and 60 rpm at 25±0.5 °C.

pH determination

The pH of SMEDDS formulation I (F1 A), before and after dilution with distilled water at (1:200) was determined using Labtronix LT-11 Digital pH meter at 25±0.5 °C.

Drug content in dry BSE and SMEDDS formulation

The drug content, total BAs in BSE and SMEDDS formulations, was determined by non-aqueous titration method. BSE/SMEDDS formulation (1 g) was transferred to a 250 mL dry iodine flask. To this, 50 mL of dimethyl formamide and 4-5 drops of thymol blue indicator was added. The solution was then titrated with 0.1 M sodium methoxide till the end point was reached. A blank titration was also performed.

HPTLC analysis of AKBA

The concentration of AKBA in the BSE and SMEDDS formulation was determined by HPTLC analysis. The HPTLC consisted of CAMAG TLC sample applicator, development glass tank-Twin Trough chamber 20 cm x 10 cm and CAMAG TLC scanner 3, stationary phase for HPTLC plates was silica gel 60 F 254 (Merck). The chromatographic conditions were mobile phase Chloroform : Methanol (9:1 v/v); application volume 10 µL and detection at 254 nm.

Estimation of AKBA in BSE and SMEDDS Formulation

Suitable amount of BSE and SMEDDS formulations were transferred to a 10 mL volumetric flask and diluted with 5 mL of methanol. The mixture was sonicated for 15 minutes and then diluted to 10 mL with methanol. The mixture was filtered through a 0.45 µm membrane filter to remove particles. The first 5 µL of the filtrate was discarded and the subsequent was collected. Appropriate aliquots of filtrates were used for the HPTLC analysis[17].

Effect of pH on release of AKBA from BSE, marketed formulation and SMEDDS formulation

The release of AKBA from the BSE, Marketed formulation and the Test formulation F1 A was determined in different media (water, HCl buffer pH 1.2, Phosphate Buffer pH 6.8) and was estimated quantitatively by HPTLC to determine the effect of pH on the release of AKBA.

***In vitro* dissolution studies**

One g of SMEDDS formulation I (F1 A) was filled in size '00' hard gelatin capsules and evaluated for *in vitro* release using USP XXIII apparatus I at 37±0.5 °C and 100 rpm. The dissolution medium was 250 mL HCl buffer pH 1.2. Aliquots (2.0 mL) were collected at 15, 30, 45, 60, 90 and 120 min, respectively, while same volume was replaced with fresh buffer. The amount of drug released was determined using HPTLC and the results were compared with those of plain BSE. For determination of the *in vitro* dissolution of plain BSE, medium was changed to HCl buffer pH 1.2 containing Tween 80 (equivalent to the amount used in the formulation)[22].

Anti-inflammatory activity-carrageenan induced rat paw edema

The anti-inflammatory activity of BSE loaded SMEDDS was compared with plain BSE using a carrageenan-induced paw edema test[23]. The study was carried out as per the protocol approved by Institutional Animal Ethics Committee (BBDNITM/IAEC/Clear/13/2009). Twenty-four albino rats (Wistar strain) of either sex (120-200 g) were randomly divided into four groups, each consisting of six rats and fasted overnight with free access to water. Group I received Tween 80 (1 %, 1 mL/kg) orally as a control group, Group II received BSE suspended in 1 % Tween 80 at a dose of 250 mg/kg administered orally, Group III received BSE loaded SMEDDS (equivalent to 250 mg/kg BSE) and Group IV received Etoricoxib (10 mg/kg) suspended in 1 % Tween 80 orally as a standard drug. After 1 h, acute inflammation (edema) was induced in all groups by injecting 0.1 mL of 1 % w/v carrageenan into the subplantar region of the right hind paw of rats. Mean paw volume was measured 1 h prior to carrageenan injection using plethysmometer and at 1, 2, 3, 4 and 5 h after the carrageenan injection. Edema was expressed as the mean increase in paw volume relative to control animals. The percentage inhibition of edema was calculated by the following equation.

Percentage inhibition of edema = $100\ (1\text{-}V_t/V_c)$

where V_c and V_t are the edema volume in control and tested group, respectively.

Results and Discussion

Solubility studies

The formulation and *in vivo* performance of SMEDDS formulation widely depends on two critical factors, first is the drug load per gram of formulation and second, avoidance of drug precipitation in the gut lumen on dilution. Both the factors are dependent on BSE solubility in various formulation components and the maximal volume reasonably encapsulated in a

unit dosage form. Therefore, a component having a high solubilisation capacity for the drug was a prerequisite for successful development of SMEDDS formulation. Results of solubility study revealed that CCTG and ethyl oleate have highest solubilisation capacity for BSE, followed by Tween 80, Cremophor EL, PEG 400 and ethanol. The solubility of BSE in CCTG and ethyl oleate was determined in terms of AKBA (standard) and was found to be 1.648±0.13 and 1.536±0.11 % w/v, respectively. Besides the higher solubility of BSE in CCTG could form a stable microemulsion when diluted with water along with lesser possibility of drug precipitation. Higher drug solubilisation in CCTG as compared to other vehicles as confirmed by solubility studies (quantified by HPLC) and also the polarity of the poorly soluble drugs favouring their solubilisation in small/medium molar volume oils, such as medium-chain triglycerides or mono- or diglycerides could be one the reasons for stability[24,25]. Further, higher solubilisation capacity of the system resulted in a stable emulsion system, which remained unaffected by dilution, thus displaying robustness to dilution.

Construction of Pseudoternary Phase Diagrams

A series of SMEDDS formulations were prepared and their self-emulsifying properties were observed visually. Pseudoternary phase diagrams were constructed in order to obtain appropriate concentration ranges of components in the areas of forming stable self emulsion/microemulsion. In the present study, both CCTG and ethyl oleate were tested for phase behaviour studies with Tween 80/PEG 400 and Cremophor EL/ethanol as the S/CoS mixture. The phase diagrams of different systems containing Tween- 80 and Cremophor EL as surfactant and different oils (CCTG and ethyl oleate) and co-surfactants (PEG 400 and ethanol) are shown in Fig. 1. CCTG gave a wider clear miroemulsion region than ethyl oleate at different S/CoS ratios. Thus, based on solubility and microemulsion region, CCTG was selected as the preferred vehicle for the further optimization of formulation. The efficiency of emulsification was good when the S/CoS concentration was more than 60 % of SMEDDS formulation. However, it was observed that increasing the surfactant ratio resulted in increased viscosity of the system and loss of flowability of system. Thus, S/CoS ratio between 3:1 and 4:1 was selected for the formulation study. Also, increasing the concentration of the co-surfactant, PEG 400, within the self-emulsifying region promoted the spontaneity of the self-emulsification process. However, higher concentration of PEG 400 is associated with two major problems, i.e., incompatibility with hard gelatin capsules[26] and loss of solvent capacity when the formulation is dispersed in water which may lead to the risk of drug precipitation[27]. Thus, while optimizing the S/CoS ratio, the concentration of PEG 400 was kept as low as possible (<15 % w/w of total formulation).

It has been reported that incorporation of drug to the SMEDDS formulation may have some adverse effect on the self-emulsification performance[28]. To identify the self emulsifying regions and to optimize the concentration of oil, surfactant and co-surfactant in the SMEDDS formulations, pseudo-ternary phase diagrams were initially constructed without BSE. When BSE was included, the self-emulsification area was found to narrow

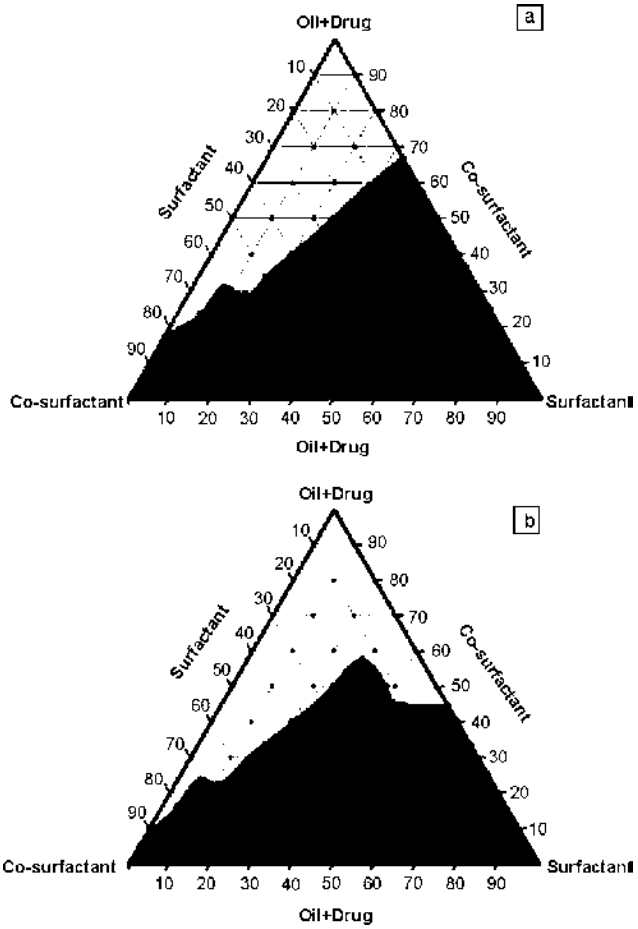

Fig. 1—Pseudoternary phase diagram of system with the different components, (a) oil (drug-enriched CCTG), surfactant (Tween 80), and cosurfactant (PEG 400), and (b) oil (drug-enriched Ethyl oleate), surfactant (Cremophor EL) and cosurfactant (Ethanol).

down. Such change in self emulsification area could possibly be due to hydrophobic nature of the drug (1og P = 7 – 10.3) which lead to net increase in concentration of the lipid phase in system. Consequently, a higher proportion of S/CoS ratio was required for stabilization.

Selection of optimized formulation

The optimized formulation was selected based on the drug loading efficiency, stability, appearance and spontaneity in emulsification (emulsification time). The composition of the optimized formulations is given in Table 1. Since, the formulation with a maximum drug load is to be selected, formulation I (F1 A) having 25 % w/w of BSE was selected. Formulation I (F1 A) was superior in terms of transparency, stability and contained less surfactant concentration than formulation IV (E2 C). Hence, formulation I (F1 A) was selected as the optimised test formulation and was further evaluated for various other *in vitro* and *in vivo* parameters.

Characterization of optimized SMEDDS formulation

Morphology and droplet size analysis

Morphology of the emulsion formed from optimized SMEDDS containing BSE was studied by TEM (Fig. 2). The emulsion droplets appeared as round and globular without aggregation. The mean droplet size of selected SMEDDS formulation I (F1 A) was found to be 494.8 nm (Plate 1).

Table 1—Composition of optimized SMEDDS formulations

Formulation composition	I (F1 A) (%w/w)	IV (E2 C) (%w/w)
Drug (BSE)	25	22.22
CCTG	25	-
Ethyl oleate	-	22.22
Tween 80	37.5	-
PEG 400	12.5	-
Cremophor EL	-	44.44
Ethanol	-	11.11

Self emulsification and precipitation assessment

Primarily SMEDDS formulation can be examined for their self-emulsification ability by visual assessment[29] by determining the rate of emulsification. An increase in the proportion of CCTG in the composition resulted in a decreased self-emulsification time, which was assumed to be due to relative decrease in proportion of surfactants.

SMEDDS containing 0-20 % (w/w) of Tween 80 did not form an emulsion, however, SMEDDS containing higher than 25 % (w/w) of Tween 80 rapidly formed an emulsion which appeared to be stable and no coalescence, phase-separation and drug precipitation were noted after centrifugation. Increase in the proportion of CCTG in the composition up to a concentration of 33 % w/w, resulted in clear dispersion beyond which a turbid dispersion resulted. CCTG at a concentration more than 57 % w/w was found to show coalescence and was thus not stable,

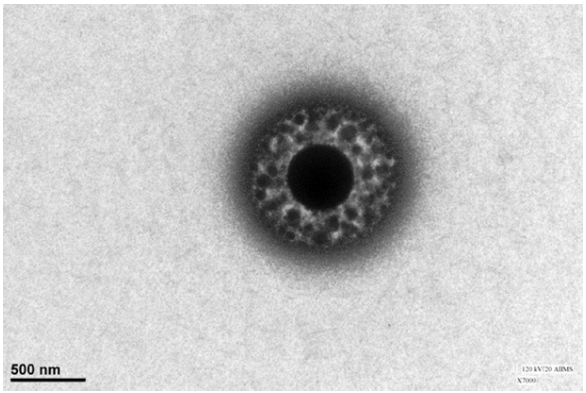

Plate 1—Image showing particle size of optimized formulation

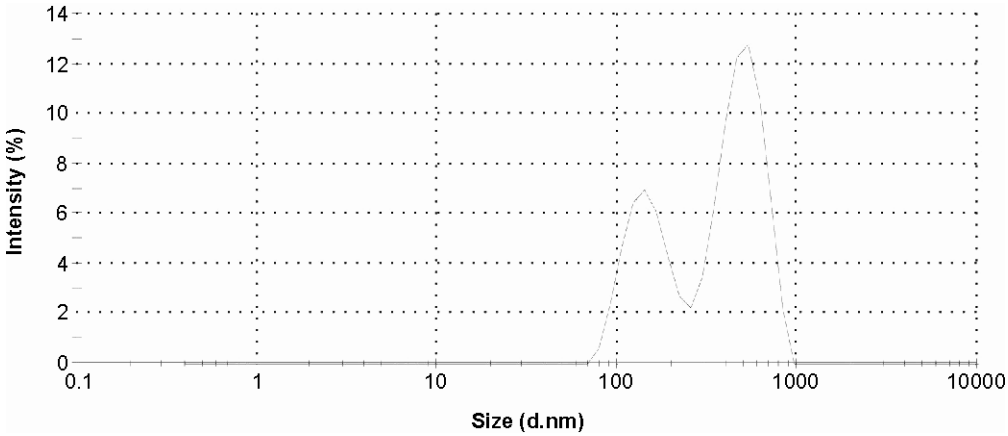

Fig. 2—Transmission Electron Microscopic image of formed microemulsion globule.

owing to the reduced surfactant concentration. This could be attributed to an increase in the free energy of system as a result of increase in surface area of smaller globules or due to lack of a physical barrier against coalescence of globules in presence of insufficient proportions of surfactant. Surfactants stabilize the emulsion system by dual mechanism, i.e., by reducing the free energy increased due to increase in surface area of system and by coating oil globules thus providing physical barrier by polar heads of surfactant molecules against the coalescence of globules.

Robustness to dilution

Self-emulsions resulting from dilution of BSE loaded SMEDDS with different dilution media were robust to all dilutions and did not show any separation/precipitation even after 24 h of storage.

Emulsification time

The rate of emulsification is an important index for assessment of the efficiency of emulsification i.e., the SMEDDS should disperse completely and quickly when subjected to aqueous dilution under mild agitation. Emulsification time study showed that all the formulae employed could emulsify within 60 seconds. Among the tested formulations, the optimized SMEDDS formulation I (F1 A) could emulsify within 30±2 S indicating rapid emulsification ability.

Transmission Test

Transmittance of light from optimized SMEDDS formulation I (F1 A) after diluting it 200 times with distilled water, HCl buffer pH 1.2 and Phosphate buffer pH 6.8 was observed at 650 nm. The clarity and transparency of the formed emulsions was not affected by the change in pH of the medium.

Determination of pH, refractive index and viscosity

The pH of the SMEDDS formulation I (F1 A) and microemulsion obtained after dilution with distilled water (1:200 w/w) was found to be 4.85±0.02. Further, refractive index of the SMEDDS formulation I (F1 A) and microemulsion obtained after dilution with distilled water (1:200 w/w) was found to be, 1.473±0.12 and 1.33±0.16, respectively, at 25±0.5 °C.

In the context of the capsule filling process of a SMEDDS formulation, viscosity of filled material is important factor. If the viscosity is too low a good seal may not be formed due to splashing which could contaminate the area of overlap between the capsule body and cap. The recommended guideline for viscosity of filling liquids/semi-solids for hard gelatin capsules at the temperature of filling is, 0.1-1 Pas[30,31]. The viscosity of SMEDDS preconcentrate was found to be 0.6167±0.16 Pas (18.5 % torque) and 0.6233±0.25 Pas (37 % torque) indicating suitability of the formulation for filling into capsules.

Drug content

The drug content, total BAs in BSE and in the test formulation F1 A was determined by non-aqueous titration method and was found to be 66.44 and 16.52±1.18 %, respectively. In terms of assay percentage, drug content (% Boswellic acid) in the test formulation F1 A was found to be 99.458 % when calculated against plain BSE indicating good loading capacity of SMEDDS formulation.

HPTLC analysis of AKBA in BSE and SMEDDS Formulation I

The concentration of AKBA in the dry BSE and test formulation F1 A was determined by HPTLC and was found to be 3.94±0.22 and 0.95±0.17 % w/w, respectively.

In the present work, main emphasis was given to the formulation of a SMEDDS system of BSE for enhanced solubility and absorption of major BAs for improved anti-inflammatory activity. AKBA was designated as one of standard marker component for identification and quantification of BAs in BSE as per United States Pharmacopoeia and Indian Pharmacopoeia (IP). Hence, official method for determination of AKBA was performed as provided in IP[32]. This is a well justified and validated method when compared to other tedious and costly methods like LC-MS.

Effect of pH on release of AKBA from BSE, marketed formulation and SMEDDS formulation I (F1 A)

The release of AKBA in water, hydrochloric acid buffer pH 1.2 and Phosphate Buffer pH 6.8 was established by HPTLC chromatogram which demonstrated that Plain BSE and Marketed formulation showed negligible release after 60 minutes in all mediums whereas, BSE loaded SMEDDS showed rapid release in all mediums, i.e., the release of AKBA from test formulation F1 A in water, HCl buffer pH 1.2, phosphate buffer pH 6.8 after 60 min was found to be 56.84±2.16, 60±1.56 and 53.68±1.64 %, respectively, showing no major difference in the percentage release (Plate 2). The SEDDS formulation showed pH independent and

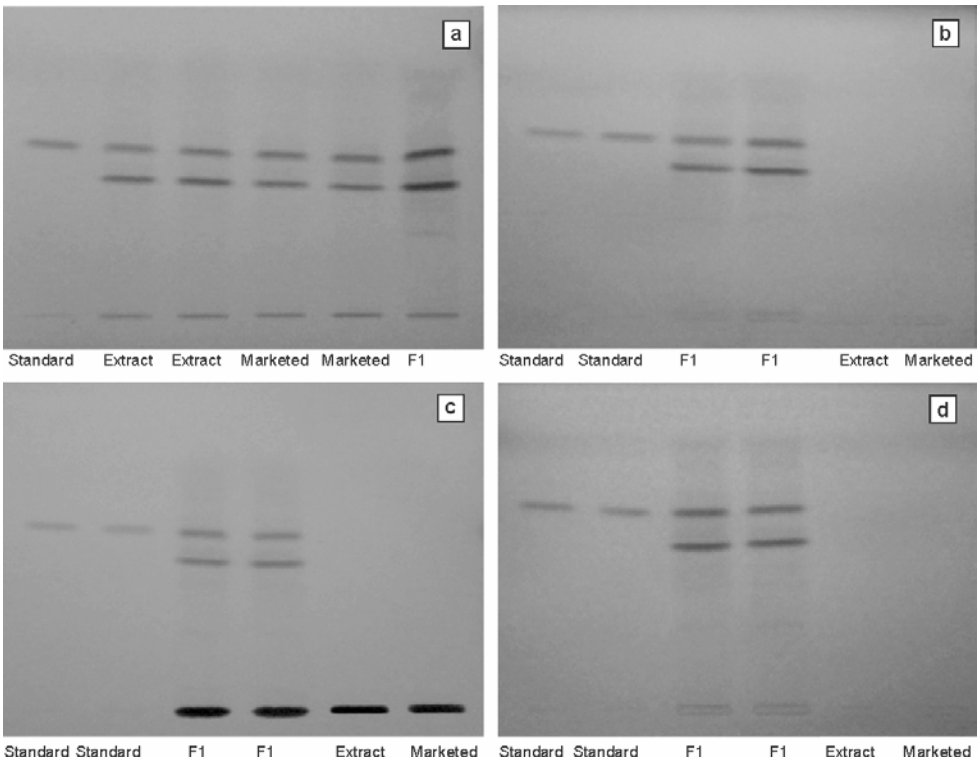

Plate 2—Identification of AKBA in a) extract, marketed formulation and SEDDS formulation I (F1); Identification of AKBA in different dilution media, b) Water, c) 0.1N HCl and d) Phosphate buffer pH-6.8 from extract, marketed formulation and SEDDS formulation I (F1) under UV light at 254 nm

higher drug release as compared to the marketed formulation and the plain BSE indicating superiority of SEDDS formulation.

In vitro drug release studies

Drug release from the SMEDDS formulation I (F1 A) was found to be significantly higher as compared with that of plain BSE. Plain BSE showed negligible release even after 120 min in 0.1 N HCl. The SEDDS formulation showed rapid dissolution in 0.1 N HCl, with more than 90 % released after 120 minutes. The spectral scan of AKBA and Formulation (F1 A) exhibited in overlapping spectra, demonstrated the release of AKBA from formulation at different time intervals, whereas no such overlapping zone was found in case of plain BSE, which showed almost negligible release of AKBA from plain BSE. It could be deduced that the SMEDDS formulation resulted in spontaneous formation of a microemulsion with a small droplet size, which permitted a faster rate of drug release than that of plain BSE. Thus, this greater availability of dissolved BSE from the SMEDDS formulation could lead to higher absorption and thus higher oral bioavailability.

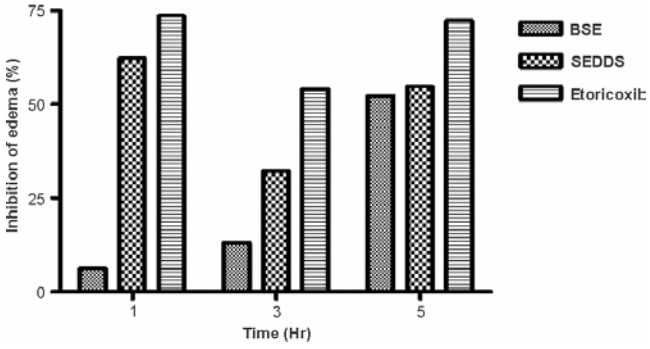

Fig. 3—Plot showing percent inhibition of carrageenan induced rat paw edema by Plain BSE and BSE loaded SEDDS and etorcoxib (standard) measured at 1, 3 and 5 h after carrageenan injection as compared to vehicle control.

Anti-inflammatory activity-carrageenan induced rat paw edema

Edema developed following injection of carrageenan serves as an index of acute inflammatory changes and can be determined from differences in the paw volume measured at 0, 1, 2, 3, 4 and 5 hours after injection. As shown in Fig. 3, BSE loaded SMEDDS significantly (p<0.001) decreased paw edema at 1 h after carrageenan injection, with inhibition level of 62.2±3.26 % when compared to the control group of animals, whereas, plain BSE showed

only 6.19±1.16 % inhibition at similar dose level. At 3 h, plain BSE showed 13.03±1.65 % inhibition of edema with a significant (p<0.01) decrease in paw volume in comparison to BSE loaded SMEDDS which showed 32.20 % inhibition. At 5th hour percent inhibition in paw edema, with values of 52.19±3.72 and 54.7 % for plain BSE and BSE loaded SMEDDS, respectively was found to be similar. Etoricoxib (standard) also showed 73.6±2.79, 59.2±3.45 and 74.6±1.95 % inhibition of edema at 1, 3 and 5 h, respectively as compared to vehicle control.

After oral administration, BSE loaded SMEDDS showed immediate effect and by 3rd hour more than 90 % drug was available for anti-inflammatory activity as supported by *in vitro* dissolution data. Only marginal effects were observed in case of plain BSE at the end of 3 h (as negligible release was observed after 2 h during *in vitro* evaluation), clearly demonstrating the enhanced solubility of BSE loaded SMEDDS over plain BSE, thus potentiating the immediate absorption of major BAs responsible for anti-inflammatory activity. Data suggests that time taken by plain BSE to demonstrate significant anti-inflammatory activity was 5 h whereas the same was attained by BSE loaded SMEDDS after 1 h, thus elucidating the importance of such a system over conventional dosage forms.

SMEDDS formulation showed immediate solubilisation and faster release as compared to the conventional marketed formulations of BSE as evident from *in-vitro* and *in-vivo* study of up to 3 hours. Conventional formulations of BSE exhibited problems of poor solubility in the GI fluid and thus poor permeability of key active components such as AKBA. A very high dose of conventional dosage form of BSE is recommended to attain therapeutic concentrations of key components KBA & AKBA (approximately 400 mg thrice daily). By formulating SMEDDS of BSE both the problems of poor solubility and poor permeability of AKBA were overcome. Moreover, reduced dosage and higher therapeutic concentration could be achieved.

Conclusion

BSE was successfully formulated as SMEDDS in an attempt to increase its dissolution rate, solubility, and oral absorption which may ultimately lead to enhanced bioavailability. The composition of the optimized formulation was as follows: 37.5 % surfactant (Tween 80), 12.5 % co-surfactant (PEG 400), 25 % oil (CCTG) and 25 % drug (BSE). When diluted with water, BSE-loaded SMEDDS spontaneously formed small droplets with an increased dissolution rate, increased solubility and ultimately, increased bioavailability of a poorly water-soluble drug, BSE. Dissolution percentage of BSE in SMEDDS, in hydrochloric acid buffer pH 1.2 was significantly higher than that of plain BSE. The developed formulation, i.e. BSE loaded SMEDDS showed better inhibition of inflammation compared to plain BSE treated group in carrageenan-induced rat paw edema model, attributed to the enhanced absorption of BSE loaded SMEDDS as compared to plain BSE. The BSE loaded SMEDDS was produced via a simple process and further, it can be used as a promising formulation for drugs that are poorly soluble and/or poorly permeable to achieve a significant improvement in the bioavailability.

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