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Formulation Development of a Transdermal Self-Microemulsifying Drug Delivery System of Bosutinib

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ABSTRACT

Tyrosine kinase inhibitors treat chronic myeloid leukemia but have many negative effects. These BCS Class II or IV medicines have low oral bioavailability due to poor solubility, permeability, or both. As P-glycoprotein substrates and hepatic presystemic metabolism inactivators, these medicines have another drawback. The present work sought to overcome the limitations of tyrosine kinase inhibitor bosutinib. A transdermal system with the medication put into a SMEDDS was created. The investigation began with solubility and emulsification studies to choose the proper oil (oleic acid), surfactant (Tween 20), and cosurfactant (Transcutol® P). The microemulsion zone was identified using ternary phase diagrams. The resultant SMEDDS exhibited a narrow polydispersity index and a droplet size of 45.66 ± 2.4 nm. The formulation's zeta potential prevented aggregation during storage, as shown by thermodynamic stability investigations under physical and thermal stress. Dispersibility and dilution experiments showed that the formulation formed the microemulsion instantly in vivo. BOS-loaded liquid SMEDDS was gelled with 2% HPMC K15M to make G-SNEDDS. The liquid SMEDDS and G-SMEDDS had similar properties. In vitro dissolution showed that near to 100% drug release could be achieved in 30 mins, compared to 5–6% for the simple medication. The formulation's in vitro permeability testing employing an artificial membrane demonstrated a 2.18-fold increase in drug penetration compared to the basic drug suspension.

Keywords: Self-microemulsifying, Bosutinib, oleic acid, Labrasol, topical gel.

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INTRODUCTION

The effectiveness of a drug largely depends upon the route by which it is administered to the patient. With the innovations over the year, the drug could be practically delivered to all parts of the body by a myriad of routes. Yet, it is the oral route that enjoys maximum success. This could be attributed to the advantages that it offers over routes, like, pain-free delivery as compared to parenterals, convenience, and self-administration is easier than rectal or ocular delivery, and most importantly its cost-effectiveness. It also offers technological advantages such as least sterility constraints, flexibility in designing, and ease of productions. Formulations can also be tailored to enhance drug delivery to specific regions in the upper or lower gastrointestinal tract (GIT)[1,2]

Self-emulsifying drug delivery systems (SMEDDS) are pre-concentrates or anhydrous form of emulsions which when introduced into an aqueous phase would form an emulsion under gentle agitation. Key components of SMEDDS are the drug along varying proportions of oils, surfactants, and hydrophilic co-surfactants. These formulations demonstrate high stability as well as generation of very large surface area due to small droplet sizes formed on dispersion which leads to an overall improvement in bioavailability by solubility enhancement. These systems have shown tremendous potential in improving the bioavailability of drug molecules falling under the BCS into class II and IV[3]. The other advantages that the system offers are ease of manufacturing scalability, long shelf life due to improved stability, palatability & patient compliance are no longer of concern, and the drugs would show a quick onset of action without being affected by food. They can be made into patient convenient dosage forms for convenience. They can be formulated into liquid, semi-solid as well as solid dosage forms[4].

Tyrosine kinase inhibitors (TKI) have shown potential as a targeted treatment for malignancies. They competitively inhibit ATP at the catalytic binding site of tyrosine kinase. The first tyrosine kinase to be clinically approved was imatinib in the year 2001[5]. Since then, in the past 20 years, 70 more drugs have been given introduced, which have proven to be effective in non-cancerous conditions as well[6]. Imatinib became the pioneering treatment for chronic myeloid leukemia (CML) with a recommended dose of 400 mg/day. Though it is well tolerated in patients but there have been reports of mild to moderate hematological toxicity like myelosuppression along with fluid retention and oedema especially eyelid oedema that can lead to ptosis, blepharoconjunctivitis, visual obstruction, or even retinal oedema, nausea, vomiting, fatigue, fever, diarrhea, muscle cramps, and skin disorders like eruptions and hypopigmentation[7]. On the more serious side, it has been reported to have caused heart failure and left ventricular dysfunction, hypophosphatemia leading to bone alteration, hepatotoxicity with elevation of liver enzymes, and peripheral neuropathy[8]. Although it has become a therapeutic standard for the newly diagnosed patients of chronic myeloid leukemia. Yet nearly one-third of patients show an inferior response to imatinib, either failing to respond to primary therapy or demonstrating resistance after an initial response. Studies by various researchers like Lavallade et al.[9] and Baccarani et al.[10] have reported a loss of complete cytogenic response in more than 30% patients. Thus, the next generation of TKI have been introduced.

Despite wide application, many TKI on the market and in development show low bioavailability after oral administration. This may reflect high first pass metabolism or low absorption, or both. Within the compound class, highly lipophilic and poorly water-soluble molecules are overrepresented and the potential for solubility limited absorption and altered pharmacokinetics when administered with food has been well documented[11]. As the majority of TKI are weakly basic with significant pH-dependent solubility, coadministration of TKI with agents that reduce gastric pH, including antacids, H_2 antagonists, and proton pump inhibitors (which are commonly prescribed in cancer therapy), can also interfere with TKI absorption[12]. These factors illustrate the biopharmaceutical complexity of this class of compounds and the potential risk of adverse effects or poorer therapeutic outcomes when drug absorption is significantly affected by food or coadministration with other medicines.

Bosutinib (BOS), is 3-Quinolinecarbonitrile, 4-[(2,4-dichloro-5-methoxyphenyl) amino]-6-methoxy-7-[3-(4-methyl-1-piperazinyl) propoxy]-, hydrate (1:1). Its chemical formula is C26H29Cl2N5O3•H2O. It has been indicated for the treatment of adult patients with chronic, accelerated, or blast phase Philadelphia chromosome-positive (Ph+) CML with resistance or intolerance to prior therapy[13]. It inhibits the Bcr-Abl kinase that promotes CML. The usual dose is 500 mg to be given once a day with food which can be escalated up to 600 mg per day with food. On regular administration, the time for peak plasma concentration is 4 to 6 hrs. it exhibits dose-dependent increase in C_{max} over than range of 200 to 800 mg dose. The average C_{max} value observed is 200 ng/mL and the area under the curve (AUC) was 3650 ng.h/mL. It was noted that when BOS was given with a high fat meal, the C_{max} and AUC increased 1.8- and 1.7-fold, respectively. BOS has a mean apparent volume of distribution of 6080 ± 1230 L[14]. It is a highly plasma protein bound molecule which did not dependent on the concentration of the drug. It is a known substrate and inhibitor of P-glycoproteins[15]. The half-life of BOS is 22.5 hrs. the absolute bioavailability was found to be 34%. It is primarily metabolized by CYP3A4 (40 – 50%) and all the metabolites are deemed inactive. BOS is associated with gastrointestinal toxicity including diarrhoea, nausea, vomiting, abdominal pain, myelosuppression, and hepatic transaminase elevations. Fluid retention occurs with BOS and may manifest as pericardial effusion, pleural effusion, pulmonary oedema, and/or peripheral oedema[16]. As per the biopharmaceutical classification system, it has been classified as a class IV molecule owing to its low aqueous solubility of 0.0095 mg/mL and a low apparent permeability[17]. Based on the studies performed using Caco-2 cell monolayer, it was found that the apparent permeability of BOS from the apical to the basolateral side is >1 X 10-6 cm/sec, but a low apparent permeability is expected that could be attributed to efflux transporters. It shows a pH dependent solubility, wherein the solubility was 11.03 mg/mL at pH 1.2 and it went on decreasing above pH 5.0, such that at pH 6.8 it was found to be 0.02 mg/mL. Thus, the aim of the present work is to develop a topical gel of bosutinib-loaded self-microemulsifying drug delivery system based on the principles of optimization.

MATERIALS AND METHOD

Bosutinib was received as a gift sample from Alembic Pharmaceuticals, Vadodara, India. Oleic acid and Tween 20 were procured from Finer Chemicals Ltd., India and Transcutol® P was obtained from Gattefosse, India. All other materials and chemicals used were of either pharmaceutical or analytical grade.

Solubility studies

Equilibrium solubility of BOS was measured in various oils, surfactants, and co-surfactants. An excess amount of BOS was added to each of the selected vehicle (10% w/w, 1 g) and vortexed for 30 secs followed by continuous shaking in a thermostatically controlled rotary shaking water bath (Remi Instruments, India) for 48 h at 25 ± 1°C. Following attainment of equilibrium, the mixture was centrifuged at 3000 rpm (88.04 × g) for 15 min and filtered through a 0.45 µ filter membrane. The filtrate was diluted appropriately using methanol, and the concentration of BOS was determined by UV-Visible spectrophotometry (UV-1800, Shimadzu, Japan) at 269 nm using methanol as the blank. Each experiment was performed in triplicate.

Preliminary screening of surfactants for their emulsifying ability

300 mg of surfactant was added to 300 mg of selected oily phase. The mixture was gently heated at 50 °C for homogenizing the components. The isotropic mixture, 50 mg, was accurately weighed into a stoppered Erlenmeyer flask and diluted with double distilled water to 50 ml to yield fine emulsion. The ease of formation of emulsions was monitored by noting the number of flask inversions required to produce a uniform emulsion. The resulting emulsions were observed visually for the relative turbidity or phase separation. The emulsions were allowed to stand for 2 h and their transmittance was assessed at 638.2 nm by UV-1800 double beam spectrophotometer (Shimadzu, Japan) using double distilled water as blank [18].

Preliminary screening of co-surfactant for their emulsifying ability

The turbidimetric method was used to assess the relative efficacy of the co-surfactant to improve the self-emulsification ability of the surfactants. Briefly, surfactant (Tween 20), 0.2 gm was mixed with 0.1 gm of cosurfactant and 0.3 gm oil (oleic acid), the mixture was homogenized with the aid of gentle heat (45–60 °C) and vortexing. The isotropic mixture, 50 mg, was accurately weighed and diluted to 50 ml with double distilled water to yield fine emulsion. The ease of formation of emulsions was noted by noting the number of flask inversions required to give uniform emulsions. The resulting emulsions were observed visually for the relative turbidity and phase separation. The emulsions were allowed to stand for 2 hrs and their transmittance was measured at 638.2 nm by UV-1800 double beam spectrophotometer (Shimadzu, Japan) using double distilled water as blank. As the ratio of co-surfactants to surfactant/s is the same, the turbidity of the resulting microemulsion will help in assessing the relative efficacy of the co-surfactants to improve the self-emulsification ability of surfactant/s[18].

Construction of ternary phase diagram

A ternary phase diagram of three-component mixtures of oil, surfactant/co-surfactant, and water was developed at ambient temperature (25 °C). A series of self-micro emulsifying system were prepared with varied weight ratios of oil, surfactant, and co-surfactant. SMEDDS with various concentration ratio of oils (5-50%), surfactant (50-95%) and co-surfactant (0-30%) were prepared. The oily phase was mixed with the surfactant/co-surfactant mixture and the dispersion was homogenized in a shaking water bath 37°C for 10 minutes. From each mixture, 1 g of the sample was diluted up to 50,100, 150, 200, or 250 mL with distilled water and mixed gently with magnetic stir bar. If a clear or bluish micro emulsion is rapidly formed of globular size 200 nm or lower, it was considered in the microemulsion region of the diagram. The studies were performed in triplicate. The effect of BOS on the self-emulsifying performance of SMEDDS was studied by adding the formulation amount (40% w/w) to the boundary composition of the self-emulsifying domain of the ternary phase plot.

Preparation of Liquid-SMEDDS

Liquid-SMEDDS (L-SMEDDS) was prepared by mixing the required amount of BOS in the oil, after it’s complete solubilization, the surfactant and co-surfactant were added. The mixture was gently stirred with the aid of a magnetic stirrer till a transparent homogenous solution was obtained.

EVALUATION OF LIQUID-SMEDDS

Measurement of % transmittance

1 mL of SMEDDS was diluted with 250 mL of distilled water and the resulting microemulsion was observed visually for any turbidity or phase separation. Thereafter, % transmittance was measured at a wavelength of 638.2 nm using UV–Vis. Spectrophotometer (UV-1800, Shimadzu, Japan).

Effect of pH

1 mL of SMEDDS was diluted with 250 mL of 0.1 N HCl (pH 1.2) and phosphate buffer pH 6.8 and the resulting microemulsion was observed visually for any turbidity or phase separation.

Dye solubilization test

A water-soluble dye (eosin) was sprinkled onto the surface of the prepared SMEDDS and observed for spontaneous dispersion to confirm the oil in water nature of the emulsion.

Time for self-emulsification

The formulation (1 mL) was dispersed in 500 mL of water in a dissolution test apparatus and gently mixed by the paddle element rotating at 50 rpm. The assembly was maintained at a temperature of 37°C. The time for self-emulsification was visually observed and noted.

Droplet size

1 mL of the prepared formulation was diluted 250-times with distilled water. The globule size of the microemulsion thus formed was determined by photon correlation spectroscopy that analyzes the fluctuations in light scattering due to Brownian motion of the particles, using a Zetatrac particle size analyser (Mictotrac, USA). Light scattering was monitored at 25°C at a 90° angle.

Cloud point

1 mL of the prepared formulation was diluted 250 times with distilled water. It was then placed in a water bath whose temperature gradually increased at the rate of 2 °C/min. The change in the appearance of the SMEDDS from clear to slightly turbid (cloudy) was observed visually and confirmed by measuring the % transmittance using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan) operating at 638.2 nm.

Thermodynamic stability studies

The motive of carrying the thermodynamic stability studies was to evaluate the effect of temperature variation on SMEDDS formulations. The thermodynamic stability study of SMEDDS formulations was evaluated by exposing them to different stress conditions through three freeze-thaw cycles (−21 and +25 °C) and heating-cooling cycles (4 and 45 °C) with the storage at each temperature of not less than 48 h. The SMEDDS formulations were also subjected to centrifugation stress by centrifugation at 3500 rpm for 30 min in a microcentrifuge (Remi, India), and the extent of phase separation or any instability problems were observed. The stability studies were each performed in triplicate.

Dispersibility test

The formulation (0.1 mL) was mixed with distilled water to cause dilutions of 100, 200, and 1000 times. A visual observation among those listed below in table 9 was made.

Table I: Visual observation and classification of self-emulsifying formulations based on their ability and speed of forming a dispersion

Sr. No.	Dispersibility and appearance	Grade	Time to self-emulsify
1	Rapidly forming nano or microemulsion having a clear or bluish appearance.	A	Within 1 min
2	Rapidly forming, slightly clear emulsion having a bluish-white appearance	B	Within 1 min
3	Fine milky emulsion that forms moderately fast	C	Within 2 min
4	Dull, greyish-white emulsion having a slightly oily appearance that is slow to emulsify	D	Within 3 min
5	Exhibit poor or minimal emulsification with large oil droplets present on the surface.	E	Within 3 min

Grade A and B formulation will remain as nano- or microemulsion in the GIT whereas those falling under Grade C would form an emulsion with a larger droplet size[19].

Physical robustness to dilution

Formulation was exposed to different fold-dilutions (50, 100, and 250) using two different media (distilled water and 0.1 N HCl). % Transmittance was measured spectrophotometrically at 638.2 nm. The diluted microemulsions was stored for 6 hours and monitored for any physical changes like precipitation or phase separation.

Drug content

BOS content in the BOS loaded L-SMEDDS was determined by dissolving a microemulsion equivalent of 10 mg of BOS in methanol (50 µg/mL). The samples were subsequently sonicated and aliquots were filtered using a 0.45 µm filter. It was then analyzed using a UV-visible spectrophotometer at a wavelength of 269 nm. The study was performed in triplicate. The drug content was determined using the following equation:

$$\%\ \text{drug content} = \frac{\text{Observed drug content}}{\text{Theoretical drug content}} \text{X}100 \quad \textbf{Equation 1}$$

Conversion of liquid SMEDDS to gel SMEDDS (G-SMEDDS)

Optimized BOS loaded L-SMEDDS was converted to G-SMEDDS by adding SMEDDS equivalent to 1 g of the drug-loaded L-SMEDDS into an aqueous solution containing 1% gelling agent and preservatives. Various gelling agents like HPMC 15CPS, HPMC 50CPS, HPMC K15M, Carbopol® 934, Carbopol® 940, and xanthan gum were screened[20]. The gelling agent was dispersed slowly in water using an overhead stirrer, following which all the formulations were allowed to swell for 30 mins. Following this the liquid SMEDDS formulation was added and was allowed to gel for 2 hours, except in the case when Carbopol was used as the gelling agent, wherein 50% v/v triethanolamine was used to neutralize the gel to cause gelation. The concentration of the gelling agent leading to the formation of the gel with the most desirable characteristics was then varied (0.5-2%) to finalize the formulation.

Evaluation of G-SMEDDS

Macroscopic evaluation

The gel of BOS loaded L-SMEDDS was visually inspected for its appearance, homogeneity, and consistency. Spreadability was assessed by placing 1 g of the gel onto a glass slab in a 1 cm circle drawn in the center, over which another slab was placed and a weight of 500 g was kept on it for 5 mins, this was done to expel the air and for the formation of a uniform film. The increase in the diameter of the circular film thus formed was used as a measure of the spreadability potential of the prepared gel[21]. Rheological assessment of the prepared gel was done using a Brookfield Viscometer (Model – RVT). A sample of 50 g of the gel was allowed to equilibrate for 10 mins before measuring the dial reading using a T-C spindle at increasing speeds from 0.5-5.0 rpm. The corresponding dial reading on the viscometer at each speed was noted. The spindle speed was successively lowered and readings were noted. The readings were then multiplied with the factor given in the manual of the viscometer to yield the viscosity (centipoise)[22]. The pH of the gel was measured using a pH meter previously standardized using standard buffers of pH 4.0 and 7.0. All the experiments were done in triplicate.

Time for self-emulsification, droplet size, and zeta potential

The BOS loaded G-SMEDDS (0.1 mg) was dispersed in 50 mL of water in a volumetric flask and gently mixed by inverting the flask. The time for self-emulsification was noted. The globule size of the microemulsion thus formed was determined by photon correlation spectroscopy that analyzes the fluctuations in light scattering due to Brownian motion of the particles, using a Zetatrac (Microtrac, USA). Light scattering was monitored at 25°C at a 90° angle.

Drug content

BOS content in the BOS loaded G-SMEDDS was determined by dissolving a microemulsion equivalent of 10 mg of BOS in methanol (50 µg/mL). The samples were subsequently sonicated and aliquots were filtered using a 0.45 µm filter. It was then analyzed using a UV-visible spectrophotometer at a wavelength of 269 nm. The study was performed in triplicate. The drug content was determined using the equation 1.

In vitro dissolution study

The in vitro dissolution test for BOS loaded G-SMEDDS and pure BOS was carried out using USP 24 type II apparatus (Electrolab, India). The dissolution test was carried out in 900 ml of 0.1 N HCl to maintain sink condition. G-SMEDDS equivalent to 100 mg BOS was used for the study. The paddle rotation was 50 rpm and the temperature was maintained at 32 ± 0.2°C. 5 ml aliquots of the sample were withdrawn and replaced with fresh dissolution media at the predetermined time points till 30 mins. The aliquots were filtered through a 0.45 µm filter. The assay was performed using a UV-visible spectrophotometer (Shimadzu, Japan) operating at 269 nm. The reported results were the mean of three measurements (± SD).

Stability studies

The BOS-loaded G-SNEDDS were filled in collapsible aluminium tubes which were crimped and stored at two different conditions viz. 30°C/ 60% RH and 40°C/ 75 % RH over 6 months. After one, three, and six months, the formulation was analyzed for droplet size, polydispersity index (PDI), and drug release (%) as reported previously.

In Vitro Permeation Study

The permeation of the drug across the membrane was tested using the Franz diffusion cells. The receptor compartment was filled with phosphate buffer pH 7.4 and maintained at 37 ± 2°C. A synthetic cellulose acetate membrane was soaked in the buffer at 37°C two hours before the experiment. It was then affixed between the donor and the receptor compartments. Gel equivalent to 70 mg BOS was placed in the donor compartment. One mL medium was withdrawn at predetermined time intervals and analyzed spectrophotometrically at 325 nm. The formulation was compared with a 2 mL suspension of the pure drug[23]. The cumulative amount ($\mu g/cm^2$) of the drug that permeates through the membrane was plotted as a function of time (hr). Flux at steady state (J_{ss}) was calculated by dividing the linear portion of the graph by the area of the membrane (3.14 cm^2). The permeability coefficient was calculated by dividing the flux at the steady state by the initial concentration of the drug taken in the donor compartment at the start of the experiment. An enhancement ratio was calculated to assess the proportional increase in the drug's permeation by dividing the flux at the steady state of the G-SMEDDS by the flux at the steady state of the pure drug suspension[24].

RESULTS AND DISCUSSION

Solubility studies

The solubility of the drug in oil is a very important criterion for maintaining the solubilized state of API after dilution. In the case of the microemulsion, there would be a risk of precipitation of drug in GIT due to decrease solvent capacity, if surfactant or co-surfactants are playing a critical role in solubilization. Hence, the selection of the oil phase with the highest solubilization characteristic is an important determinant in the self-emulsifying formulation. As shown in table II, oleic acid provided the highest solubility of BOS (33.35 ± 3.2 mg/mL) and was chosen for further investigations. Safety is a major determining factor in choosing a surfactant as a large amount of surfactants may cause GI irritation. Typically, non-ionic surfactants are less toxic than ionic surfactants viz. anionic and cationic. The utility of non-ionic surfactants in o/w microemulsion is likely to provide better in vivo stability. Nonionic surfactants, Labrasol® (HLB 14) and Tween 20 (HLB 16.7) with an HLB value higher than 10 provided highest solubility of BOS (65.25 ± 3.67 and 21.65 ± 1.33 mg/mL respectively) and thus were selected for further study. Dring the flask inversion study it was observed that the number of inversions required to form a transparent emulsion using Tween 20 were lower than that required to form a transparent emulsion of oleic acid with Labrasol®. Thus, Tween 20 was finalized as the surfactant of choice for the further studies. Transient negative interfacial tension and the fluid interfacial film are rarely achieved by the use of a single surfactant, usually necessitating the addition of a co-surfactant. The presence of co-surfactants decreases the bending stress of the interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form microemulsion over a wide range of compositions. Co-surfactant, Transcutol P, was found to be an effective solubilizer for BOS with a solubility of 337.8 ± 3.17 mg/mL, therefore it was used in the SMEDDS development for improving drug loading. The selection of surfactant and co-surfactant was not only based on solubility studies but also was dependent on their emulsification ability.

Table II: Solubility of BOS in various vehicles at 25°C (n=3)

Vehicle	Component	Solubility (mg/mL) ± S.D, n=3
Isopropyl palmitate	Oil	18.69 ± 1.13
Labrafil® M 2125		20.44 ± 0.81
Capryol® 90		19.77 ± 0.17
Isopropyl myristate		28.21 ± 0.93
Oleic acid		33.09 ± 2.19
Castor oil		9.28 ± 0.76
Maisine®		8.83 ± 1.1
Labrafil® M 1944 CS		16.84 ± 1.18
Peceol®		27.31 ± 1.76

Labrafac® PG		15.61 ± 0.67
Tween 20	Surfactant	21.65 ± 1.33
Tween 80		20.59 ± 0.67
Labrasol®		65.25 ± 3.67
Acrysol® K140		11.54 ± 1.1
Acrysol® K150		7.26 ± 0.31
Acrysol® K160		4.94 ± 0.55
Tween 60		18 ± 0.8
Lauroglycol® FCC		15.97 ± 0.37
Transcutol® P	Co-surfactant	337.8 ± 3.17
Polyethylene glycol 400		31.06 ± 0.57
Propylene glycol		39.51 ± 1.02

Preliminary screening of surfactant for their emulsifying ability

The %transmittance (%T) values of various dispersions are given in table III. Emulsification studies clearly distinguished the ability of various surfactants to emulsify oleic acid. The study reveals Tween 20 had a very good capability to emulsify the lipid component, oleic acid. Whilst, the surfactant HLB used in the study ranged from 10-16, there is huge discrimination between surfactant's emulsification ability. The probable reason behind such behavior of surfactant is structural differences and chain length. Tween 20 rendered the shortest time for emulsification with lesser flask inversions and highest %T and thus was further investigated.

Table III: Emulsification efficiency of various surfactants

Surfactant	%Transmittance (n=3)	No. of inversions
Labrasol®	70.26 ± 1.20	26 ± 1.5
Tween 20	97.60 ± 0.60	16 ± 1.3
Acrysol® K150	89.9 ± 1.15	20.5 ± 1.2
Tween 80	50.47 ± 1.38	27.2 ± 1.5
Acrysol® K140	57.62 ± 0.81	29.4 ± 1.2

Preliminary screening of co-surfactant for their emulsifying ability

The addition of a co-surfactant to the formulation containing surfactant has been reported to improve self-emulsification and dispersibility[25]. Co-surfactants were selected based on %transparency and ease of emulsification as depicted in table IV. Transcutol® P, as well as propylene glycol, exhibited good emulsification, whereas, the emulsification potential of polyethylene glycol 400 wasn't as desired. Transcutol® P showed almost instant emulsification with only three flask inversions, highest % transmittance and maximum solubilization potential for BOS. This could assert the importance of co-surfactant incorporation to a dispersion containing surfactant. All experiments were done in triplicate.

Table IV: Emulsification studies on surfactant/co-surfactant combinations

Co-surfactant	%Transmittance	No. of inversions
Transcutol P	97.63 ± 0.19	3.1 ± 1.1
Polyethylene glycol 400	94.41 ± 0.23	5.2 ± 0.5
Propylene glycol	97.13 ± 0.30	4 ± 0.7

Construction of ternary phase diagram

A series of ternary phase diagrams were constructed to identify micro/microemulsion regions based on their turbidity measurements and visual transparency. Care was taken to ensure that observations are not made on metastable systems, although the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous. The relationship between the phase behavior of a mixture and its composition can be captured with the aid of a phase diagram. The incorporation of API in the SMEDDS system might affect the performance of the self-emulsifying system. To identify the self-emulsifying region with maximum drug loading, ternary phase diagrams were constructed in the absence and presence of BOS. A total of 54 batches having varying degrees of oleic acid, Labrasol®, and PEG 400 were formulated (Data not shown). It was observed that increasing the drug incorporation decreases the self-emulsifying region. Thus, the formulation was optimized to 40% w/w of drug incorporation. The ternary diagram of the system containing oil (oleic acid), surfactant (Tween 20), and co-surfactant (Transcutol® P) with no drug and 40% w/v loading of BOS is shown in Figure 1 A and B respectively. It can be observed that when Labrasol® was used in a very high proportion (Smix ratio 0.9:0.1), a very less amount of oil (10 %w/w) could be solubilized at a high concentration of surfactant (85 %w/w). It was observed that the incorporation of co-surfactant, PEG 400, increased the spontaneity of the self-emulsification process. The gel-like region was found to increase with increasing Labrasol ® concentration, while the self-emulsifying region expanded with the increasing amount of the PEG 400. The selected mixture of 20 % w/w oleic acid, 30 % w/w of Tween 20, and 50 % w/w of Transcutol® P was selected to load the drug.

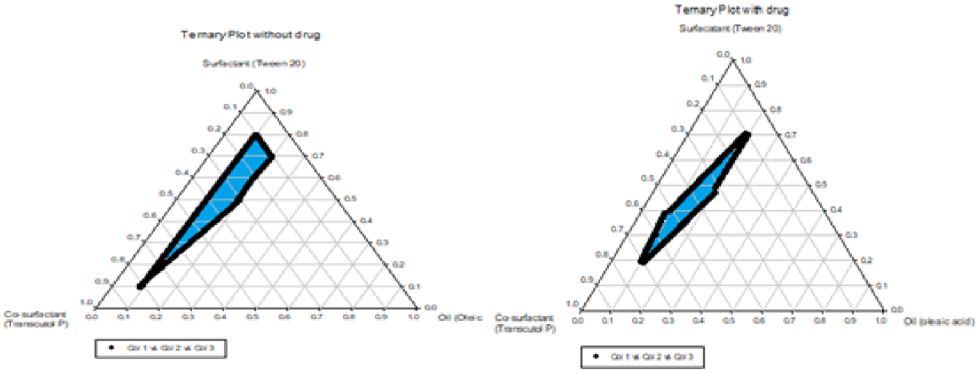

Figure 1: Ternary phase diagram in the A) absence of BOS and B) presence of BOS

EVALUATION OF LIQUID-SMEDDS

Measurement of % transmittance

The optimized batch showed a % transmittance of 99.30 ± 0.45 % suggesting the formation of a clear and transparent microemulsion without any precipitation of the drug.

Effect of pH

A 250-fold dilution with media mimicking the stomach (0.1 N HCl, pH 1.2) and the small intestine (phosphate buffer, pH 6.8) did not cause any turbidity or phase separation in the liquid SMEDDS indicating that the SMEDDS would remain stable at all physiological pH of skin.

Dye solubilization test

The rapid disappearance of the red colour of the oil soluble dye, eosin, indicated that the emulsion formed was indeed oil in water.

Time for self-emulsification

The time required for self-emulsification of the formulated liquid SMEDDS was found to be 9.51 ± 1.36 secs.

Droplet size

The average globule size was 45.66 ± 2.4 nm (after drug loading) as seen in figure 2 and a PDI value of 0.264. The lower PDI value (near to zero) indicates the formation of uniform globules that had narrow size distribution. The zeta potential was found to be −18.56 mV, suggesting the formation of a stable microemulsion.

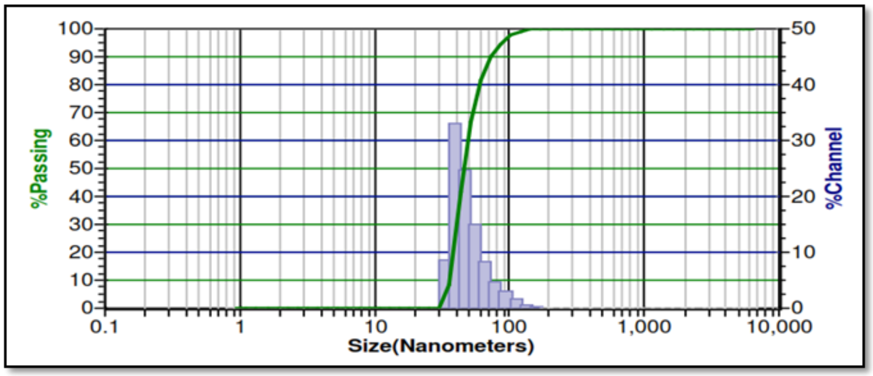

Figure 2: Particle size distribution of the prepared liquid SMEDDS

Cloud point

Cloud point was measured as the temperature at which there was a sudden appearance of cloudiness visually. The temperature at which the turbidity first starts appearing was noted to be 75-80 °C. this indicates that the precipitation of the drug or the phase separation would occur at a temperature higher than the cloud point. Such a situation wouldn't be encountered practically and hence confirming that the SMEDDS would remain stable during the shelf life of the product when stored appropriately.

Thermodynamic stability studies

The optimized L-SMEDDS did not show any signs of precipitation, cracking, creaming, or turbidity when subjected to various high-stress conditions like centrifugation, and three consecutive freeze-thaw as well as heating-cooling cycles. This leads us to the conclusion that the BOS loaded SMEDDS have an innate resistance to physical or temperature induces stress.

Dispersibility test

The optimized formulation remained in the A category at all three dilutions levels, indicating less probability of precipitation of the drug in vivo.

Physical robustness to dilution

The globule size and % transmittance values remained unaffected upon dilutions with water as well as 0.1 N HCl. The appearance of the diluted mixture remains unaffected by pH as indicated by the formation of stable and transparent microemulsion formation

Drug content

The drug content of BOS loaded L-SMEDDS was found to be 99.76 % ± 0.35%.

Conversion of liquid SMEDDS to gel SMEDDS (G-SMEDDS)

Various gelling agents from varied origins were screened to formulate the gel of the optimized BOS loaded L-SMEDDS. The results of the preliminary screening are shown in tables V and VI.

Table V: Preliminary screening of gelling agents

Gelling agent	Concentration of the gelling agent (%w/w)	Observation
Carbopol® 934	1	A highly viscous gel was formed.
Carbopol® 940	1	A highly viscous gel was formed.
Xanthan gum	1	A turbid, sticky, low viscosity gel was formed.
HPMC 15CPS	1	A turbid gel with no viscosity was obtained.
HPMC 50CPS	1	A turbid gel with no viscosity was obtained.
HPMC K15M	1	A transparent gel with slight viscosity was obtained.

Table VI: Preliminary screening of gelling agents

Sr. No.	Concentration of HPMC K15M (% w/w)	Observation
	0.5	Gel-like consistency not obtained
	1	Transparent gel formed but with less viscosity than desired

1.5	Transparent gel formed but with less viscosity than desired
2	Viscous gel-like consistency with uniform transparent appearance obtained.

Carbopol was not found to be suitable to form the gel of the liquid SMEDDS. This could be attributed to a high proportion of oleic acid in the formulation which would make the mixture acidic, thereby requiring a higher volume of triethanolamine to neutralize and form the gel. Xanthan gum formed a gel with high adhesiveness and low viscosity, this could be attributed to the helicoidal bonds in its structure[26]. Hydroxypropyl methylcellulose was able to form gels but the grades HPMC 15 cps and HPMC 50 cps did not yield gels of the desired viscosity. On the other hand, HPMC K15M at 2% w/w concentration was successfully able to form a transparent gel with the desired viscosity to cause spreading of the formulation without any drag. Thus, this gelling agent at 2% concentration was finalized. Additionally, HPMC is known to be an adhesive polymer, and this would provide an added benefit, as this would allow for the formulation to retain on the skin for a longer time, to allow better permeation of the drug[27].

Evaluation of G-SMEDDS

Macroscopic evaluation

The prepared gel was found to be shiny, transparent, without any lumps and minimal air entrapment. Spreadability is an important criterion for any topical formulation as that guarantees ease of application when used by the patients. The spreadability of G-SMEDDS was found to be 4.75 ± 0.3 cm. The pH of the formulation was found in the range of 5.5 – 6. Though the gel was brought to the neutralization pH by the use of triethanolamine but the decrease in the pH could be attributed to the presence of acidic ingredients like oleic acid in the formulation. This pH also corroborates the non-irritancy of the formulation. On rheological evaluation, the gel was found to show a pseudoplastic behavior with a viscosity of 1.58 X 10^6 mPa s. This kind of non-Newtonian behavior is an indication of the decrease in viscosity when the shear is increased[28]. This would prove to be beneficial for a topical formulation, wherein the patient would be able to apply the formulation with minimum drag to the skin due to the increase in the spreadability of the formulation during rubbing[29].

Time for self-emulsification, droplet size, and zeta potential

The self-emulsification time was also found to be 15 ± 1.5 secs showing that it was not significantly affected upon conversion of BOS loaded L-SMEDDS to BOS loaded G-SMEDDS. There was no change in the classification of the formulation under dispersibility test, it remained a type A formulation that are known to form microemulsions rapidly on dilution. The globule size of

G-SMEDDS was found to be 48.67 ± 3.14 nm with a PDI of 0.317 ± 0.08 as shown in figure 3. Both of these values confirm that gel formation did not affect the properties of the optimized SMEDDS consequentially.

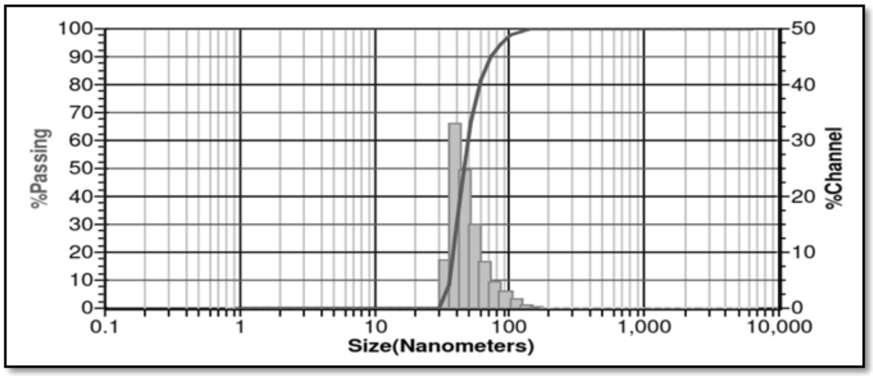

Figure 3: Particle size distribution of the BOS loaded G-SMEDDS

Drug content

The drug content of BOS loaded G-SMEDDS was found to be 98.95 ± 0.26 % which did not show any noteworthy difference from that observed in the liquid SMEDDS.

In vitro dissolution study

The pure drug showed a release of 6.34 ± 0.40 % whereas that observed from G-SMEDDS was 99.40 ± 1.56 % at the end of 30 mins as can be seen in figure 4. This indicates 15.68 times increase in the release of the drug, bosutinib. This proves that the incorporation of BOS into a SMEDDS has improved its solubility drastically.

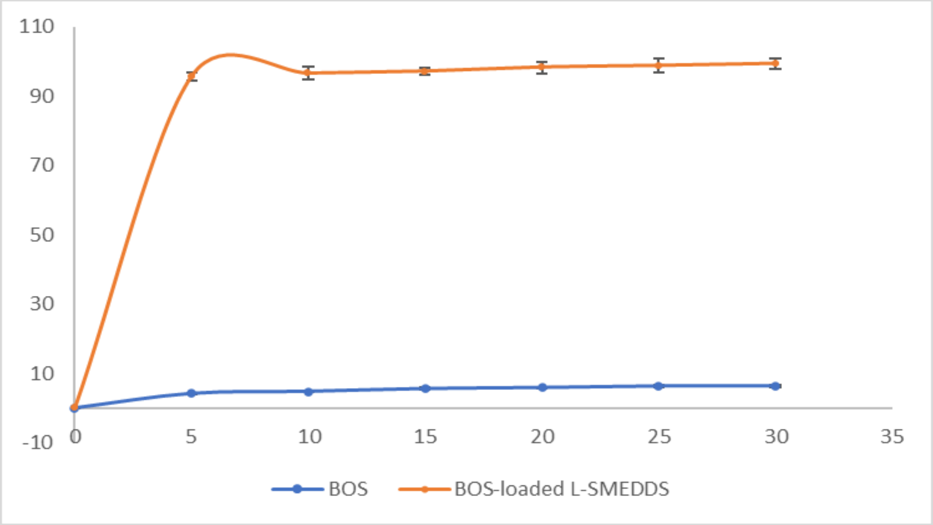

Figure 4: In vitro drug release of BOS loaded G-SMEDDS and pure BOS in 0.1 N HCl

Stability studies

The accelerated stability study was performed as per the storage conditions and timepoints specified under the ICH guidelines Q1A(R2). The samples were evaluated for droplet size and polydispersity index to confirm that the droplets did not aggregate as this could lead to the precipitation of the drug. The drug release of the sample was also assessed to attest to the aforementioned assumption. The results obtained are summarized in table VII.

Table VII: Observations made during stability studies of BOS-loaded G-SMEDDS

Timepoint	**Storage condition**	**Droplet size (nm)**	**Polydispersity index**	**% Drug release at the end of 30 mins**
Initial	NA	48.67 ± 3.14	0.317 ± 0.08	99.40 ± 1.56
1 month	30 ± 0.5°C/ 65 ± 2% RH	49.34 ± 1.58	0.322 ± 0.06	99.25 ± 1.66
	40 ± 0.5°C/ 75 ± 2% RH	49.64 ± 1.13	0.325 ± 0.03	99.47 ± 2.52
3 months	30 ± 0.5°C/ 65 ± 2% RH	50.21 ± 1.54	0.334 ± 0.04	98.23 ± 2.18
	40 ± 0.5°C/ 75 ± 2% RH	51.08 ± 1.95	0.338 ± 0.06	98.91 ± 1.75
6 months	30 ± 0.5°C/ 65 ± 2% RH	52.74 ± 1.65	0.345 ± 0.03	98.59 ± 1.39
	40 ± 0.5°C/ 75 ± 2% RH	53.31 ± 2.22	0.342 ± 0.05	1.24 2.67

In vitro permeation study

The observations made during the percutaneous penetration study of the G-SMEDDS and the pure BOS suspension are depicted in figure 5. The flux at steady state ($\mu g/cm^2/hr$) of pure BOS and G-SMEDDS was found to be 0.53 and 2.51 respectively. The permeability coefficient of G-SMEDDS was found to be 3.58×10^{-5} cm/h as opposed to that of pure BOS which was deduced to be 7.58×10^{-6} cm/h, showing a 2.18-times enhancement in the permeation of the drug. This could be attributed to both the lipophilic and the hydrophilic compartments in the systems. The surfactant and cosurfactant in the SMEDDS affect the structure of the stratum corneum and reduce the diffusional barrier, leading to their action as "permeation enhancers"[30]. Once the formulation enters into the stratum corneum, it starts affecting both the polar and the lipid pathways of drug absorption. The lipids in the system affect the stratum corneum in multiple ways. It may the direct partition of BOS into the stratum corneum, or destabilizing the lipid struct of the stratum corneum by intercalating between them[31]. Surprisingly the aqueous portion of the SMEDDS can also wreak havoc in the stratum corneum in myriad ways. It can hydrate the skin increasing the interlamellar volume of the lipid bilayers and leading to their disruption. It may alternatively hydrate the corneocytes to which lipid chains in the stratum corneum are covalently attached which would also lead to a disordered structure[32]. All of this could aid in the permeation of the drug into deeper layers of the skin as could be observed from the results of the study.

CONCLUSION

The study attempted to improve the solubility and permeability of the tyrosine kinase inhibitor, bosutinib. The drug has been classified under Class IV of the Biopharmaceutical Classification System, indicating that both poor aqueous solubility and low membrane permeability were responsible for the poor oral bioavailability of the drug. Studies have also shown the involvement of presystemic hepatic metabolism to be one of the reasons for the poor oral bioavailability of this drug. This leads to the administration of a high dose of the drug to achieve the desired anti-cancer effect. This has led to serious systemic side effects. Thus, the research endeavored to improve the solubility and permeability of BOS with the aim to reduce its dosage requirement which would, in turn, reduce the incidence and severity of side effects. The study was focused on improving the solubility, permeability, and reducing the presystemic metabolism of the drug by preparing a self-microemulsifying system converted into a gel-based dosage form. The research brought to light this approach had the merit in improving the bioavailability of both the drugs. Thus, we can conclude beyond doubt that the aim envisaged at the start of the work could be accomplished and that SMEDDS-based transdermal drug delivery have the potential to improve the solubility and permeability of anti-cancer agents like tyrosine kinase inhibitors.

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