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Formulation, Development of a Transdermal Self-Microemulsifying Drug Delivery System of Dasatinib

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ABSTRACT

Tyrosine kinase inhibitors have shown to be effective in the treatment of chronic myeloid leukemia. These drugs generally belong to the Biopharmaceutical Classification System Class II or IV, indicating that their oral bioavailability is less due to its poor solubility, permeability, or both. Dasatinib is a tyrosine kinase inhibitor and the present study was aimed to overcome these limitations associated with it. Thus, a transdermal system comprising of the drug loaded into a self-microemulsifying drug delivery system was formulated. The study started by selecting the right combination of the oil (oleic acid), surfactant (Labrasol®), and cosurfactant (polyethylene glycol 400) by performing solubility and emulsification ability studies. The ternary phase diagrams were constructed to identify the microemulsion region. The liquid system was converted into an easy-to-handle gel form using 1% w/w Carbopol ETD 2020 as the gelling agent. The final system had a droplet size of 76.42 ± 1.2 nm, a narrow polydispersity index, and a high zeta potential. The thermodynamic stability studies, dispersibility and dilution tests proved that the formulation would be unaffected and would form the microemulsion instantaneously. In vitro dissolution confirmed close to 100% drug release could be obtained within 30 mins as opposed to the plain drug showing only 50 – 60 % release. *In vitro* permeability studies, revealed a 1.6-times enhancement in the permeation of the drug. Preparation of a self-microemulsifying drug delivery system and loading it into a topical gel could address the issues associated with the effective delivery of dasatinib.

Keywords: SMEDDS, dasatinib, solubility, permeability, bioavailability, tyrosine kinase inhibitor, topical gel.

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INTRODUCTION

In large part, the effectiveness of a drug depends upon how it is administered to the patient. As a result of innovations over the past year, the drug could be delivered to all parts of the body in a variety of ways. Nevertheless, it is the oral route that has the greatest impact. Among the benefits of this delivery method are the ease of self-administration (as opposed to rectal or ocular delivery) and the pain-free delivery over parenteral administration. Additionally, it is an extremely cost-effective method. Additionally, it is technologically advantageous, such as offering less sterility constraints, greater design flexibility, and ease in manufacturing. There is also the option of tailoring formulations so that they deliver drugs more effectively to specific regions in the 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. SMEDDS typically contains the drug along with oils, surfactants, and hydrophilic cosurfactants in varying proportions. As a result of small droplet sizes that form on dispersion, these formulations have high stability as well as a very large surface area, enhancing solubility and therefore improving bioavailability. Bioavailability of drug molecules falling into classes II and IV of the BCS has been significantly enhanced by these systems.[3] The system also offers ease of manufacturing scalability, extended shelf life due to improved stability, palatability and patient compliance are no longer concerns, and the drugs have a quick onset of action without being affected by food. The dosage forms can be made patient-friendly for convenience. A liquid, semi-solid or solid dosage form can be created.[4]

As a targeted treatment for malignancy, Tyrosine Kinase Inhibitors (TKI) have shown promise. They competitively inhibit ATP at the catalytic binding site of tyrosine kinase. In 2001, imatinib became the first tyrosine kinase drug to be approved for clinical use.[5] The past 20 years have brought 70 more drugs that have been shown to be effective for non-cancerous conditions too.[6] The dose of 400 mg/day for imatinib has become the standard of care for people with chronic myeloid leukemia (CML). 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 a more serious note, it has caused heart attacks and left ventricular dysfunction, hypophosphatemia affecting bones, hepatitis with elevated liver enzymes, and peripheral neuropath.[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.

Many TKI on the market and in development show low bioavailability after oral administration despite their wide application. Either a high first pass metabolism or a low absorption may be responsible for this. Among the compound class, highly lipophilic and poorly water-soluble molecules are overrepresented, and administration with food is likely to limit solubility-induced absorption and alter pharmacokinetics.[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] This emphasizes the biopharmaceutical complexity of this class of compounds, as well as the possibility of adverse effects or worse therapeutic outcomes when food or other medicines significantly alter drug absorption.

Dasatinib (DAS), N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)piperazin-1-yl]-2-methylpyrimidin-4-yl]amino]-1,3-thiazole-5-carboxamide monohydrate, a tyrosine kinase inhibitor, inhibiting both the active and inactive conformation of breakpoint cluster region-Abl (Bcr-Abl) kinase along with several non-receptor tyrosine kinases in the sarcoma family.[13] It is the drug of choice against imatinib-resistant chronic myeloid leukemia. The pharmacokinetic profile of DAS following oral administration in humans showed that the drug suffers from very low bioavailability of 34% in dogs and 14 – 51% in mice.[14] Absolute bioavailability in human subjects has still not been determined. Various reports suggest that DAS has been classified as a BCS Class II molecule, indicating that though it would cross the intestinal membrane easily, its poor aqueous solubility would be a limiting factor leading to its low bioavailability. Another factor contributing to its low oral bioavailability is the hepatic first pass metabolism due to the CYP3A4.[15] Dasatinib and its metabolites are primarily excreted via the faeces. The half-life of the drug is 3–5 hours.

DAS has also reported to show a pH dependent decrease in solubility ranging from 18.4 mg/mL at pH 2.6 to < 0.001 mg/mL at pH 7. DAS exhibits pH-dependent aqueous solubility with two basic ionization constants at 6.8 and 3.1, and one weakly acidic ionization constant at 10.8.[16] It has a reported diffusion coefficient of 0.7287 X 10-4. It has a molecular weight of 488 g/mol, a polar surface area of 135Å2, and a log P value of 3.82.[17] Absorption following oral administration of DAS is solely governed by the solubility of dose in the intestinal milieu and permeability through

the intestinal membrane. Besides, it is also a substrate for two ATP-binding cassette transporter proteins, ABCB1 and ABCG2. This could play an important role in altering the pharmacokinetics of DAS.[13] Though the pharmacokinetic significance of this has proven to be insignificant. Treatment using DAS has been connected to side effects such as neutropenia, bleeding disorders, fluid retention, QT prolongation, pulmonary arterial hypertension, severe skin reactions, and tumour lysis syndrome.[18, 19] Due to the low oral bioavailability of DAS, a high dose (140 - 200 mg in divided doses, twice a day) needs to be incorporated into the dosage form to achieve the desired anti-leukemic activity, the incidences of the side effects are commonplace. Till date the therapeutic benefits of DAS have been limited which could largely be attributed to poor formulation design. Thus, the present study aims to develop a system based on the principles of self-microemulsification to address the issue of low bioavailability of DAS by improving its aqueous solubility. The other parameter leading to its low oral bioavailability i.e., high hepatic first pass metabolism would be addressed by formulating the DAS-loaded self-microemulsifying system into a transdermal formulation like a gel. Thus, the aim of the present work is to develop a transdermal gel of dasatinib-loaded self-microemulsifying drug delivery system.

MATERIALS AND METHODS

Dasatinib was received as a gift sample from Alembic Pharmaceuticals, Vadodara, India. Oleic acid and polyethylene glycol 400 were procured from Finer Chemicals Ltd., India and Labrasol® was obtained from Gattefosse, India. All other materials and chemicals used were of either pharmaceutical or analytical grade.

Solubility Studies

Equilibrium solubility of DAS was measured in various oils, surfactants, and co-surfactants. An excess amount of DAS 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 DAS was determined by UV-Visible spectrophotometry (UV-1800, Shimadzu, Japan) at 323 nm using methanol as the blank.[20] Each experiment was performed in triplicate.

Preliminary Screening of Surfactant for their Emulsifying Ability

An oily phase of 300 mg was mixed with 300 mg of surfactant. The mixture was gently heated at 50°C to homogenize the components. In a stoppered Erlenmeyer flask, 50 mg of the isotropic mixture was accurately weighed and diluted with double distilled water to yield fine emulsion. A

measure of the ease of emulsion formation was the number of flask inversions necessary to achieve a uniform emulsion. Visually, the emulsions were assessed for 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 .[21]

Preliminary Screening of Co-Surfactant for their Emulsifying Ability

Using the turbidimetric method, we assessed the relative effectiveness of the co-surfactant in improving the self-emulsification ability of the surfactants. Briefly, surfactant (Labrasol® and Tween 80), 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 emulsion. The resulting emulsions were observed visually for the relative turbidity and phase separation. The emulsions were allowed to stand for 2 h 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.[21]

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) using the water titration method. A series of pseudo ternary diagrams were constructed with the varied ratio of surfactant/co-surfactant (3:1, 2:1, 1:1, 1:2, and 1:3). Oil and surfactant/co-surfactant (Smix) were mixed thoroughly in different volume ratios (1:9, 2:8, 3:7, 4:6, 5:5, 4:6, 3:7, 2:8, 9:1) and titrated with dropwise addition of water under gentle agitation. Slow aqueous titration was done to each weight ratio of oil and Smix and generated samples that were clear or slightly bluish in appearance were determined as a microemulsion. Ternary plots were constructed using Chemix software. The studies were performed in triplicate. The self-emulsifying power was also assessed using infinite dilution using purified water. The effect of DAS 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) were prepared by mixing the required amount of DAS, oil, surfactant, and cosurfactant to achieve a transparent homogenous solution by heating at 25 °C for 15 mins.

Evaluation of Liquid-SMEDDS

Time for Self-Emulsification and Droplet Size

The formulation (0.1 mL) 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 particle size analyzer (Microtrac, USA). Light scattering was monitored at 25 °C at a 90° angle.

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 1 was made.

Table 1: Visual observation and classification of self-emulsifying formulations based on their ability and speed of forming a dispersion

Dispersibility and appearance	Grade	Time to self-emulsify
Rapidly forming nano or microemulsion having a clear or bluish appearance.	A	Within 1 min
Rapidly forming, slightly clear emulsion having a bluish-white appearance	B	Within 1 min
Fine milky emulsion that forms moderately fast	C	Within 2 min
Dull, greyish-white emulsion having a slightly oily appearance that is slow to emulsify	D	Within 3 min
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.[22]

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) was performed using the FTIR model Shimadzu 8400, Japan attached to an attenuated total reflectance (ATR) accessory. ATR was fitted with a single bounce diamond at 45° angle, internally reflected incident light providing a sampling area of 1 mm in diameter with a sampling depth of several microns. Pure DAS (solid) and DAS loaded SMEDDS (liquid) were analyzed. A small amount of DAS (solid) was directly placed on the diamond disk, and DAS loaded SMEDDS (liquid) sample was kept in the liquid sample holder. The sample was scanned for absorbance over the range from 4,000 to 400 wavenumbers (per centimeter) at a resolution of 1 cm^{-1}.

Drug Content

DAS content in the DAS loaded L-SMEDDS was determined by dissolving a microemulsion equivalent of 10 mg of DAS 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 323 nm. The study was performed in triplicate. The drug content was determined using the following Equation 1:

$$\% \textbf{ drug content} = \frac{\textbf{Observed drug content}}{\textbf{Theoretical drug content}} \textbf{X100} \qquad \textbf{Equation 1}$$

Conversion of Liquid SMEDDS to Gel SMEDDS (G-SMEDDS)

Optimized DAS 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% w/w gelling agent and preservatives. Various gelling agents like HPMC 15CPS, HPMC 50CPS, HPMC K15M, Carbopol® 934, Carbopol® 940, and xanthan gum were screened.[23] 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 DAS 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.[24] 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).[25] 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 DAS 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

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In Vitro Dissolution Study

The in vitro dissolution test for DAS loaded G-SMEDDS and pure DAS 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 DAS 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 DAS-loaded G-SNEDDS were filled in collapsible aluminum 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 DAS 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.[26] The cumulative amount (µg/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.[27]

RESULTS AND DISCUSSION

Solubility Studies

The selection of the oil phase with the highest solubilization characteristic is an important determinant in the self-emulsifying formulation. As shown in Table 2, oleic acid provided the highest solubility of DAS (146.6 ±3.2 mg/mL) and was chosen for further investigations. Nonionic surfactant, Labrasol® (HLB 14) provided the highest solubility of DAS (25.1 ± 3.8 mg/mL) and thus were selected for further study. Co-surfactant, polyethylene glycol 400 (PEG 400), was found to be an effective solubilizer for DAS with a solubility of 82.3 ± 2.2 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 2: Solubility of DAS in various vehicles at 25°C (n=3)

Vehicle	Component	Solubility (mg/mL) ± SD, n=3
Gelucire® 44/14	Oil	88.1 ± 2.5
Capryol® 90		30.9 ± 1.6

Precirol®		32.2 ± 1.6
Labrafac® PG		8.5 ± 0.5
Oleic acid		146.6 ± 3.2
Isopropyl myristate		20.2 ± 1.8
Maisine® CC		30.7 ± 1.8
Isopropyl palmitate		13 ± 2.2
Olive Oil		11.1 ± 1.5
Labrafil®M2125	Surfactant	10.9 ± 1.7
Span 20		20.6 ± 2.3
Tween 80		19.7 ± 2.1
Lauroglycol® 90		11.2 ± 1.4
Labrasol®		25.1 ± 3.8
Acrysol®K-150		10.2 ± 0.7
Labraglycol® FCC		8.5 ± 0.3
Labrafil® 1944		11 ± 2.3
Span 80		11.4 ±2.6
Tween 20		8.1 ±1.1
Polyethylene glycol 400	Co-surfactant	82.3 ±2.2
Transcutol® HP		29.4 ±1.5
Glycerine triacetate		9.1 ±0.7
Propylene glycol		23.4 ±2.9

Preliminary Screening of Surfactant for their Emulsifying Ability

The %transmittance (%T) values of various dispersions are given in Table 3. Emulsification studies clearly distinguished the ability of various surfactants to emulsify oleic acid. The study reveals Labrasol® had the very good capability to emulsify the lipid component, followed by Tween 80, while Span 20 appeared to be a poor emulsifier for 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 reasons behind such behavior of surfactants are structural differences and chain length. Labrasol® rendered the shortest time for emulsification with lesser flask inversions and highest %T and thus were further investigated.

Table 3: Emulsification efficiency of various surfactants

Surfactant	%Transmittance (n=3)	No. of inversions
Span 20	92.27 ±0.23	22.7 ±1.5
Labrasol®	97.60 ±0.60	8.3 ±1.5
Tween 80	97.43 ±0.20	11.3 ±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.[28] Co-surfactants were selected based on %transparency and ease of emulsification as depicted in Table 4. Transcutol® HP, as well as propylene glycol, exhibited good emulsification, whereas, PEG 400 showed maximum %T (99.83%), instant emulsification with only three flask inversions, and highest solubility. This could

assert the importance of co-surfactant incorporation to a dispersion containing surfactant. All experiments were done in triplicate.

Table 4: Emulsification studies on surfactant/co-surfactant combinations

Co-surfactant	%Transmittance	No. of inversions
Polyethylene glycol 400	99.83 ± 0.22	2.7 ± 1.1
Transcutol HP	96.93 ± 0.38	4.7 ± 0.5
Propylene glycol	97.43 ± 0.21	8.3 ± 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 DAS. 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 (Labrasol®), and co-surfactant (PEG 400) with no drug and 40% w/v loading of DAS 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 17 % w/w oleic acid, 37 % w/w of Labrasol®, and 46 % w/w of PEG 400 was selected to load the drug.

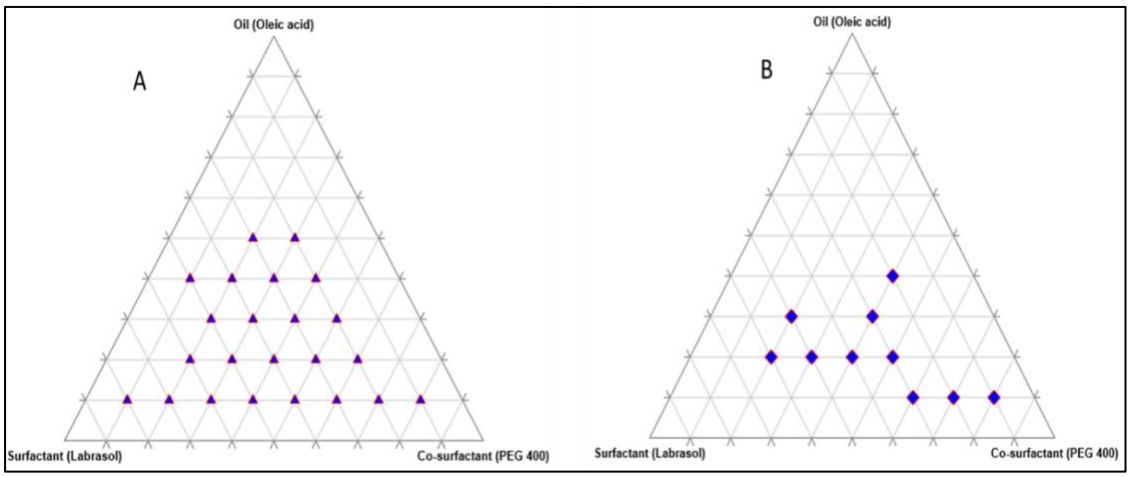

Figure 1: Ternary phase diagram in the A) absence of DAS and B) presence of DAS

Evaluation of Liquid-SMEDDS

Time for Self-Emulsification and Droplet Size

The time required for self-emulsification of the formulated liquid SMEDDS was found to be 57 ± 3 secs. The average globule size was 76.42 ± 1.2 nm (after drug loading) as shown in Figure 2 and a PDI value of 0.170. The lower the PDI value (near to zero) indicates the formation of uniform globules that had narrow size distribution. The percent transmittance was 95.05 %, suggesting the formation of a clear and transparent microemulsion. The zeta potential was found to be −21.7 mV, suggesting the formation of a stable microemulsion.

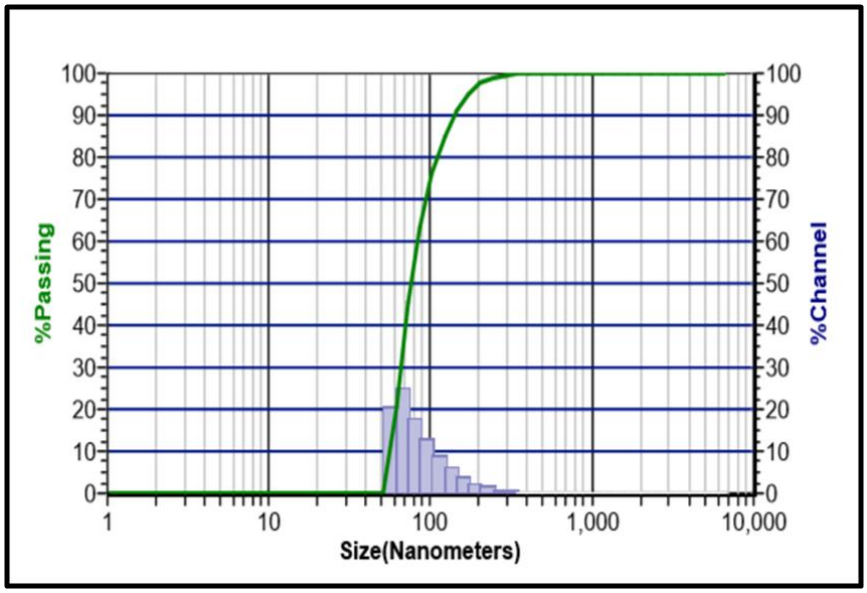

Figure 2: Particle size distribution of the prepared liquid SMEDDS

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 DAS loaded SMEDDS have an innate resistance to physical or temperature induces stress.

Dispersibility Test

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

Fourier Transform Infrared Spectroscopy (FTIR)

To check the compatibility of the DAS with selected excipients, FTIR of DAS API with the mixture of the excipients was carried out and the recorded spectra in the region of 400– 4,000 cm^{-1} as shown in Figure 3. Looking at the scans, significant changes in the shape and intensity of the major peaks were observed at 3243 cm^{-1} (O-H stretching), 1609 cm^{-1} (amide C=O stretching), 1575 cm^{-1} (C-C strain of the aromatic ring), 1446 cm^{-1} (HC=CH, aryl), 1196 cm^{-1} (C-N stretching), and 1004 cm^{-1} (aromatic C-H bending), suggesting the H-bond formation of DAS with the excipients used in the formulation.

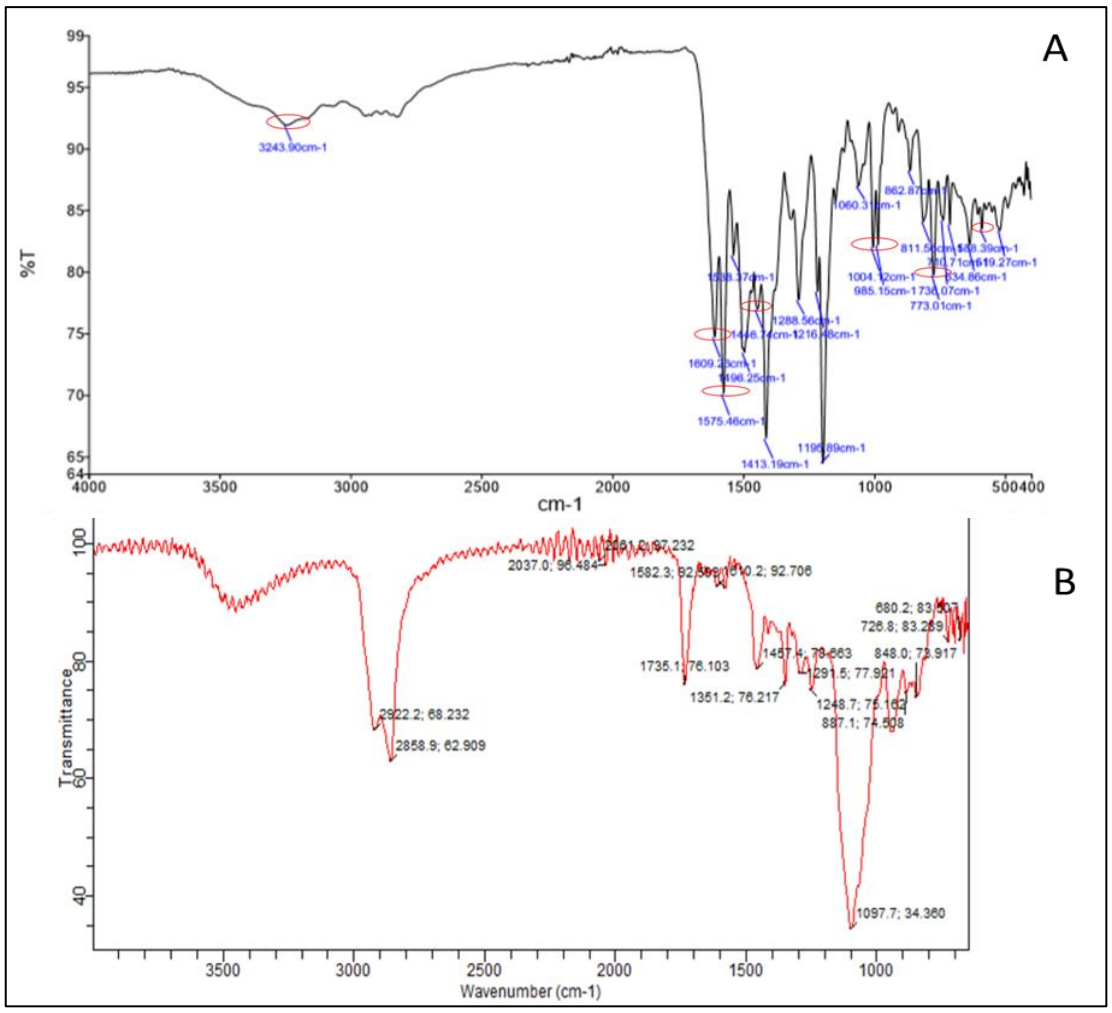

Figure 3: FTIR spectrum of A) Plain DAS and B) DAS with oleic acid, Labrasol®, and PEG 400

Drug Content

The drug content of DAS loaded L-SMEDDS was found to be 99.37 ± 0.71%.

Conversion of Liquid SMEDDS to Gel SMEDDS (G-SMEDDS)

Various gelling agents from varied origins were screened to formulate the gel of the optimized DAS loaded L-SMEDDS (Tables 5 and 6). Carbopol ETD 2020 at 1% w/w concentration was successfully able to form a transparent gel with the desired viscosity for suitable spreadability of the gel.

Table 5: Preliminary screening of gelling agents

Gelling agent (1 % w/w)	Observation
Xanthan gum	Gel-like consistency not obtained
Sodium alginate	Separation of oil observed
HPMC	Gel formed but it was opaque
Carbopol ETD 2020	Transparent gel formed

Table 6: Preliminary screening of gelling agents

Sr. No.	Concentration of Carbopol ETD 2020 (% w/w)	Observation
1	0.5	Gel-like consistency not obtained
2	1	Transparent gel formed
3	1.5	Transparent gel formed
4	2	The transparent gel formed but spreading was relatively difficult

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 the ease of application when used by the patients. The Spreadability of G-SMEDDS was found to be 4.82 ± 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 9.2 X 10^6 mPa s.

Time for Self-Emulsification, Droplet Size, and Zeta Potential

The self-emulsification time was also found to be 65 ± 2 secs showing that it was not significantly affected upon conversion of DAS loaded L-SMEDDS to DAS loaded G-SMEDDS. There was no change in the classification of the formulation under dispersibility test, it changed from a type A formulation to a type B formulation, that is not considered detrimental, as type B formulations are

also considered to form microemulsions rapidly on dilution. The globule size of G-SMEDDS was found to be 77.62 ± 2.01 nm with a PDI of 0.256 ± 0.04 as shown in Figure 4.

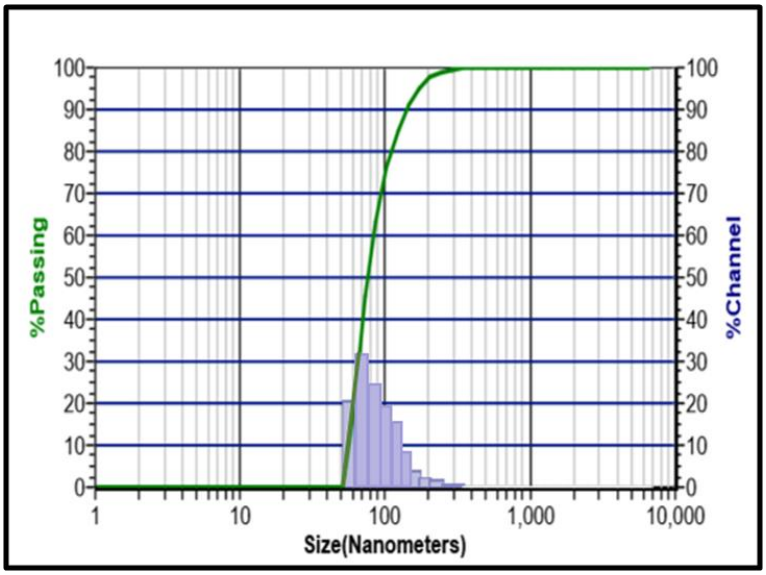

Figure 4: Particle size distribution of the DAS loaded G-SMEDDS

Drug Content

The drug content of DAS loaded G-SMEDDS was found to be 99.45 ± 0.04 % 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 66.39 ± 2.67% whereas that observed from G-SMEDDS was 99.87 ± 2.75 % at the end of 30 mins as can be seen in Figure 5. This validates the rationale behind the study to improve the solubility of DAS by preparing it as a self-emulsifying system.

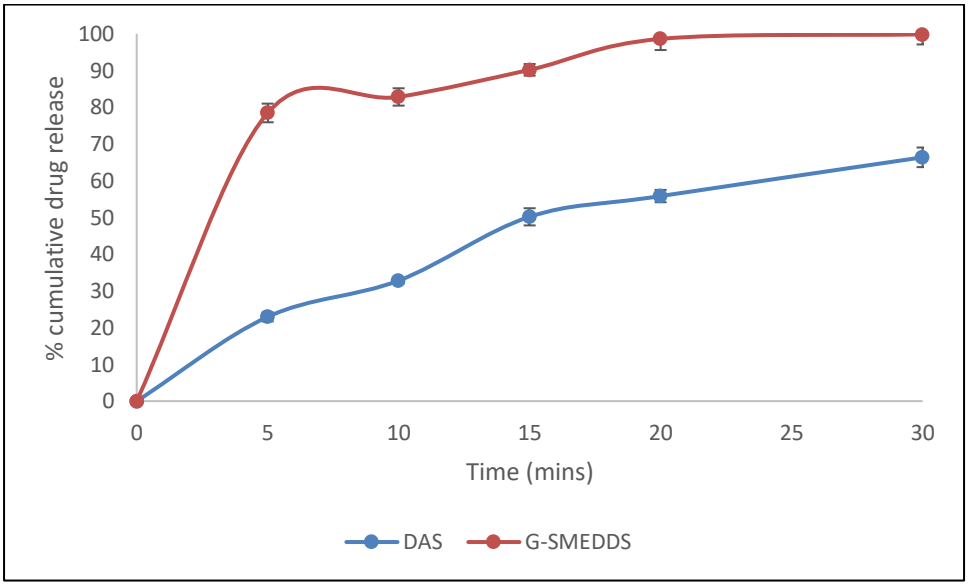

Figure 5: In vitro drug release of DAS loaded G-SMEDDS and pure DAS in 0.1 N HCl

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 7.

Table 7: Observations made during stability studies of DAS-loaded G-SMEDDS

Timepoint	Storage condition	Droplet size (nm)	Polydispersity index	% Drug release at the end of 30 mins
Initial	NA	77.62 ±2.01	0.256 ± 0.04	99.87 ± 2.75
1 month	30 ± 0.5°C/ 65 ± 2% RH	82.43 ± 1.97	0.253 ± 0.02	99.54 ± 2.66
	40 ± 0.5°C/ 75 ± 2% RH	83.64 ± 2.13	0.259 ± 0.03	99.62 ± 2.43
3 months	30 ± 0.5°C/ 65 ± 2% RH	83.51 ± 2.68	0.263 ± 0.02	98.95 ± 2.14
	40 ± 0.5°C/ 75 ± 2% RH	83.47 ± 2.53	0.278 ± 0.02	98.74 ± 1.59
6 months	30 ± 0.5°C/ 65 ± 2% RH	85.62 ± 2.24	0.284 ± 0.03	98.32 ± 2.48
	40 ± 0.5°C/ 75 ± 2% RH	86.14 ± 1.83	0.289 ± 0.02	98.44 ± 2.93

In Vitro Permeation Study

The observations made during the percutaneous penetration study of the G-SMEDDS and the pure DAS suspension are depicted in Figure 6. The flux at steady state (µg/cm^2/hr) of pure DAS and G-SMEDDS was found to be 1.35 and 2.18 respectively. The permeability coefficient of G-SMEDDS was found to be 3.58 X 10^{-5} cm/h as opposed to that of pure DAS which was deduced to be 3.11 X 10^{-5} cm/h, showing a 1.6-times enhancement in the permeation of the drug.

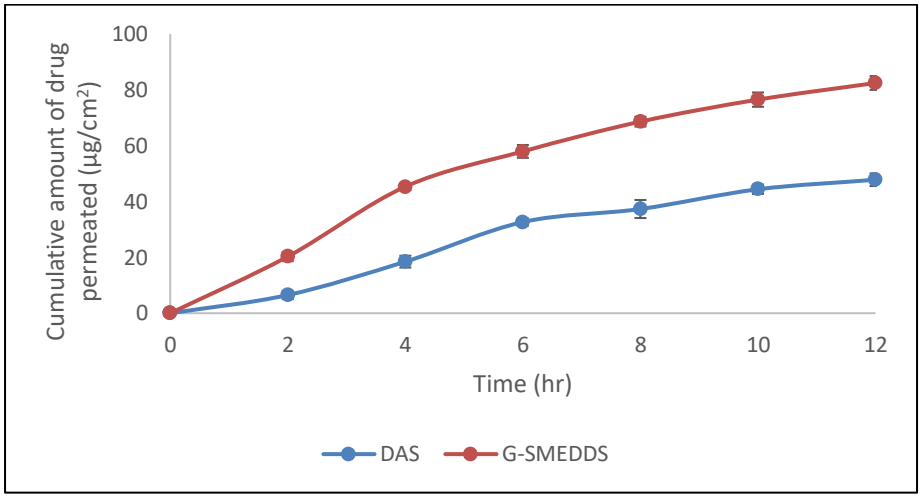

Figure 6: Cumulative amount of DAS permeated in µg/cm^2 versus time in hours

DISCUSSION:

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 the drug exhibited maximum solubility in oleic acid, it was selected as the oil phase. 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. Based on the solubility of the drug in the surfactant and the number of inversions needed to form a transparent emulsion, Labrasol® was shortlisted as the surfactant. 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. Based on experiments conducted, PEG 400 was selected to play the role of the co-surfactant.

The ternary phase diagrams constructed helped in selecting a combination of 17 % w/w oleic acid, 37 % w/w of Labrasol®, and 46 % w/w of PEG 400 for effective drug loading and spontaneous formation of a transparent, thermodynamically stable emulsion. Evaluation of the liquid SMEDDS thus formed, revealed that the system had a droplet size of 76.42 ± 1.2 nm with a sufficiently low PDI of 0.170. Negative zeta potential value was obtained owing to the use of oleic acid. A similar report was made by Midha et al who prepared the SMEDDS of atorvastatin using oleic acid as the oil phase.[29] FTIR studies demonstrated that there was no chemical interaction between the drug and the selected excipients. The stability of the liquid SMEDDS was confirmed from the results of the thermodynamic stability studies.

To convert the liquid SMEDDS to a topical gel, various gelling agents were screened and the one showing the optimum gelling ability was explored further to finalize the concentration of the gelling agent that would form the gel with suitable organoleptic properties. Sodium alginate caused the oil phase to separate, this could be attributed to it being a salt that could have affected the structure and stability of the microemulsion. A similar observation was made by S. Tenjarla.[30] Of the gelling agents screened, Carbopol ETD 2020 was found to form a gel with the desired features of transparency and easy spreadability. Thus, the next part of the study was directed towards finalizing the concentration of the gelling agent suitable to form the gel. From the results, it was observed that both 1 and 1.5% w/w could form acceptable gels, thus 1% w/w Carbopol ETD 2020 was finalized as the gelling agent. Carbopol 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.

The prepared gel was found to be organoleptically suitable, and the rheological investigation revealed that the gel exhibited a pseudoplastic behavior. This kind of non-Newtonian behavior is an indication of the decrease in viscosity when the shear is increased.[31] 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.[32] There was no significant difference seen between the droplet size and PDI of the liquid and gel SMEDDS. Both of these values confirm that gel formation did not affect the properties of the optimized SMEDDS consequentially. The in vitro drug release of the SMEDDS-loaded gel was found to be significantly greater than the release from the drug suspension. This proves that the incorporation of DAS into a SMEDDS has improved its solubility. A 1.6-fold increase was observed from the SMEDDS-loaded gel as opposed to the plain 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."[33] 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 DAS into the stratum corneum, or destabilizing the lipid struct of the stratum corneum by intercalating between them.[34] 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 [34]. 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.

CONCLUSIONS

The study attempted to improve the solubility and permeability of the tyrosine kinase inhibitor, dasatinib. The drug has been classified under Class II 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 DAS 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.

DECLARATIONS

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Conflict of Interest: The authors declare that they have no conflicting interests to conduct the study

Ethical Approval: Not applicable

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