

Development of Dasatinib Loaded Self-Micro Emulsifying Drug Delivery System: In-Vitro Characterization

Nitin V. Kokare^{*1}, Dr. Rohit R. Shah²,

1. Department of Pharmaceutical Chemistry, Appasaheb Birnale college of Pharmacy, Sangli.

2. Department of Pharmaceutics, Appasaheb Birnale college of Pharmacy, Sangli.

***Author for Correspondence**

Mr. Nitin V. Kokare

ORCID: 0009-0009-0247-3761

Department of Pharmaceutical Chemistry, Appasaheb Birnale college of Pharmacy, Sangli. MH, India. 416416.

Mob. No. +91-9975631841

E-Mail ID- nitinkokare111@gmail.com

Abstract

Dasatinib is a BCS Class II tyrosine kinase inhibitor indicated for the treatment of chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia. However, its poor aqueous solubility and pH-dependent absorption significantly limit its oral bioavailability. The present study aimed to develop and characterize a Dasatinib-loaded Self-Micro Emulsifying Drug Delivery System (SMEDDS) to enhance solubility and improve in-vitro anticancer activity. Solubility screening facilitated the selection of Capryol 90 as the oil phase, Tween 40 as the surfactant, and Transcutol HP as the co-surfactant. A pseudo-ternary phase diagram was constructed to identify the optimal microemulsion region. The optimized SMEDDS formulation was prepared by dissolving Dasatinib in the preconcentrate mixture and evaluated for cytotoxic activity using the MTT assay against the HL-60 human leukemia cell line. The results demonstrated dose-dependent inhibition for all tested samples. The standard drug, 5-Fluorouracil, exhibited an IC₅₀ value of 33.91 µg/ml, while the Dasatinib API showed an IC₅₀ of 44.29 µg/ml. Notably, the SMEDDS formulation exhibited improved cytotoxic activity with an IC₅₀ value of 38.56 µg/ml, indicating enhanced therapeutic potential compared to the pure drug. The enhanced performance of the SMEDDS may be attributed to improved solubilization and cellular uptake. Overall, the developed SMEDDS formulation represents a promising lipid-based strategy for enhancing the solubility and in-vitro anticancer efficacy of Dasatinib.

Keywords: Dasatinib, SMEDDS, Self-Micro Emulsifying Drug Delivery System, Solubility Enhancement, HL-60 Cell Line, MTT Assay

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Introduction

Oral drug delivery is the most favored route due to its ease, cost-effectiveness, and high patient compliance. However, many orally administered drugs exhibit poor aqueous solubility, resulting in low and variable bioavailability. Conventional immediate-release dosage forms often fail to maintain consistent plasma drug concentrations. Dasatinib, a second-generation tyrosine kinase inhibitor used in chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia, suffers from poor solubility and pH-dependent absorption. It is classified as a BCS Class II drug, where dissolution is the rate-limiting step for absorption. These limitations necessitate the development of advanced formulation strategies. Lipid-based drug delivery systems have shown promise in improving solubility and oral absorption of lipophilic drugs. Among them, self-microemulsifying drug delivery systems (SMEDDS)

are widely investigated. SMEDDS spontaneously form fine oil-in-water microemulsions in the gastrointestinal tract. The small droplet size enhances drug dissolution and absorption. SMEDDS also promote lymphatic transport and reduce first-pass metabolism. Additionally, surfactants used in SMEDDS can inhibit P-glycoprotein-mediated efflux. Formulating Dasatinib as a SMEDDS can overcome solubility-related challenges and improve bioavailability. The Quality by Design approach further ensures formulation robustness and reproducibility. Thus, SMEDDS represents a promising strategy for effective oral delivery of Dasatinib.

Present study aimed to enhance solubility of dasatinib and in-vitro characterization.

Materials and method
Chemicals and Polymers

Dasatinib powder (99.45% w/w) was obtained from Shilpa Medicare Ltd., Raichur, Karnataka, India. Capryol 90 was obtained from Gattefosse Pvt. Ltd., Mumbai, India. Tween 40 was obtained from Mohini organics Pvt. Ltd. Transcutol HP was obtained from S.D. Fine-Chem Limited, Mumbai, India.

Cell lines and reagents

HL-60 human promyelocytic leukemia cell line was obtained from NCCS Pune, India. 10% fetal bovine serum (FBS) was obtained from NCCS Pune, India. 1% penicillin-streptomycin solution was obtained from NCCS Pune, India. MTT Reagent was obtained from NCCS Pune, India.

Method

Construction of pseudo ternary phase diagram

Dasatinib's solubility profile was evaluated to identify suitable components for the SMEDDS formulation, leading to the selection of Tween 40 as the surfactant, Transcutol HP as the co-surfactant, and Capryol 90 as the oil phase. A ternary phase diagram (Figure 2) was constructed to optimize the proportion of these constituents. In Formulation I, a mixture of 6 g of surfactant and co-surfactant blend, 4 g of oil, and 50 mg of dasatinib was prepared. Formulation II consisted of 7 g of surfactant and co-surfactant blend, 3 g of oil, and 50 mg of dasatinib. Both formulations were stored in tightly closed glass tubes at room temperature (25°C) until further use.

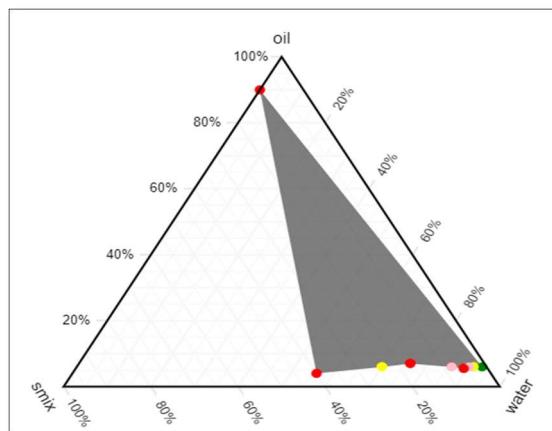


Figure 1: Ternary Phase Diagram for preparation of SMEDDS of Dasatinib

Preparation of SMEDDS

A blank SMEDDS was formulated by blending the oil, surfactant, and co-surfactant, followed by vortex mixing until a clear and homogeneous solution was obtained. For the drug-loaded formulation, 10.5 mg of dasatinib was incorporated into 100 μ L of the prepared blank SMEDDS and mixed thoroughly to ensure uniform dispersion.

In-vitro anti-cancer activity against HL-60 cell lines

The MTT cell proliferation assay is a colorimetric assay for assessing cell viability. It can be used for measurement of cell proliferation and analysis of cytotoxic and cytostatic compounds, such as anticancer drugs and other pharmaceutical compounds. The assay is based on the cleavage of the tetrazolium salt MTT in the presence of an electron-coupling reagent.

activity carried out according to the method described by D. Seenaiiah et al. 2014. HL-60 cells were seeded in 96-well plates with 7.5×10^3 /well in 0.1 mL of RPMI 1640 medium supplemented with 10% FBS and cultured in a humidified incubator (at 37 °C in 5% CO₂) for 24 h. The tested compounds were dissolved in DMSO, and ethanol and serial dilutions were made for the compounds. Different concentrations of dilutions were added to cells in 96 well plates and the cells were incubated further for 48 h.

In each well plate run, wells for solvent control (medium + cells + [DMSO and ethanol]), blank (medium + solvent [DMSO and ethanol]), and growth control (medium + cells) were also included.

MTT (3-(4,5-Dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) solution (20 μ L of 5 mg/mL) was added to each well and the incubation was continued for an additional 4 h. The medium was removed and 100 μ L of DMSO was added to dissolve the formazan crystals. The dark blue formazan crystals formed within the viable cells were solubilized with DMSO and the absorbance (OD) of each well was measured using an ELISA plate reader (Molecular Devices) at 492 nm. The percent survival of the cells was calculated by using non-linear regression. Cell survival was measured as the absorbance percentage compared with the control (non-treated cells with the compounds). The proliferation inhibitory effects of the compounds on cancer cells were expressed as IC₅₀, which is the compound concentration required to inhibit cell growth by 50%. IC₅₀ values of the compounds were obtained by plotting the percentage of survival versus the concentrations. We used 5-Fluorouracil as the standard anticancer drug against HL-60 cells in our experiments.

The MTT cell cytotoxicity experiments were performed in triplicates; and each point on the graphs was given with standard deviation of the mean value. IC₅₀ values of the compounds were determined by regression analysis using GraphPad Prism software version 8.0 (GraphPad Software Inc., San Diego, CA). Data are shown as a mean $\pm$ SD (standard deviation) of three independent experiments.

Apoptosis detection

The cells (1×10^6) were harvested and washed twice with ice cold PBS. Subsequently, the cells were labelled with Annexin V and Dead Cell assay kit according to the manufacturer's instructions. This

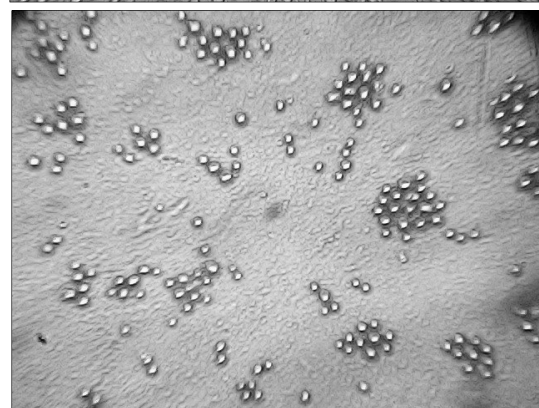
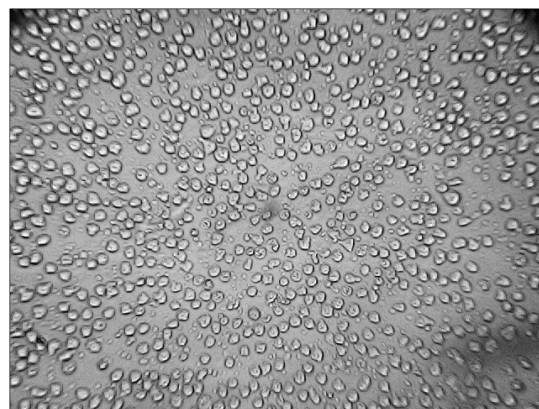
assay is based on the phosphatidylserine (PS) detection on the apoptotic cells surface, using fluorescently labelled Annexin V. The samples were determined by the Muse Cell Analyzer (Millipore, USA) and analyzed by software provided by Merck Millipore

Cell cycle analysis

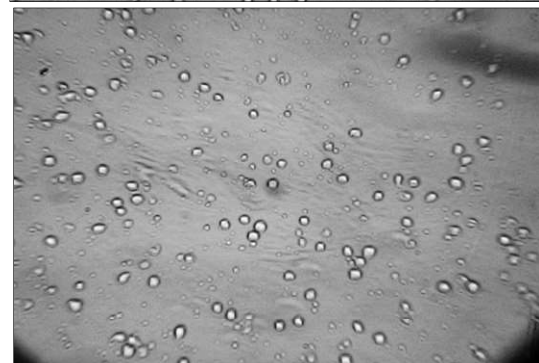
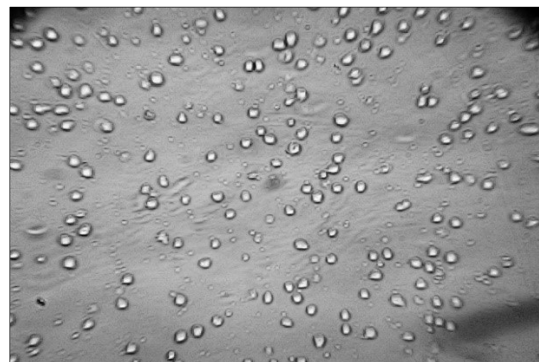
Analysis of DNA content and cell cycle distribution was done using muse cell cycle kit by flow cytometry. HL 6 cell line was cultured as per standard procedures described earlier and treated with LD₅₀ concentration of sample was added and incubated for 24 hours. Non treated control was also maintained and then incubated at 37°C in a humidified 5% CO₂ incubator for 24 hours. For fluorescence activated cell sorting (FACS) analysis, the treated HL 6 cells were incubated for 24 h. After overnight incubation cells were detached and pipetted out and spun at 300 × g for 5 min and washed once with 1 × Phosphate-buffered saline (PBS). The cells were fixed with 1 ml of ice-cold ethanol and incubated at -20 °C for overnight. The ethanol fixed cells were washed once with PBS followed by 200 mL of Muse cell cycle reagent. The tubes were incubated for 30 min at dark and analyzed on Muse flow cytometer (Millipore, USA)

Table No. 1 IC₅₀ data of Control Standard-5FU, Sample API and SMEDDS formulation

Sr. no.	Sample	concentration (µg/ml)	Absorbance (O D)	% Inhibition	IC ₅₀
1.	Control		2.137		
2.	Standard-5FU	10	0.996	53.40	33.91
		40	0.746	65.07	
		100	0.403	81.12	
3.	Dasatinib-API	10	1.361	36.32	44.29
		40	0.985	53.88	
		100	0.683	68.03	
4.	SMEDDS Formulation	10	1.219	42.96	38.56
		40	0.945	55.75	
		100	0.516	75.83	



HL-60- Control
STD 5FU. HL-60



Drug API Dasatinib
SMEDDS Formulation

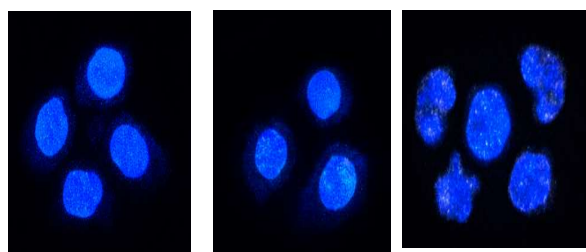


Figure No. 2 Showing cells of control, standard drug, Drug API and formulation

The MTT assay was conducted to evaluate the cytotoxic effects of different samples on the HL-60 human leukemia cell line. The tested samples included a standard drug (5-Fluorouracil, 5-FU), an active pharmaceutical ingredient (API), and a formulated version of the API. The percentage of inhibition and IC₅₀ values were determined for each sample at different concentrations (10, 40, and 100 µg/ml), providing insights into their relative efficacies. (Bhavikatti *et al.*, 2021)

The control group showed the highest absorbance, confirming the viability of untreated HL-60 cells. The standard drug, 5-FU, demonstrated a dose-dependent increase in cytotoxicity, with an inhibition rate of 53.40% at 10 µg/ml, increasing to 81.12% at 100 µg/ml. The IC₅₀ value for 5-FU was recorded at 33.91 µg/ml, indicating its potent anticancer activity.

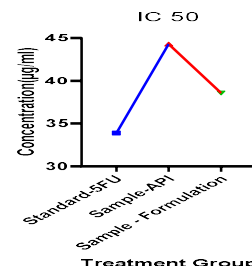
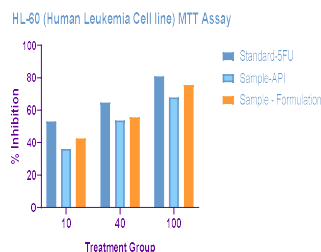
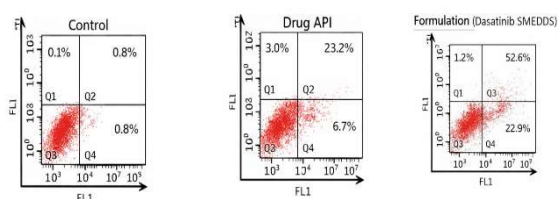
The API sample exhibited a similar trend of dose-dependent inhibition, with a 36.32% inhibition rate at 10 µg/ml and 68.03% at 100 µg/ml. The IC₅₀ value for the API was determined to be 44.29 µg/ml, which is higher than that of 5-FU, suggesting that the API is less potent than the standard drug.

The formulated version of the API also showed dose-dependent cytotoxic effects, with inhibition rates of 42.96%, 55.75%, and 75.83% at 10, 40, and 100 µg/ml, respectively. The IC₅₀ value for the formulation was found to be 38.56 µg/ml, which is lower than that of the API but higher than that of 5-FU, indicating an improvement in cytotoxic activity compared to the raw API.

DAPI staining

Formulation
Control
Dasatinib SMEDDS

Drug API



DAPI Staining Analysis

DAPI staining was performed to evaluate nuclear morphology and chromatin condensation as an indicator of apoptosis. In the control group, cells exhibited normal nuclear morphology with uniformly stained, round nuclei, indicating intact chromatin structure and absence of apoptotic changes.

The Drug API-treated group showed mild nuclear alterations, including slight chromatin condensation and reduced nuclear size in some cells, suggesting the initiation of apoptotic changes.

In contrast, the Formulation (Dasatinib SMEDDS)-treated group demonstrated pronounced apoptotic features, including intense DAPI fluorescence, chromatin condensation, nuclear shrinkage, and fragmented nuclei (apoptotic bodies). These observations indicate enhanced induction of apoptosis compared to the pure drug.

Cell cycle analysis

Cell cycle distribution and apoptosis were analyzed using flow cytometry following Annexin V/Dead Cell assay. The dot plots represent different quadrants corresponding to viable, early apoptotic, late apoptotic, and necrotic cell populations.

In the control group, the majority of cells were viable, with minimal apoptosis observed (0.1–0.8%), indicating normal cell cycle progression and low basal cell death.

The Drug API-treated group showed a significant increase in apoptotic cell population, with 23.2% cells in early apoptosis and 6.7% in late apoptosis, suggesting that the pure drug induces apoptosis to a moderate extent compared to control.

In contrast, the Formulation (Dasatinib SMEDDS)-treated group demonstrated a marked increase in apoptotic cells, with 52.6% early apoptotic and 22.9% late apoptotic populations. This indicates enhanced

apoptotic induction and possible cell cycle arrest compared to the Drug API alone.

Conclusion

The present investigation demonstrated the successful development of a Dasatinib-loaded Self-Micro Emulsifying Drug Delivery System (SMEDDS) to address solubility-related limitations of Dasatinib. The optimized formulation composed of Capryol 90, Tween 40, and Transcutol HP efficiently enhanced drug solubilization. In-vitro cytotoxicity studies using the HL-60 cell line revealed improved anticancer activity of the SMEDDS formulation compared to the pure drug, as indicated by a reduced IC₅₀ value. Apoptosis studies through DAPI staining and flow cytometric analysis further confirmed enhanced apoptotic induction and increased early and late apoptotic cell populations in the formulation-treated group.

These findings suggest that SMEDDS is an effective lipid-based delivery approach for improving the therapeutic potential of poorly water-soluble anticancer drugs like Dasatinib. However, further in-vivo pharmacokinetic and bioavailability studies are necessary to establish its clinical applicability.

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