OPEN ACCESS

Pharmaceutical Communications
DOI: org/10.55627/pharma.002.02.0573

Research Article

Enhancing Oral Solubility and Permeability of Edoxaban Tosylate via Development of Self Micro-Emulsifying Drug Delivery System

Syeda Aneela Azad[1]*, Umair Ilyas[1], Reem Altaf[2], Kalsoom Saleem[1] and Shaista Gul[1]

[1]Department of Pharmaceutical Sciences, Riphah Institute of Pharmaceutical Sciences, Riphah International University Islamabad, Pakistan.
[2]Department of Pharmacy, Iqra University, Islamabad, Pakistan
***Correspondence:** aneelaazad3@gmail.com

Abstract

Edoxaban tosylate monohydrate (EDTM) is a class IV biopharmaceutical classification system (BCS) drug, as it is poorly soluble and permeable, limiting its bioavailability. The objective of this study was to improve the permeability and solubility of EDTM by incorporating it into a self-micro-emulsifying drug delivery system (SMEDDS). Suitable excipients were selected and evaluated for their compatibility. The solubility of EDTM was evaluated for all excipients (oil, surfactant, and co-surfactants) at different ratios. Subsequently, olive oil, Kolliphor RH40, and PEG-400 were selected as the oil, surfactant, and co-surfactants, respectively; furthermore, they were developed into SMEDDS by adding 15 mg of EDTM. The optimized EDTM-SMEDDS (F1, F2, F3, and F4) were characterized for particle size, polydispersity index (PDI), zeta potential, stability, *in vitro* release, and *ex vivo* permeation. F2 showed a particle size of 56.43 ± 1.78, PDI of 0.190, and -3.30 ± 0.56 mV of zeta-potential. The enhanced release and permeability were observed for all other EDTM-SMEDDSs and raw EDTM dispersions. Following storage under continuous temperature and accelerated stability conditions, F2 showed no signs of phase separation and was visually clear while retaining the percentage encapsulation efficiency and drug loading, indicating stability. We conclude that SMEDDS significantly improved the oral solubility and permeability of EDTM which indirectly may improve its bioavailability.

Keywords: Self-emulsification, BCS class-IV, solubility, permeability, micro-emulsion, SMEDDS

1. Introduction

Oral administration of medicines is the most convenient route for the delivery of active pharmaceutical ingredients (API). However, one of the major hurdles in oral dosing is adequate systemic exposure, accomplished after the optimization of oral bioavailability (Aungst 2017). To achieve an adequate systemic concentration of a drug, solubility plays an important role (Chaudhari and Dugar 2017). Up to 90% of newly discovered/synthesized drug candidates have low aqueous solubility and permeability per the biopharmaceutical classification system (BCS) and are included in Class II and IV (Lei et al. 2011). This prompted pharmaceutical manufacturers and researchers to design delivery systems to overcome these challenges (Odenwald and Turner 2017). Currently, tremendous efforts have been made to combat challenges like decreased drug solubility, poor drug permeability, and enzymatic degradation. These efforts include efflux pump inhibitors, permeation promoters, and drug development in nanoparticles (Son, Lee, and Cho 2017).

Edoxaban tosylate monohydrate (EDTM) was developed and clinically administered targeting selective factors in the coagulation cascade instead of inhibiting multiple factors (Partida and Giugliano 2011). EDTM is an oral small molecule

that reversibly and directly inhibits FXa, a non-vitamin K antagonist acting as a direct oral anti-coagulant (DOACs) and anti-thrombin drug. Researchers were successful in developing EDTM that reduced warfarin-associated adverse effects. For instance, compared to warfarin, EDTM required less monitoring and dietary restrictions, with a wide therapeutic window (Rashid et al. 2021). This breakthrough was beneficial for prevention and treatment of venous thromboembolism in patients suffering from atrial fibrillation. Following EDTM oral administration, peak plasma concentration was achieved approximately 1-2 hours later. The absolute bioavailability of EDTM is 62%, whereas its volume of distribution is 107 liters. *In vitro* protein binding is approximately 55%. The clinical significance of EDTM was also demonstrated by Rashid and colleagues (Rashid et al. 2021).

EDTM is a BCS Class IV drug, indicating low solubility and permeability. To increase its clinical efficacy, it is crucial that its permeability be enhanced in order to increase its transport across the gastrointestinal membrane and subsequent distribution (Wang and Skolnik 2013). It is also important to increase the solubility, as poorly soluble drugs are eliminated rapidly from the gastrointestinal tract, leading to poor blood dissolution and distribution. Hence, increasing the solubility and permeability of the drug has great significance for its utilization in drug discovery and development.

Based on the above-mentioned concept, the objective of this study was to increase the permeability and solubility of EDTM to ultimately enhance its bioavailability. To achieve this aim, EDTM-loaded SMEDDS suitable for oral delivery were developed. First, SMEDDS with different concentrations of excipients (oils, surfactants, and co-surfactants) were formulated. The effect of EDTM-SMEDDS on solubility and pemeability was investigated via *in vitro* and *ex vivo* studies to ascertain the diffusion of the EDTM from optimized SMEDDS. The stability of formulated EDTM-SMEDDS was also investigated to check the effect of harsh environmental conditions on EDTM-SMEDDS.

2. Materials and Methods

2.1. Materials

EDTM was donated by Vision Pharmaceuticals (Pvt) Ltd. Islamabad, Pakistan. Tween 80 (polyoxyethylene sorbitan monooleate) was purchased from Hangzhou Zhongbao Corp. Ltd, Hangzhou, China; Tween 20 from Avantor ™ Performance Materials Inc, PA, USA; Span 20, 80 (sorbitan monooleate) and Sodium Stearate from Techno Pharmchem, Phase III, Delhi, India; Benzalkonium Chloride, Corn Oil from Acros Organics, Janssen Pharmaceuticalaan 3a, Geel, Belgium; Sodium Lauryl Sulphate (SLS) from Shanghai Auway Daily Chemicals Co., Ltd, China; Crodamol Oil, Senamon Oil, Clove Oil (oil of *Cinnamomum zeylanicum*), Soybean Oil (Glycine max) from Brighton, BNI 3TN, UK; Propylene glycol (1,2-propanediol) was purchased from Shinghwa Amperex Technology (Dongying) Co. Ltd. China; PEG-4000 (polyethylene glycol) from Hangzhou Lingeba Technology Co. Ltd., China; Castor oil was purchased from Astral Ltd., India; Kolliphor RH40 (polyoxyl 40 hydrogenated castor oil), olive oil from Agricultural & Environmental Testing & Research Laboratories, South Africa. Trifluoroacetic acid, Ammonium dihydrogen phosphate, Acetic Acid, Acetonitrile (HPLC grade) from ChemLab NV, Industriezone, Belgium. No further purification of chemicals was done prior to use as all chemicals were of analytical grades.

2.1. Calibration Curve of EDTM

15mg of EDTM was dissolved in 20 mL of diluent [ACN: Acetonitrile (10): water (90)] in a 50mL volumetric flask, acting as standard stock solution. Sonication was carried out for 5-10 minutes for complete solubility of the drug. The volume of the drug solution was then made up to 50mL by adding diluent to obtain an eventual concentration of 300 µg/mL. From the stock solution, further

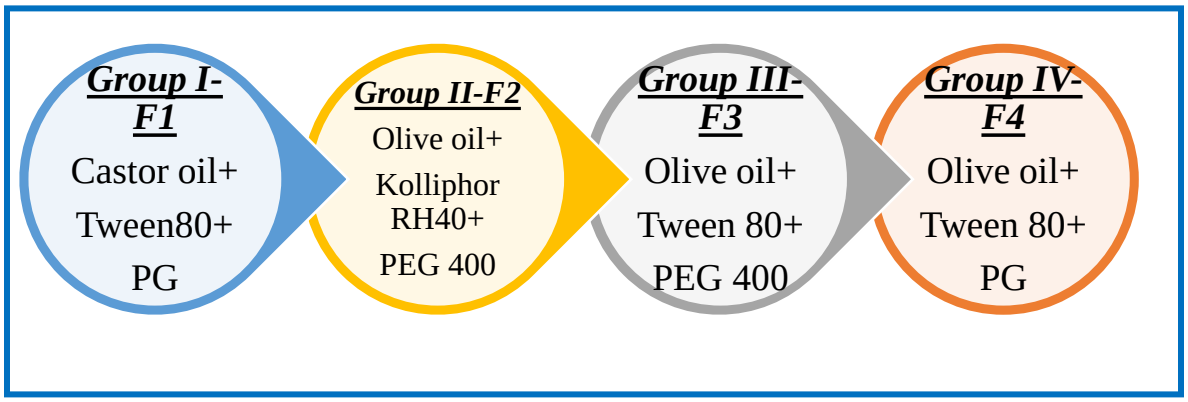

Figure 1. Groups for Combinations of Oil, Surfactants and Co-surfactant for Emulsification Test, F1-Group I, F2-Group II, F3-Group III, F4-Group IV. PG-Propylene glycol, PEG-400-Polyethylene glycol 400

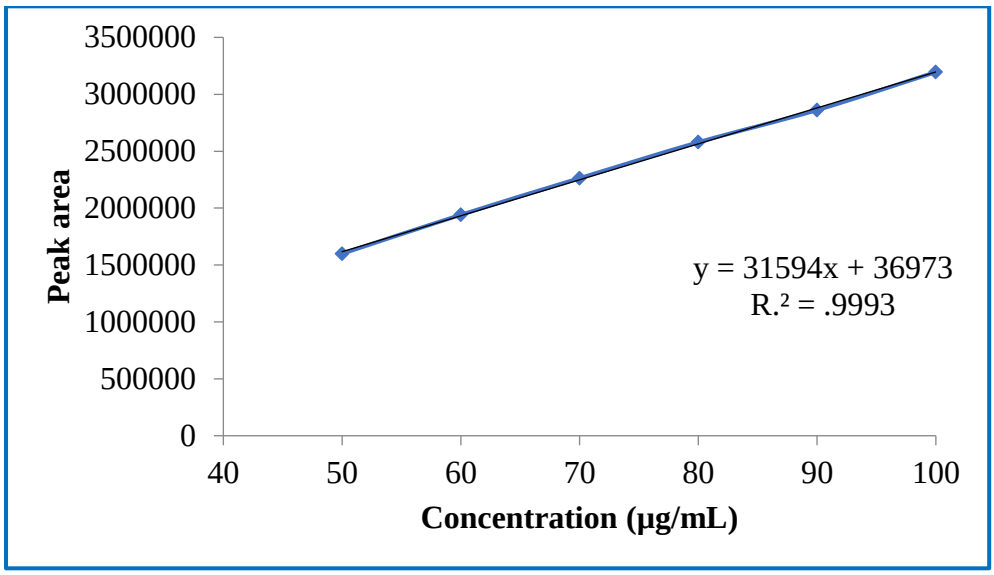

Figure 2. Calibration curve of Edoxaban tosylate (EDTM) in ACN:Water diluent at ambient temperature.

serial dilutions were made with diluent to get working standard solutions of 100 µg/mL, 90 µg/mL, 80 µg/mL, 70 µg/mL, 60 µg/mL, and 50 µg/mL (Sankar et al. 2021). The assay was conducted using HPLC at 290 nm as λ_{max}, column size of 4.6 mm x 250mm, 5 µm packing C18 with column temperature at 35°C, maintained at a flow rate of 1.2mL/min and injection volume of 10 µL. The mobile phase used was a mixture of acetonitrile and buffer [dissolve 2.3g of ammonium dihydrogen phosphate in 1000 mL of water, adjust to pH 2.0 with phosphoric acid, added 100µL of trifluoroacetic acid, filtrate] (ACN: Buffer, 22:78 ratio). The readings were plotted and value for R^2 was obtained. All measurements were taken in triplicate.

2.2. Compatibility Tests

Compatibility between surfactants and oils were determined by slight modifications in the method used previously (Jianxian et al. 2020). Mixture of oil, surfactant and co-surfactant at selected weight ratios were made by using oscillated mixing. The appearance of resulting solutions was physically evaluated.

Table 1. Surfactant-cosurfactant mixtures (Smix) with weight ratios.

Smix	Smix Code with Weight Ratios
Tween 80:PG	T_{80}-PG (1:1, 2:1, 3:1)
Kolliphor RH-40:PEG-400	T_{80}-PEG (1:1, 2:1, 3:1)
Tween 80:PEG-400	T_{80}-PEG-400 (1:1, 2:1, 3:1)
Tween 80:PG	T_{80}-PG (1:1, 2:2, 3:1)

Notes: PEG = polyethylene glycol, PG = propylene glycol.

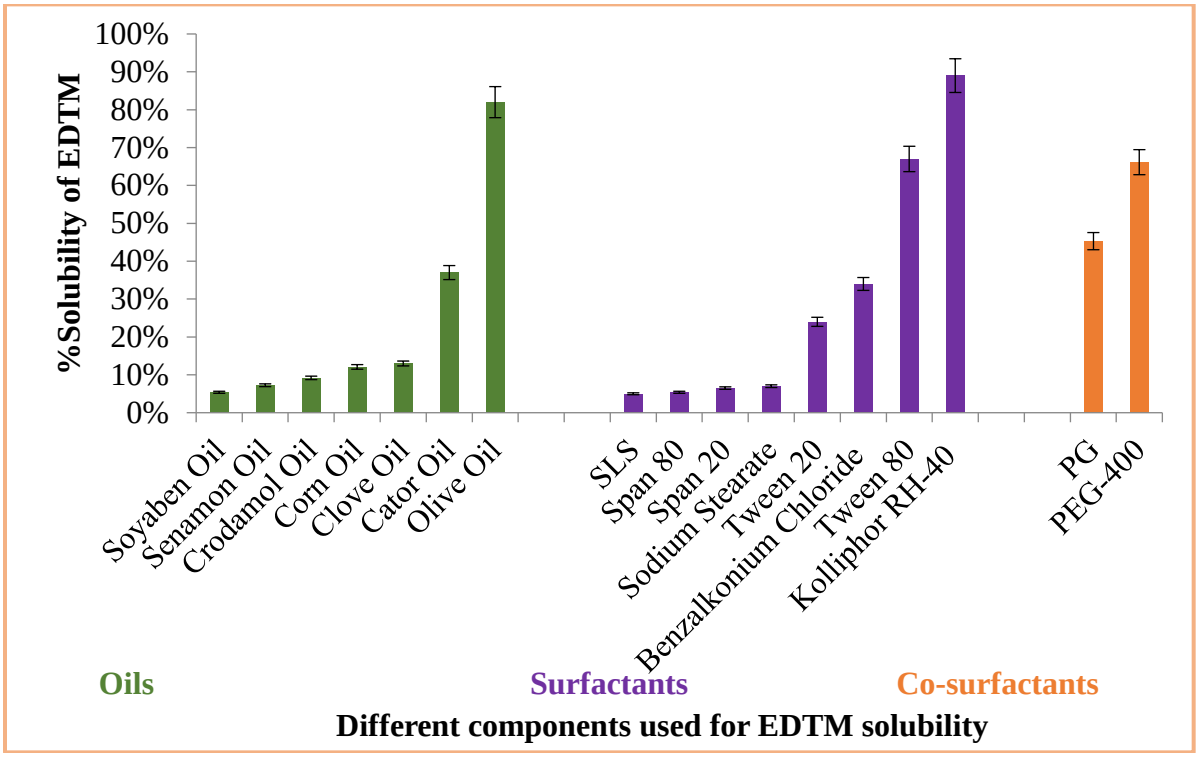

Figure 3. Comparative solubilities of EDTM in different components (oils, surfactants and co-surfactants) evaluated at 25°C. SLS-Sodium lauryl sulphate.

2.3 Solubility Studies of EDTM

All the excipients of the optimized SMEDDS formulations were selected based on solubility studies of EDTM. To find out appropriate compositions of SMEDDS, the solubility of drug in various oils, surfactants and co-surfactants was measured (castor oil, Olive oil, Corn oil, Soyabean oil, Cinnamon oil, Crodamol oil and clove oil as oils, Tween 80, Kolliphor RH40, Span 20, Sodium Stearate, Benzalkonium Chloride as surfactants and PEG-400 and PG as co-surfactants). An excess amount of EDTM was added into 5 mL of each vehicle in separate glass vials. After sealing, the mixtures were shaken at 37°C, 100 rpm for 72 h, followed by ultrasonic treatment (sonicator) for 20 min and centrifuged at 3000 rpm for 10 min. After achieving equilibrium, the supernatants were filtered through a PTFE membrane filters (0.45 µm). The concentration of EDTM was then

quantified by HPLC at 290nm with suitable dilutions (Mahmood et al. 2023).

Olive oil and castor oil were chosen to be the oil phase, Tween 80 and Kolliphor RH40 as surfactants, and PEG-400 and PG to be co-surfactants. Four combinations of selected lipid excipients were analyzed to select the optimal mixture for drug delivery. Figure 1 shows four group combinations that were tested for emulsification properties.

The selected surfactants and co-surfactants were blended at ambient temperature in three weight ratios (1:1, 2:1 and 3:1, with an increasing concentration of surfactants) making surfactant-cosurfactant mixtures (Smix), also known as Km, to determine the effect of Smix ratios on micro-emulsion formation **(Table 1).** Oil and Smix were thoroughly stirred at different weight ratios (1:1 till 1:9) in separate glass vials. Moreover, 45 new mixtures of varying ratios were made using this method.

2.4. Preliminary Studies of Self-micro-emulsification Efficiency

The mixtures (oil+Smix) (2 mL) were slowly stirred, using magnetic stirrer, avoiding any bubble formation, while addition of aqueous phase was done using micro pipette. Briefly, 10µL of liquid mixtures/systems were added dropwise into 100 mL of distilled water in separate beakers, maintained at 37°C and 100 rpm (supplementary data). The resultant emulsions were assessed through naked eye for rate of emulsification and appearance. They were graded as micro-emulsions based on the following criteria.For better visual examination, any air bubbles formed were removed by sonication for 1-2 minutes.

1. Nano emulsion (oil in water) (NE): clear, transparent, isotropic emulsion, easily flow ability.
2. Nano emulsion gel (NEG): clear and highly viscous gel-like emulsion.
3. Micro-emulsion (ME): milky, cloudy mixture or nearly transparent homogenous emulsion without phase separation.
4. Emulgel (EG): milky and highly viscous gel (Ansari et al. 2023, Kim et al. 2023, Jianxian et al. 2020)

Systems/mixtures that formed micro-emulsion upon dilution were subjected to emulsification time determination. The self-emulsification efficiency was tested by using the protocols similar to those used for the determination of micro-emulsions. 50 µL of selected systems/mixtures were stirred in 100 mL of distilled water kept at 37±0.5°C and 50 rpm. Furthermore, the systems were then visually assessed for emulsification time and final appearance of systems based on following the grading scale:

- Grade A: clear nano-emulsion (<1 min.)
- Grade B: translucent/less clear nano-emulsion (>1 min)
- Grade C: clear or slightly translucent micro-emulsion with bluish white appearance (either within 2 mins or <1min)
- Grade D: bright milky/white emulsion (>2 mins)
- Grade E: emulsions with either poor emulsification, possessing large oil globules on the surface or no emulsion formation (Nasr, Gardouh, and Ghorab 2016, Ansari et al. 2023, Jianxian et al. 2020).

2.5. Preparation of EDTM-loaded SMEDDS

Based on the solubility and compatibility data, liquid ETDM-SNEDDS were prepared. The selected systems were then loaded with EDTM. Each 2 mL of the system was loaded with 15 mg of EDTM, the concentration of the drug was kept constant (15mg/2mL). The systems were mixed thoroughly in glass vials to dissolve the drug completely under constant stirring at 1200 rpm at 37±0.5°C for 15 mins and stored at 25°C until further analysis.

2.6. Estimation of Drug Content and Encapsulation Efficiency

The EDTM-SMEDDS were then filtered using 0.45µm membrane filter prior to analysis for entrapment efficiency and drug-loading to ensure

the removal of un-incorporated drug. ETDM-SMEDDS were then diluted up to the required concentration and dissolved in ACN: Water to let the drug release in the solvent, followed by entrapment efficiency and drug loading analysis on HPLC at a λ_{max} of 290nm. Each sample was analysed in triplicate. These EDTM-SMEDDS were then kept under observation, at ambient temperature for 2 days, for any cloudiness or phase-separation before further characterization studies (Ansari et al. 2023, Kim et al. 2023).

2.7. Droplet Size and Zeta-potential Analysis

The droplet size and zeta-potential analysis for selected EDTM-SMEDDS formulations was performed by Zetasizer ZS 90 (Malvern Instruments, Malvern, Worcestershire, U.K). For this purpose, 100µL of EDTM-SMEDDS formulation were made up till 1mL with distilled water. The detection range was 2 nm-5000 nm. Each sample was analysed in triplicate.

2.8. Fourier Transformed Infrared Spectroscopy (FTIR)

FTIR was employed to detect chemical changes and possible interactions of EDTM-SMEDDS. FTIR for all excipients, raw drug and optimized EDTM-SMEDDS was measured. All FTIR spectra were recorded in range of 200 cm^{-1} to 2000 cm^{-1} at a resolution of 2 cm^{-1}.

***2.9. In vitro* Release Behavior of Loaded EDTM-SMEDDS**

The *in vitro* dissolution studies were carried out using USP dissolution testing apparatus type II. The dialysis membranes with molecular weight cut off of 3500 Da (Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) were soaked in distilled water 24 hours prior to use. Furthermore, 2 mL of freshly prepared EDTM-SMEDDS (containing 15 mg EDTM) and 2 mL EDTM dispersion (containing 15 mg EDTM) was sealed in dialysis membranes and suspended in 250 mL SIF (pH 6.8) and SGF (pH 1.2). Notably, release medium was maintained at 37±0.5°C at 100 rpm. Aliquots (5 mL) were withdrawn periodically at predetermined time intervals (0 min, 15 mins, 30 mins, 45 mins, 1h, 1.5h, 2h, 2.5h up till 24h). Equal volume (5 mL) of fresh release medium was added to maintain a constant volume. Aliquots were filtered with 0.45 µm PTFE membrane filters and the content of the EDTM was analyzed by HPLC at 290nm. Each formulation was analysed in triplicate (Thota et al. 2023, Ansari et al. 2023).

***2.10. Ex vivo* Drug Permeation Studies**

Sprague–Dawley male rats (250–300 g) were sacrificed to study the duodenal permeability. Duodenal part of intestine was cut (≈3.5 cm). To remove the excess fats, mucus, and luminal contents, intestines were washed with normal saline. Freshly prepared EDTM-SMEDDS were made. EDTM dispersion (control) containing 15 mg/2mL was prepared by mixing 15 mg of drug in diluent (ACN: Water) on magnetic stirrer. The formulations and control were injected into the duodenum with the help of a syringe. To avoid any leakage of formulations the other side of duodenum was tied with a thread. The prepared duodenum with different formulations were placed in different chambers of organ bath at constant temperature of 37±0.5°C. The compartment was filled with 50 mL of PBS and aliquots of 5 mL were withdrawn at predetermined time interval and immediately replaced with fresh medium. The samples were analysed for drug dialyzed across the membrane with HPLC. Each sample was analysed in triplicates and the results were calculated as mean±S.D (Jianxian et al. 2020).

2.11. Stability Studies

In order to evaluate the stability of the optimized EDTM-loaded SMEDDS, stability studies were conducted as per ICH guide lines (Huynh-Ba and Zahn 2009). To assess the stability, optimized EDTM-SMEDDS formulations were added to capped glass vials and stored at on-going conditions of 25°C±2°C, 60%±5% RH and accelerated conditions of 40°C±2°C, 75%±5% RH. Later, samples were withdrawn from formulations fter one month and analysed for drug loading content, entrapment efficiency,

visual clarity, and phase separation using the HPLC method (Shafiq un Nabi et al. 2007, Qureshi, Mallikarjun, and Kian 2015, Jianxian et al. 2020).

3. Results

3.1. Calibration Curve of EDTM

After making working standard solutions, a calibration curve was plotted (Figure 2). This curve was then utilized to find the unknown concentration of EDTM for solubility studies, encapsulation efficiency, and drug loading. The value for R^2 obtained was 0.9993.

3.2. Solubility Studies

The solubility of EDTM was assessed in different components of the system i.e. oils, surfactants and co-surfactants for selection of the best components, compatible with the drug (Figure 3). Among the oils selected, EDTM showed highest solubility in castor oil (0.82±0.11 mg/mL), and olive oil (0.82±0.11 mg/mL), which were selected as oil phases. Amongst various surfactants investigated, EDTM exhibited maximum solubility in Kolliphor RH40 (0.89±1.02 mg/mL) as compared to Tween 80 (0.67±0.005 mg/mL), hence selected as surfactant phase. Among various co-surfactants studies, PG (0.6615±0.996 mg/mL) showed greater solubility in comparison to PEG-400 (0.453±0.238 mg/mL) both of these co-surfactants were selected for further studies. Solubility of different components of SMEDDS formulations is combined in figure 3.

3.3. Selection of Excipients Based on Solubility Studies

Excipients were selected on the basis of solubility studies. Castor oil and olive oil were chosen to be the oil phases, Tween 80 and Kolliphor RH40 as surfactants, and PEG-400 and PG selected as co-surfactants. The compatibility among selected surfactants, co-surfactants and oils was studied to choose the best components. All selected surfactants, co-surfactants and oils resulted in clear and homogenous mixtures in combinations. These combinations (Smix) were then further mixed in different weight ratios ranging from 1:1 to 1:9. Ratios of 13:43:5 of oil: surfactant: co-surfactant resulted in larger emulsification region, as shown in Table 2. The micro-emulsion region narrows down as the ratios of Smix and oil increases. These systems were observed visually for emulsification region and characterized according to the grading system described in section 2.5.

3.4. Self-emulsification time Determination

The *in vitro* performance of systems selected, was visually assessed based on the grading system mentioned in section 2.4, results are presented in Table 3. Visual observations showed that all SMEDDS systems were of grade A and B. These results agree with some previous studies (Mahmoud, Bendas, and Mohamed 2009, Nasr, Gardouh, and Ghorab 2016).

3.5. Preparation of EDTM-Loaded SMEDDS

After the selection of suitable excipients based on previous tests, EDTM loaded SMEDDS were prepared (table 4).

3.6. Estimation of Drug Content & Encapsulation Efficiency

The drug loading and entrapment efficiency of optimized EDTM-SMEDDS is presented in table 5. A high percentage of drug entrapment and a significant amount of loading was achieved.

3.7. Particle Size, Zeta-potential and Polydispersibility Index (PDI) Analysis

All optimized EDTM-SNEDDS showed high negative ζ potential values. The ζ potentials of EDTM-SMEDDS were found to be in range between -11.1 mV and -3.26 mV. The average globule size ranged between 56 nm and 664.4 nm. The average particle size in F2 was 56.43 nm. The ζ potential of F2 was -3.26 mV. The ζ potential of all optimized EDTM-SMEDDS is presented in Figure 4, Table 6.

3.8. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR studies were conducted to find out interactions as well as incompatibility between EDTM-SMEDDS and excipients. The functional group peak of PEG-400 was observed at 1956 cm^{-1}.

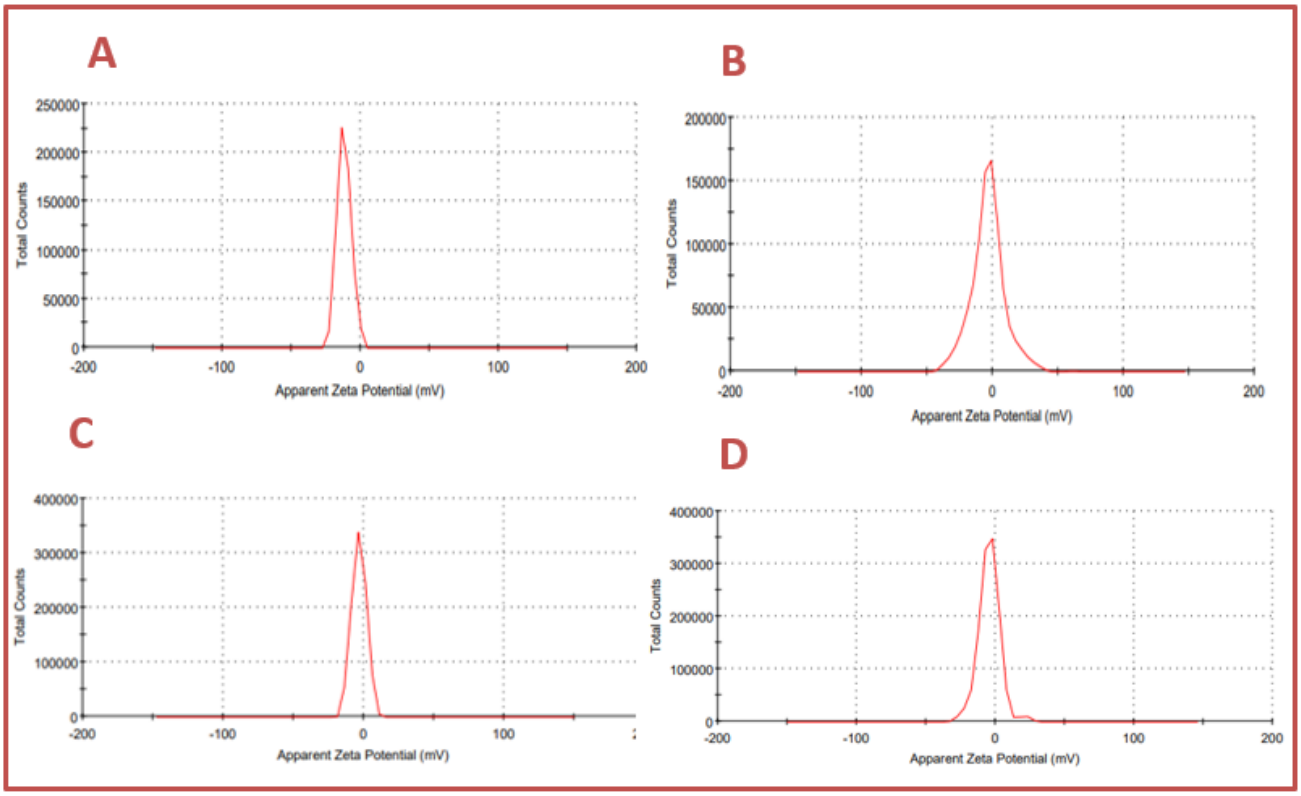

Figure 4. ζ-potentials of optimized EDTM-SMEDDS conducted at room temperature for different formulations. A-F1, B-F2, C-F3, D-F4.

Moreover, PG functional group peaks were observed between 1500-2000 cm^{-1} for C=O bending, between 1000-1500 cm^{-1} we found C-O-C stretching and Si-O functional group peak, from 500-1000 cm^{-1} Si-C functional group peak was present. For Kolliphor RH40 showed a specific peak at 1105 cm^{-1}, which is associated with OH stretching vibrations of C=O groups in its structure. Meanwhile, castor oil showed a peak at 1742 cm^{-1} (-C=O stretching of triglyceride ester carbonyls) and olive oil showed an absorbance peak at 1163 cm^{-1}. Distinct peaks of EDTM at 1618 cm^{-1} and 1500 cm^{-1} were observed, which were related to the band stretching of C=O of amide group and C=C, respectively. The peaks at 1375 cm^{-1}, 1217 cm^{-1}, 1158 cm^{-1}, 1010 cm^{-1} are attributed to CH_3 deformation. The peak at 684 cm^{-1} is due to CH out of plane aromatic band. There is no shift or change in characteristic peaks of EDTM before and after SMEDDS formation. After formulation development with excipients, the characteristic peaks at 1618 cm^{-1} (C=O), 1500 cm^{-1} (C=C) did not change, indicating the stability of the EDTM in SMEDDS with no evidence of drug-excipient interaction.

3.9. *In vitro* Drug Release Study

The *in vitro* release profile of different optimized EDTM-SMEDDS in SGF and SIF along with pure EDTM dispersion (control) is presented in Figure 6 and 7. The results signify that *in vitro* release profile of EDTM from SMEDDS produced a continuously superior drug release in SGF and SIF as compared to pure EDTM dispersion. Within the initial 4 hours of *in vitro* release study, a 'burst release' effect was observed from all EDTM-SMEDDS as well as the control (raw drug dispersion) in SGF and similar results were obtained in SIF as well. However, the F2 EDTM-SMEDDS showed a comparable release with F1 in SIF; also, had faster and higher release in SGF.

Table 2. Grading of systems for different ratios of all four group combinations at ambient temperature

Sr.#	Group I		Group II		Group III		Group IV	
	Time (sec)	Visual Grade	Time (sec)	Visual Grade	Time (sec)	Visual Grade	Time (sec)	Visual Grade
1	05	Milky	30	Clear	1 min	Clear	05	Clear
2	07	Milky	11	Milky	01	Clear	49	Clear
3	17	Clear	22	Clear	<2 mins	Milky	38	Clear
4	<2 mins	Clear	07	Milky	22	Milky	29	Gel
5	20	Clear	11	Clear	27	Clear	42	Clear
6	10	Milky	15	Milky	19	Clear	44	Gel
7	15	Clear	28	Clear	22	Milky	12	Gel
8	12	Milky	30	Clear	19	Milky	11	Clear
9	17	Gel	24	Gel	40	Gel	40	Gel
10	20	Gel	06	Gel	25	Gel	25	Gel
11	19	Gel	02	Gel	30	Gel	30	Gel
12	28	Gel	30	Gel	51	Gel	51	Gel

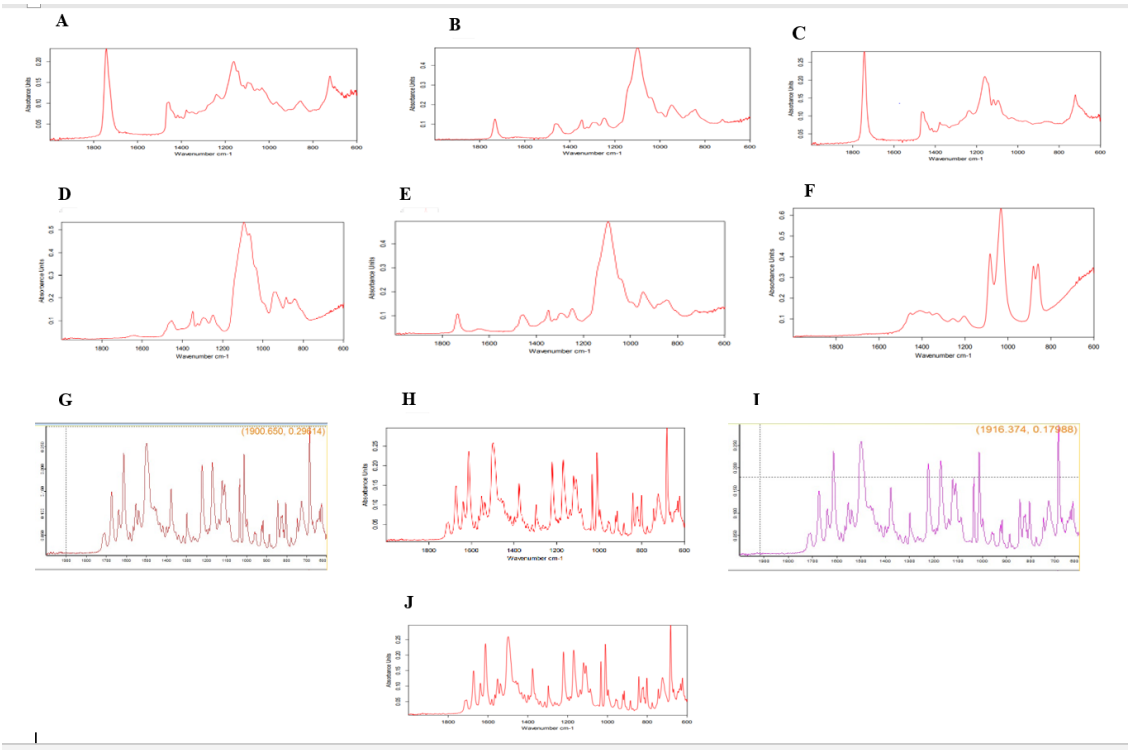

Figure 5. FTIR images of A (Castor Oil), B (Kolliphor RH 40), C (Olive Oil), D PEG 400), E (PG), F (Tween 80), G (EDTM Raw Drug), H (F1), I (F2), J (F3).

Notably, both dispersions exhibited approximately similar release within first few hours of initiation of study. The remaining SMEDDS released lesser than F1 and F2 in SIF and SGF. Despite this, all EDTM-SMEDDS showed 100% release up to 24 hours in both media. The raw drug was not completely released in both the media till 24 hours (20.00% in SIF and 22.90% in SGF), whereas, SMEDDS formulations showed controlled release which lasted till 12 hours. Of all the EDTM-SMEDDS, F2 showed superior release which was extended till 12 hours in SIF and SGF.

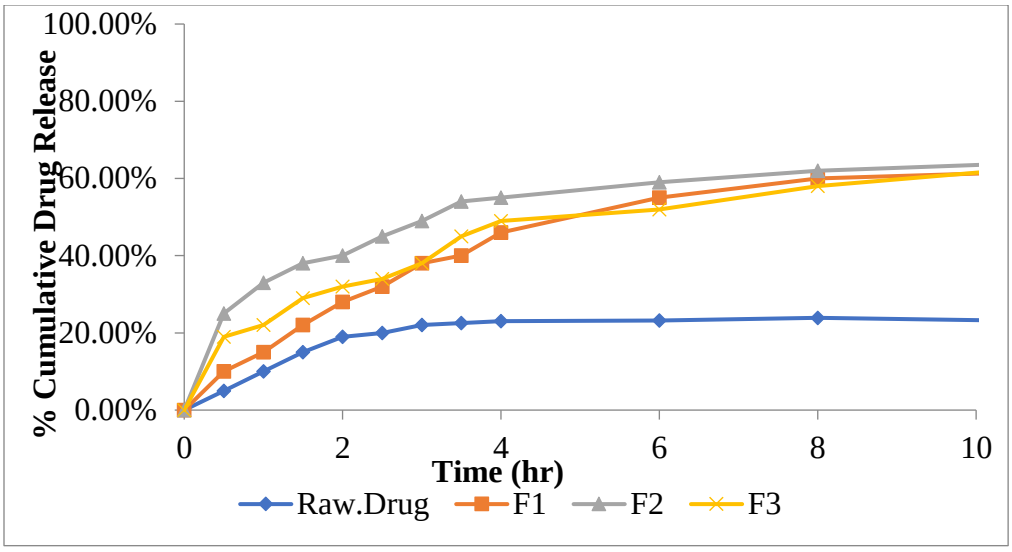

Figure 6. ***In vitro*** **elease profile of EDTM-SMEDDS formulations (F1, F2, F3) and pure EDTM dispersion (Raw Drug) in SGF (pH 1.2) at 37±0.5°C for 24 hr. Results displayed are only for 12 hrs.**

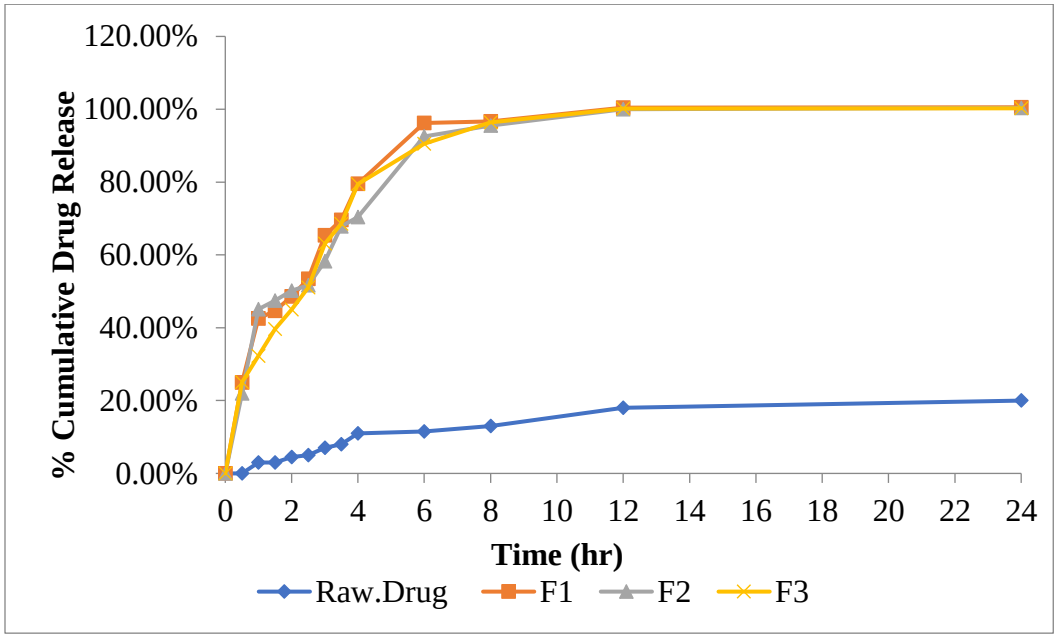

Figure 7. ***In vitro*****release profile of EDTM-SMEDDS formulations (F1, F2, F3) and EDTM dispersion (C) carried out for 24 hr at 37±0.5°Cin SIF (pH 6.8). Results displayed are for 12 hrs only.**

For raw drug, the plateau phase was detected only within 8 hours of initiation of study with 80% release lasting till 24 hours in both media, while all the EDTM-SMEDDS had their plateau phase in 12 hours of initiation of study in SIF and SGF. The release studies were continued for 24 hours to detect plateau phase.

3.10. *Ex vivo* Drug Permeation Studies

The drug perfused through rat's intestine. It was found that cumulative drug diffused through rat's intestine from all EDTM-SMEDDS was higher than cumulative drug diffused through pure EDTM dispersion. The amount of drug diffused through rat intestine from all SMEDDS can be

Table 3. Visual observations for dispersibility test for various SMEDDS systems.

System Code	Emulsion Appearance	Time (s)	Grade
Group I	Rapid forming milky emulsion	12	B
Group II	Rapid forming clear emulsion	28	A
Group III	Rapid forming milky emulsion	19	B
Group IV	Rapid forming clear emulsion	4	A

Table 4. Percent (%) composition of successful EDTM-SMEDDS

Formulation code	Oil (mL)	Surfactant (mL)	Co-surfactant (mL)
F1	Castor Oil (0.26 mL, 13%)	Tween 80 (0.87 mL, 43.5%)	PG (0.87 mL, 43.5%)
F2	Olive Oil (0.26 mL, 13%)	Kolliphor RH-40 (0.87 mL, 43.5%)	PEG 400 (0.87 mL, 43.5%)
F3	Olive Oil (0.26 mL, 13%)	Tween 80 (0.87 mL, 43.5%)	PEG 400 (0.87 mL, 43.5%)
F4	Olive Oil (0.26 mL, 13%)	Tween 80 (0.87 mL, 43.5%)	PG (0.87 mL, 43.5%)

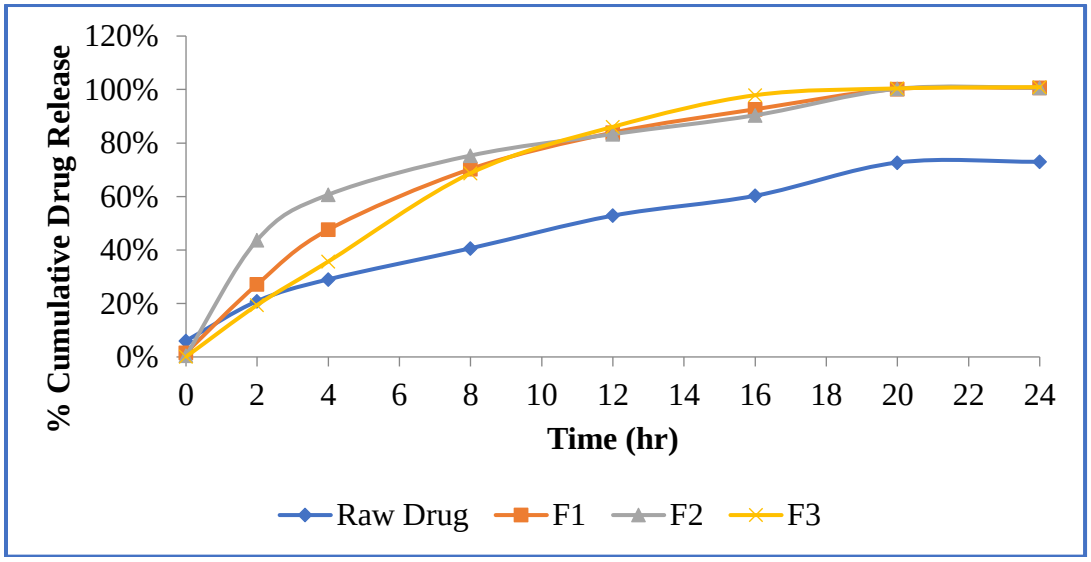

Figure 8. *Ex vivo*diffusion study for reconstituted EDTM-SMEDDS formulations (F1, F2, F3) and pure EDTM dispersion (C) in PBS, pH 6.8 at 37±0.5°C using rat intestine.

arranged in a descending order i.e., F2>F1>F3>C. The cumulative amount of drug diffused after 20 hours of initiation of study from F2 was 100%. F2 rapidly diffused into buffer medium following a sustained release of EDTM. In comparison, all other SMEDDS formulations also showed a cumulative drug release of 100% but had less drug diffusion relative to F2. While the raw drug (control) released the least into buffer medium and only 72.98% was released during 24-hour period (Figure 8).

3.11. Stability Studies

Stability of micro-emulsions is a key parameter to ensure the stability under several stressful and

Table 5. %Entrapment and drug loading of different formulations of EDTM-SMEDDS.

Formulation code	Entrapment Efficiency (%)	Drug loading (%)
F1	92.20	7.79
F2	93.02	6.98
F3	94.38	5.62
F4	92.01	4.07

non-stressful conditions. All EDTM-SMEDDS showed no signs of phase separation, phase inversion/change or any other sign of instability when observed visually. For ongoing stability testing, F1 and F2 retained approximately same encapsulation efficiency (%EE) and drug loading (%DL), while F3 had %EE decreased from 94.038% to 90.77%. Similarly, for accelerated stability testing, F1 and F2 retained approximately same %EE and %DL while F3 had %EE dropped to 90.88%. This showed that F1 and F2 were stable formulations but F3 had a decrease in its physicochemical parameters and was not stable for a period of three months (Table 7).

4. Discussion

EDTM, a non-vitamin K antagonist, a member of novel oral anti-coagulant (NOACs), is an anti-thrombin drug. EDTM was initially developed as a selective factor Xa-inhibitor, an alternative of vitamin K antagonist, such as warfarin, to overcome its adverse effects (Rashid et al. 2021). The aim of this study was to develop EDTM-SMEDDS in order to increase its permeability and solubility. Since it belongs to BCS class IV, it was necessary to develop some novel drug delivery system for EDTM in order to overcome issues associated with drugs of BCS Class IV. Furthermore, SMEDDS were chosen as drug delivery system or a carrier for EDTM as they have the ability to present the drug to GIT in a solubilized and micro-emulsified form. Due to small particle size of SMEDDS, an increase in surface area enables more efficient drug transport through intestinal aqueous layer and brush borders, enhancing bioavailability. Moreover, good thermodynamic stability and easy manufacturing makes this delivery system more suitable for incorporation of poorly soluble and poorly permeable EDTM.

The composition of SMEDDS include different surfactant to co-surfactants (Smix) ratios and blending them with oils. Surfactants create a film around each micro-emulsion particle that help in reducing the interfacial tension and provide a barrier to aggregation, thereby, preventing precipitation of drugs within the gastrointestinal lumen. Notably, negative interfacial tension and interfacial film required for creation of repulsion for particles are hardly attained with the use of single surfactant, hence, SMEDDS requires co-surfactants. They are also a valuable component of SMEDDS system as they determine the globule size, stability, and crucial for stabilizing the SMEDDS formulations (Ijaz et al. 2016). Additionally, co-surfactants decrease the bending stress at the interface, providing elasticity for interfacial film formation in the micro-emulsion for varying compositions i.e. (oil/water, water/oil, bi-continuous). Oils are considered to be the main component of SMEDDS system. They are important for increasing the solubility of lipophilic drugs by increasing the fraction of drug transported via lymphatic system, therefore, enhancing the absorption of lipophilic drugs (Jianxian et al. 2020).

Upon visual assessment of the compatibility studies results, all excipients were compatible with each other and did not result in any phase separation or coagulation. Afterwards, they were moved to selection of excipients step. Selection of excipients is a vital step in the development of SMEDDS as the drug loading capability of EDTM depends upon its solubility in different excipients

Table 6. Particle size, Zeta potential and PDI of 2 mL optimized EDTM-SMEDDS (mean ±SD)

Formulation code	Particle size (±σ) (nm)	PDI	ζ potential (±σ) (mV)	Conductivity (mS/cm)
F1	175.5±7.5	0.225	-11.7±5.07	0.0369
F2	56.43±1.78	0.190	-3.30±0.56	0.0304
F3	177.6±9.1	0.272	-3.26±0.6	0.0229
F4	664.4±100.7	1.000	-4.44±0.07	0.0251

of SMEDDS. Selection of excipients was based on the solubility studies to find a suitable oil, surfactant and co-surfactant with maximum solubilizing capacity for achieving maximum drug loading (Jianxian et al. 2020). Comparing the results for selection of excipients, it appeared that the self-microemulsifying properties of systems with surfactant Kolliphor RH40 were better than those with Tween 80; however, the solubility of EDTM in Kolliphor RH40 and Tween 80 was superior to all the other surfactants considered within this study. Kolliphor RH40 is an hydrophilic surfactant that helps induce the self-emulsification of oil phase in aqueous media (Eleftheriadis et al. 2019), while Tween 80 acted as drug carrier (Dannenfelser et al. 2004). Among the co-surfactants, PEG-400 and PG showed enhanced solubility, therefore, were selected. Furthermore, olive oil and castor oil were selected based on their maximum EDTM solubility. Olive oil is reported to have beneficial effects on thwarting hypercholesterolemia prognosis due to its capacity in reducing cholesterol (Balata et al. 2016). Castor oil possess a long chain of fatty acids that contain carbon chain with more than 12 carbon atoms. Due to these carbon chains, castor oil can transport micro-emulsion to the lymphatic system while bypassing the hepatic metabolism (Sharma et al. 2011). In addition, the visual clarity of formed systems may be attributed to the increasing concentration of surfactant, decreased concentration of oil and co-surfactant. The surfactants potentially decrease the oil content at interface that results in decrease in size of formed emulsion which is reflected by visual clarity. imilar observations were reported in another study (Jianxian et al. 2020). Based on these results, castor oil and olive oil were chosen as oil phases, Kolliphor RH40 and Tween 80 were chosen as surfactants, and PEG-400 and PG were selected as co-surfactant phases.

Emulsification is the key parameter for determination of effectiveness of self-micro emulsification property of developed SMEDDS systems. We measured the self-micro emulsification efficiency of SMEDDS systems by the rate of emulsification. Notably, emulsification is considered to be rate limiting step in drug absorption. Shorter self-microemulsification time indicated easy and spontaneous emulsification upon interaction with gastrointerstinal fluids. The results also depicted that the self-microemulsification is dependent upon the composition of SMEDDS system and ratios of oil: surfactant: co-surfactant. Moreover, the findings indicated that the self-micro emulsification process for the developed SMEDDS system was spontaneous and decreased the time for self-emulsification with an increase in surfactant concentration (Table 3). This showed that the formed SMEDDS systems could disperse quickly under moderate agitation through the help of surfactants with the ability to decrease the interfacial tension, diffuse water into oil phase, producing interfacial disruption and releasing the particles into aqueous media. Consistent with previous studies, the high concentration of

Table 7. Stability testing of selected EDTM-SMEDDS under different conditions.

Stability Testing	F1		F2		F3	
	%EE	%DL	%EE	%DL	%EE	%DL
Zero	92.20	7.79	93.02	6.98	94.04	5.62
Accelerated 40°C	91.85	8.14	92.25	7.74	90.88	9.10
Ongoing 25°C	91.72	8.27	92.26	7.74	90.77	9.22

surfactant with respect to co-surfactant have led to stable self-microemulsifying system and the smaller particle size was achieved (see Table 6 or particle size distribution). An excess amount of co-surfactant led the system to be less stable owing to its high intrinsic aqueous solubility resulting in increase in globule size (Craig et al. 1995). Many SMEDDS systems showed phase separationdepisted by a gel phase upon dilution when the oil content was more In short, it is expected that these SMEDDS systems remain as SMEDDS upon dispersion in gastrointestinal fluids. However, the test is qualitative and subjected to only limited interpretation, as it provided information on ease of dispersion or emulsification of these system but not the quality of emulsion (Jianxian et al. 2020, Craig et al. 1995). Moreover, globule size is also considered as an essential factor in identifying the extent and rate of drug release and absorption. A smaller globule size allows faster dissolution and/or emulsification and provide larger surface area for absorption of drug, which can be achieved by employing suitable surfactant-co-surfactant ratios. The Smix, in defined ratios, also formed a strong barrier between formed particles that prevented them from coagulating. The droplet size decreased with reducing the oil content. When Smix: oil was low the particles formed were larger in size, as reported previously (Suresh and Sharma 2011). Besides, the presence of surfactant also presents particles for absorption, stabilize them and condenses closely packed interfacial film, which further improves the thermodynamic stability of SMEDDS (Qureshi, Mallikarjun, and Kian 2015). The particle size of selected EDTM-SMEDDS was 56.43±1.78 nm. The fact that the particle size did not increase upon the addition of surfactants may prove to be beneficial from drug delivery aspect as the larger surface area dispersion would be available for improving drug absorption (Hintzen et al. 2014). Another crucial factor in measuring particle homogeneity is polydispersibility index (PDI) that was used as a measure of homogeneity. F2 EDTM-SMEDDS had PDI of 0.190 showing that the F2 particles were homogenous and had enhanced physical stability.

Zeta-potential indicates the stability of emulsions. Greater positive or negative ζ-potential values means that a high degree of repulsion is present between particles, which results in emulsion stability. The ζ-potential of optimized EDTM-SMEDDS F2 was -3.30±0.56 mV, showing F2 to be more stable. Furthermore, multiple ions of different strengths are present in gastrointestinal tract that help minimize the surface charge of micro-emulsions generated from self-microemulsifying formulations. For this reason, SGF shielded the negative charge of SMEDDS. The ζ-potential of near to zero points to insignificant repulsion among particles, leading to coalescence or aggregation, confirming the larger particle formation in SGF in comparison to water. Likewise, the negative charge of gastric mucus layer will repel the same charged particles of SMEDDS formulations, therefore, shortening the gastric emptying time. Moreover, reduction in gastric emptying time results in rapid passage of

EDTM within SMEDDS systems from stomach and a reduced release of EDTM. The reduced drug release in stomach leads to fewer gastric side effects (Jianxian et al. 2020). Negative ζ-potential also shows the presence of non-ionic surfactants, which might be due to presence of free fatty acids. Intestinal cells exhibit negative charge due to mucosal fluid that allows positive charged particles a chance for better interaction with gastrointestinal mucosa. Thus, at physiological pH, F2 EDTM-SMEDDS would reach a positive ζ-potential. Generally, it is considered that the high ζ-potential value particles do not coalesce due to electrostatic repulsion but this may not be strictly true for systems with non-ionic surfactants (Mahmood et al. 2023). In addition, stability testing findings indicated that SMEDDS formulations were stable and did not exhibit any sign of aggregation or phase separation.

Additionally, FTIR spectra of all EDTM-SMEDDS showed broad OH bands, decreased intensities of C=O peaks and shifting of these absorbance peaks towards higher wavelengths, indicating the H-bonding between EDTM and excipients. Presence of specific EDTM peaks in the spectra of different SMEDDS formulations indicate that the molecular structure of EDTM remained intact. In addition, no chemical interactions occurred between EDTM and excipients in EDTM-SMEDDS formulations.

The spontaneous release of EDTM *in vitro* and *ex vivo* can be attributed to the formation of micro-emulsion during release studies. Thus, dissolution of EDTM from EDTM-SMEDDS can lead to boosting absorption and increasing bioavailability of EDTM. A high correlation of particle size and release was also observed. F2 EDTM-SMEDDS with least particle size showed enhanced release in comparison to all other SMEDDS as well as raw EDTM dispersion. This may be attributed to the presence of larger surface area by particles for dissolution and permeability of EDTM. The poor performance of raw EDTM can contribute to poor aqueous solubility and wettability. Among all EDTM-SMEDDS, F2 showed highest EDTM release in SIF than SGF, though there was minimal difference with F1. The good release profile of EDTM-SMEDDS in SIF media indicated that the EDTM would be released efficiently in basic media (Mahmood et al. 2023, Jianxian et al. 2020).

The results from stability studies for all selected EDTM-SMEDDS showed encouraging results under ongoing stability as well as accelerated stability conditions. There were only small changes observed in %EE and % DL of all EDTM-SMEDDS except F3. Due to appropriate amount of oil and Smix, the phase change/separation and other instability issues (crystallization, flocculation, and phase inversion) were prevented.

5. Conclusion

A simple, easy to scale up to industrial level, EDTM-SMEDDS was developed in this study using different ratios of oil: surfactant: co-surfactant (Olive Oil: Kolliphor RH-40: PEG-400). This improved the EDTM dissolution and permeability when tested for *in vitro* and *ex-vivo* studies. Among all EDTM-SMEDDS, F2 were selected to be optimized SMEDDS for EDTM based on smaller size, lesser PDI, zeta-potential and stability studies. EDTM- SMEDDS boosted its solubility and permeability, which may also lead to improvement in bioavailability, resulting in improved therapeutic efficacy and lower toxicity profile. Moreover, no phase separation, aggregation or phase change was observed showing SMEDDS exhibited excellent stability under various environmental and storage conditions. In addition, SMEDDS held their integrity upon contact with gastrointestinal fluids and rapidly emulsified. In conclusion, these results suggest that EDTM-SMEDDS holds promise as a novel system for enhancing the solubility and permeability of this BCS Class-IV drug.

Conflict of Interest

The authors declare that they have no conflicts of

interest to disclose.

Funding
There was no specific funding available for this project.

Study Approval
There are no human subjects involved so, this study requires no institutional or ethical review board approval.

Consent Forms
NA.

Authors Contributions
SAA was responsible for conceptualization, methodology, validation, formal analysis, investigation, writing of original draft, and visualization. SG , KS, and RA were responsible for conceptualization, methodology, investigation, writing – review & editing. UI was responsible for conceptualization, methodology, supervision, writing – review & editing, and project administration.

Acknowledgments
None

References

Ansari, Muhammad Mohsin, Dang-Khoa Vo, Ho-Ik Choi, Jeong-Su Ryu, Yumi Bae, Nadeem Irfan Bukhari, Alam Zeb, Jin-Ki Kim, and Han-Joo Maeng. 2023. "Formulation and Evaluation of a Self-Microemulsifying Drug Delivery System of Raloxifene with Improved Solubility and Oral Bioavailability." *Pharmaceutics* no. 15 (8):2073.

Aungst, Bruce J. 2017. "Optimizing oral bioavailability in drug discovery: an overview of design and testing strategies and formulation options." *Journal of Pharmaceutical Sciences* no. 106 (4):921-929.

Balata, Gehan F, Ebtessam A Essa, Hanan A Shamardl, Samira H Zaidan, Mohammed AS %J Drug design Abourehab, development, and therapy. 2016. "Self-emulsifying drug delivery systems as a tool to improve solubility and bioavailability of resveratrol."117-128.

Chaudhari, Smruti P, and Rohit P Dugar. 2017. "Application of surfactants in solid dispersion technology for improving solubility of poorly water soluble drugs." *Journal of Drug Delivery Science and Technology* no. 41 (1):68-77.

Craig, D. Q. M., S. A. Barker, D. Banning, and S. W. Booth. 1995. "An investigation into the mechanisms of self-emulsification using particle size analysis and low frequency dielectric spectroscopy." *International Journal of Pharmaceutics* no. 114 (1):103-110. doi: https://doi.org/10.1016/0378-5173(94)00222-Q.

Dannenfelser, Rose-Marie, Handan He, Yatindra Joshi, Simon Bateman, and Abu T. M. Serajuddin. 2004. "Development of clinical dosage forms for a poorly water soluble drug I: Application of polyethylene glycol–polysorbate 80 solid dispersion carrier system." *Journal of Pharmaceutical Sciences* no. 93 (5):1165-1175. doi: https://doi.org/10.1002/jps.20044.

Eleftheriadis, Georgios K., Panagiota Mantelou, Christina Karavasili, Paschalina Chatzopoulou, Dimitrios Katsantonis, Maria Irakli, Aggeliki Mygdalia, Ioannis S. Vizirianakis, and Dimitrios G. Fatouros. 2019. "Development and Characterization of a Self-Nanoemulsifying Drug Delivery System Comprised of Rice Bran Oil for Poorly Soluble Drugs." *AAPS PharmSciTech* no. 20 (2):78. doi: 10.1208/s12249-018-1274-y.

Hintzen, Fabian, Glen Perera, Sabine Hauptstein, Christiane Müller, Flavia Laffleur, and Andreas Bernkop-Schnürch. 2014. "In vivo evaluation of an oral self-microemulsifying drug delivery system (SMEDDS) for leuprorelin." *International Journal of Pharmaceutics* no. 472 (1-2):20-26.

Huynh-Ba, Kim, and Manuel Zahn. 2009. "Understanding ICH Guidelines Applicable to Stability Testing." In *Handbook of Stability Testing in Pharmaceutical Development: Regulations, Methodologies, and Best Practices*, edited by Kim Huynh-Ba, 21-41. New York, NY: Springer New York.

Ijaz, Muhammad, Sonja Bonengel, Ožbej Zupančič, Muhammad Yaqoob, Markus Hartl, Shah Hussain, Christian W Huck, and Andreas Bernkop-Schnürch. 2016. "Development of oral self nano-emulsifying delivery system (s) of lanreotide with improved stability against presystemic thiol-disulfide exchange reactions." *Expert on Drug Delivery* no. 13 (7):923-929.

Jianxian, Chen, Kalsoom Saleem, Muhammad Ijaz, Masood Ur-Rehman, Ghulam Murtaza, and Mulazim Hussain Asim. 2020. "Development and in vitro evaluation of gastro-protective aceclofenac-loaded self-emulsifying drug delivery system." *International journal of nanomedicine* no. 1 (1):5217-5226.

Kim, Yong-Han, Seong-Bo Kim, Se-Hee Choi, Thi-Thao-Linh Nguyen, Sung-Hoon Ahn, Kyung-Sun Moon, Kwan-Hyung Cho, Tae-Yong Sim, Eun-Ji Heo, Sung Tae Kim, Hyun-Suk Jung, Jun-Pil Jee, Han-Gon Choi, and Dong-Jin Jang. 2023. "Development and Evaluation of Self-Microemulsifying Drug Delivery System for Improving Oral Absorption of Poorly Water-Soluble Olaparib." *Pharmaceutics* no. 15 (6):1669.

Lei, Y., Y. Lu, J. Qi, S. Nie, F. Hu, W. Pan, and W. Wu. 2011. "Solid self-nanoemulsifying cyclosporin A pellets prepared by fluid-bed coating: preparation, characterization and in vitro redispersibility." *International Journal of Nanomedicine* no. 6:795-805.

Mahmood, Arshad, Laraib Khan, Muhammad Ijaz, Imran Nazir, Mahrukh Naseem, Muhammad Azam Tahir, Muhammad Naeem Aamir, Masood Ur Rehman, and Mulazim Hussain Asim. 2023. "Enhanced Intestinal Permeability of Cefixime by Self-Emulsifying Drug Delivery System: In-Vitro and Ex-Vivo Characterization." *Molecules* no. 28 (6):2827.

Mahmoud, Enas A, Ehab R Bendas, and Magdy I Mohamed. 2009. "Preparation and evaluation of self-nanoemulsifying tablets of carvedilol." *AAPS pharmscitech* no. 10 (1):183-192.

Nasr, Ali, Ahmed Gardouh, and Mamdouh Ghorab. 2016. "Novel solid self-nanoemulsifying drug delivery system (S-SNEDDS) for oral delivery of olmesartan medoxomil: design, formulation, pharmacokinetic and bioavailability evaluation." *Pharmaceutics* no. 8 (3):20.

Odenwald, Matthew A, and Jerrold R Turner. 2017. "The intestinal epithelial barrier: a therapeutic target?" *Nature Reviews Gastroenterology and Hepatology* no. 14 (1):9.

Partida, R. A., and R. P. Giugliano. 2011. "Edoxaban: pharmacological principles, preclinical and early-phase clinical testing." *Future Cardiol* no. 7 (4):459-70. doi: 10.2217/fca.11.28.

Qureshi, Mohd Javed, Chitneni Mallikarjun, and Wong Gan Kian. 2015. "Enhancement of solubility and therapeutic potential of

poorly soluble lovastatin by SMEDDS formulation adsorbed on directly compressed spray dried magnesium aluminometasilicate liquid loadable tablets: a study in diet induced hyperlipidemic rabbits." *Asian journal of pharmaceutical Sciences* no. 10 (1):40-56.

Rashid, Md Abdur, Saiqa Muneer, Jayanti Mendhi, Mohammad Zaidur Rahman Sabuj, Yahya Alhamhoom, Yin Xiao, Tony Wang, Emad L. Izake, and Nazrul Islam. 2021. "Inhaled Edoxaban dry powder inhaler formulations: Development, characterization and their effects on the coagulopathy associated with COVID-19 infection." *International Journal of Pharmaceutics* no. 608:121122. doi: https://doi.org/10.1016/j.ijpharm.2021.121122.

Sankar, P Ravi, MM Eswarudu, P Siva Krishna, D Srikanth, P Srinivasa Babu, and N Rohith. 2021. "Novel validated RP-HPLC method for determination of edoxaban tosylate monohydrate in bulk and its pharmaceutical dosage form." *Journal of Pharmaceutical Sciences Research* no. 13 (5):232-237.

Shafiq un Nabi, Sheikh, Faiyaz Shakeel, Sushma Talegaonkar, Javed Ali, Sanjula Baboota, Alka Ahuja, Roop K. Khar, and Mushir Ali. 2007. "Formulation development and optimization using nanoemulsion technique: A technical note." *The American Association of Pharmaceutical Scientists* no. 8 (2):1-6.

Sharma, Shiwani, AD Sharma, M Naseer, and R Singh. 2011. "Formulation and evaluation of self emulsifying drug delivery system of ibuprofen using castor oil." *Int J Pharm Pharm Sci* no. 3 (4):299-302.

Son, Gi-Ho, Beom-Jin Lee, and Cheong-Weon Cho. 2017. "Mechanisms of drug release from advanced drug formulations such as polymeric-based drug-delivery systems and lipid nanoparticles." *Journal of Pharmaceutical Investigation* no. 47 (4):287-296.

Suresh, Preeti K, and Sudhanshu %J drugs Sharma. 2011. "Formulation and in-vitro characterization of selfnanoemulsifying drug delivery system of cinnarizine." *Drugs* no. 11:12.

Thota, S. K., N. Dudhipala, V. Katla, and K. Veerabrahma. 2023. "Cationic Solid SMEDDS of Efavirenz for Improved Oral Delivery: Development by Central Composite Design, In Vitro and In Vivo Evaluation." *AAPS PharmSciTech* no. 24 (1):38. doi: 10.1208/s12249-022-02495-3.

Wang, J., and S. Skolnik. 2013. "Permeability diagnosis model in drug discovery: a diagnostic tool to identify the most influencing properties for gastrointestinal permeability." *Curr Top Med Chem* no. 13 (11):1308-16. doi: 10.2174/15680266113139990035.