

Original Research

Received 24 June 2026

Revised 29 July 2026

Accepted 13 Aug 2026

Natural Resources for Human Health 2026, Vol. 6 (16s): 817–833

KEYWORDS:

Metolazone; Self-microemulsifying drug delivery system; BCS class II; Pseudo-ternary phase diagram; Simplex lattice mixture design; Solubility enhancement.

Formulation, Optimization and Evaluation of Self-Microemulsifying Drug Delivery System (SMEDDS) for Solubility Enhancement of Metolazone

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ABSTRACT: Background: Metolazone (MTZ), a quinazoline-sulphonamide thiazide-like diuretic, is a Biopharmaceutical Classification System (BCS) class II drug with poor aqueous solubility, which results in its low oral bioavailability (65%). The purpose of this study was to develop and optimize a Self Micro-Emulsifying Drug Delivery System (SMEDDS) of Metolazone for enhanced solubility and dissolution.

Method: Pre-formulation studies like Fourier Transform Infrared Spectroscopy (FTIR), identification of λ_{max} and melting point determination were performed to confirm drug identity. Ultraviolet (UV) spectrophotometric was developed for the quantification of MTZ. Equilibrium solubility studies revealed Capmul MCM PG-8-NF, Tween 80 and Transcutol P as oil, surfactant and co-surfactant. A pseudo-ternary phase diagram was constructed to identify the self-micro emulsification region, followed by optimization by simplex lattice mixture design. Quadratic models were developed for droplet size, equilibrium solubility, self-emulsification time and percentage transmittance. The optimized SMEDDS was evaluated for physicochemical characteristics, thermodynamic stability and in-vitro studies and was compared with marketed tablet.

Results: The optimized SMEDDS exhibited $95.3 \pm 0.31\%$ transmittance, no phase separation, globule size of 46.05 ± 0.34 nm, and an emulsification time of 42 ± 0.76 s. Equilibrium solubility was increased to 74.2 ± 0.87 mg/mL. The formulation was thermodynamically stable with a cloud point of $68.5 \pm 0.11^\circ\text{C}$. The quadratic models were statistically significant ($p < 0.05$), with $R^2 > 0.92$ and adequate precision >4 . In vitro drug release demonstrated faster and pH-independent release. The optimized SMEDDS retained its performance after 6 months of accelerated stability testing, indicating its potential for enhancing MTZ solubility and dissolution.

1. INTRODUCTION

Oral administration continues to be the most widely preferred and patient-compliant route of drug delivery on account of its ease of administration, cost-effectiveness, and flexibility in dosage form design. However, nearly 40% of new chemical entities and a large proportion of existing drug molecules exhibit poor aqueous solubility, and their oral absorption is consequently

dissolution rate-limited, resulting in erratic and incomplete bioavailability. Among the various formulation strategies devised to overcome this limitation—such as solid dispersion, complexation, salt formation, particle size reduction and co-crystal formation—lipid-based drug delivery systems have emerged as one of the most versatile and clinically translatable approaches, particularly for Biopharmaceutical Classification System (BCS) class II drugs whose absorption is limited by dissolution rather than membrane permeability^[1-5].

Self-microemulsifying drug delivery systems (SMEDDS) are isotropic mixtures of oil, surfactant and co-surfactant (or a hydrophilic co-solvent) that, upon mild agitation with an aqueous medium such as gastrointestinal fluid, spontaneously form fine oil-in-water microemulsions with a droplet size generally below 100 nm^[6-7]. By presenting the drug already dissolved in a fine emulsified state, SMEDDS circumvent the dissolution step, which is frequently the rate-limiting step for oral absorption of hydrophobic drugs, and can additionally promote intestinal lymphatic transport, thereby reducing hepatic first-pass metabolism. These systems can be filled directly into soft or hard gelatin capsules as a liquid formulation or subsequently converted into a free-flowing solid dosage form by adsorption onto suitable solid carriers, granulation or spray drying, which improves the physical and chemical stability of the formulation while retaining the self-emulsification behaviour^[8-10]. Rational development of a SMEDDS formulation typically follows a sequence of solubility screening of the drug in a panel of oils, surfactants and co-surfactants, construction of a pseudo-ternary phase diagram to identify the self-emulsification region, and optimization of the relative proportions of the selected components using a suitable statistical design of experiments (DoE), most commonly a simplex-lattice or simplex-lattice mixture design, which allows the simultaneous evaluation of the effect of the three formulation components on multiple critical quality attributes with a minimum number of experimental trials^[10-15].

Metolazone (MTZ) is a synthetic quinazoline-sulphonamide derivative that is pharmacologically classified as a thiazide-like diuretic. It acts primarily by inhibiting sodium reabsorption at the cortical diluting segment of the distal convoluted tubule, and to a lesser degree at the proximal convoluted tubule, thereby promoting natriuresis and diuresis and reducing intravascular volume^[15-20]. It is used either alone or in combination with loop diuretics for the management of hypertension and oedema associated with congestive heart failure and renal disease, and it is particularly valued as an add-on therapy to overcome loop-diuretic resistance in decompensated heart failure through the mechanism of sequential nephron blockade. Despite its clinical utility, Metolazone is a BCS class II drug with low aqueous solubility (log P 5.12; aqueous solubility of approximately 0.0407 mg/mL), and this poor wettability and dissolution behaviour is reflected in an oral bioavailability of only about 65%, with the rate and extent of absorption reported to be formulation dependent^[21-25].

A limited number of solubility- and bioavailability-enhancement approaches have previously been explored for Metolazone, including a solvent-free intravenous emulsion formulation, co-crystal-loaded oral fast-dissolving films that achieved nearly two-fold higher relative bioavailability than a marketed tablet in rabbits, comparative evaluation of parenteral formulations for their diuretic action, and solid dispersion-based fast-dissolving tablets employing natural superdisintegrants. Given that SMEDDS technology has been successfully applied to enhance the solubility and dissolution of several other structurally related and poorly soluble drugs using a comparable design-of-experiments strategy, the present work was undertaken to develop and systematically optimize a liquid SMEDDS of Metolazone using a Simplex lattice mixture design, with droplet size, equilibrium solubility, self-emulsification time and percentage transmittance as the critical dependent responses, to enhance its solubility and provide a platform for subsequent conversion into a solid dosage form^[26-30].

2. MATERIALS AND METHODS

2.1 Materials

Metolazone was procured as a gift sample. Capmul MCM C8, Capmul MCM PG-8-NF, Captex 355, Captex 200 P, Acconon MC 8 and Acrysol EL135 were obtained as gift samples from Abitec Corporation. Miglyol 812 N, Labrafac PG and Transcutol P (diethylene glycol monoethyl ether) were procured from Gattefosse. Castor oil, oleic acid, Tween 20, Tween 40, Tween 80, Span 80, glycerin and Lauroglycol 90 were of analytical grade and procured from standard commercial suppliers. Methanol (analytical grade) and the reagents used for preparation of phosphate buffer pH 7.4 were of analytical grade. Double-distilled water was used throughout the study.

2.2 Instruments

A double-beam UV-visible spectrophotometer was used for spectral scanning and absorbance measurements. Fourier transform infrared (FTIR) spectra were recorded on a Shimadzu FTIR spectrometer. Melting point was determined using a capillary melting point apparatus. A digital vortex mixer, bath sonicator, and a cooling centrifuge were used for the solubility studies. The pseudo-ternary phase diagram was constructed using Chemix School software. Design-Expert® software (Stat-Ease Inc., Minneapolis, USA) was used for generation of the simple lattice mixture design, statistical analysis of the experimental data and construction of contour and three-dimensional response surface plots.

2.3 Preformulation studies

2.3.1 Determination of wavelength of maximum absorption (λ_{max}): A qualitative solution of Metolazone was prepared in methanol and scanned spectrophotometrically over the wavelength range of 200-400 nm against methanol as a blank to ascertain the wavelength of maximum absorption [7].

2.3.2 Fourier transform infrared (FTIR) spectroscopy: The identity of the procured drug and the characteristic functional groups present in Metolazone were confirmed by FTIR spectroscopy. Spectra were recorded in the wavenumber range of 400-4000 cm^{-1} , and the observed peaks were compared with the reported frequencies of the corresponding functional groups [8].

2.3.3 Melting point determination: The melting point of Metolazone was determined by the capillary tube method. A small quantity of the powdered drug was filled into a thin, sealed capillary tube, which was placed in the melting point apparatus along with a thermometer, and the temperature at which the drug melted was recorded in triplicate [9].

2.4 Solubility studies

The saturation solubility of Metolazone in various oils, surfactants and co-surfactants was determined by the equilibrium shake-flask method. An excess amount of drug (initially 20 mg) was added to 2 mL of each vehicle in a glass vial and vortex-mixed for 10 minutes, followed by sonication at 37°C for a few minutes to ensure uniform mixing. The mixtures were then centrifuged at 3000 rpm for 15 minutes at room temperature. Where the sample remained clear without visible settling of undissolved drug, the process of excess drug addition was repeated to ascertain the maximum solubility. Once no further drug could dissolve, the sample was centrifuged, and the clear supernatant was separated, diluted appropriately with methanol, and filtered through a Whatman filter paper. The concentration of Metolazone solubilized in each vehicle was determined by UV-visible spectrophotometry ($n = 3$) [10].

2.5 Preliminary screening for selection of oil, surfactant and co-surfactant

Based on the solubility data, the two oils, two surfactants and two co-surfactants showing the highest solubilizing capacity in each category were shortlisted for preliminary screening of self-emulsification efficiency. Eight possible combinations (oil-surfactant-co-surfactant) were evaluated, with each vehicle taken in an equal volume of 1 mL (total 3 mL) and a fixed dose of 5 mg of Metolazone incorporated into each batch. The prepared mixtures were visually assessed for ease and rate of self-emulsification upon dilution with water and graded on an A-E scale (A = rapid emulsification, <1 min, clear/slightly bluish appearance; E = poor emulsification with visible oil droplets) and were further evaluated for percentage transmittance (%T, measured spectrophotometrically after dilution with distilled water) and visual drug precipitation on standing [11].

2.6 Construction of pseudo-ternary phase diagram

On the basis of the preliminary trials, the oil, surfactant and co-surfactant combination showing the highest %T, most rapid emulsification and absence of drug precipitation was selected for construction of the pseudo-ternary phase diagram [12]. A series of batches with varying proportions of oil, surfactant and co-surfactant (in different weight ratios, keeping the total at 100%) were prepared, and each mixture was titrated with water with continuous stirring at ambient temperature until the appearance changed. The mixtures were assessed for emulsification efficiency, %T and rate of emulsification (time taken, in seconds, for the mixture to form a homogeneous dispersion on dilution), and the boundaries of the microemulsion existing region were plotted using Chemix School software to delineate the self-microemulsification zone.

2.7 Experimental design and optimization

A simplex-lattice mixture design was employed using Design-Expert® software to optimize the composition of the Metolazone SMEDDS. The concentration ranges of the three independent mixture components- A: concentration of oil (Capmul MCM PG-8-NF), B: concentration of co-surfactant (Transcutol P), and C: concentration of surfactant (Tween 80)-were selected based on the boundaries of the self-emulsification region identified from the pseudo-ternary phase diagram. Droplet size (R1, nm), equilibrium solubility (R2, mg/mL), self-emulsification time (R3, s) and percentage transmittance (R4, %) were evaluated as the dependent responses. The experimental runs generated by the software were prepared, and each was characterized for the four responses [13]. Data were fitted to linear and special cubic/quadratic mixture models, and the model showing the best fit (highest R², adjusted R², and non-significant lack-of-fit) was selected for each response. Analysis of variance (ANOVA) was performed to determine the statistical significance of the fitted models and individual model terms ($p < 0.05$ considered significant), and contour plots and three-dimensional response surface plots were generated to visualize the influence of the mixture components on each response.

2.8 Validation of the design and selection of the optimized batch

The predictive ability of the fitted mixture models was validated by preparing two checkpoint batches at compositions falling within the design space but distinct from the batches used to generate the model. The observed (actual) values of the four responses for the checkpoint batches were compared with the values predicted by the model, and the closeness of the predicted and actual values was used as a measure of the model's validity. Subsequently, numerical optimization based on the desirability function approach (with droplet size and emulsification time minimized, and equilibrium solubility and percentage transmittance maximized) was used to identify the composition offering the highest overall desirability, which was prepared and characterized as the optimized liquid SMEDDS batch [14].

2.9 Evaluation of optimized liquid SMEDDS

2.9.1 Measurement of Percentage Transmittance

The optical clarity of the SMEDDS formulation was evaluated as an indirect indicator of microemulsion formation. Following a 1:250 dilution with distilled water, transmittance was measured spectrophotometrically at 650 nm, with deionized water serving as the blank. A transmittance close to 100% suggests the formation of a clear and stable microemulsion [15,16].

2.9.2 Freeze-Thawing

Freeze-thawing was used to evaluate the stability of formulations. The formulations were subjected to 3 to 4 freeze-thaw cycles. It included freezing at -4°C for 24 hours, which was followed by thawing at 40°C for 24 hours. Centrifugation was performed at 3000 rpm for 5 minutes. The formulations were observed for phase separation [17,18].

2.9.3 Droplet Size Analysis

100 µl of SMEDDS formulation was diluted to 250 mL in a beaker and gently mixed using a glass rod. The resultant emulsion was then subjected to particle size analysis (using Malvern Mastersizer (Worcestershire, UK) equipped with 2000 Hydro MU) with a particle size measurement range of 0.02 to 2000 µm. Particle size was calculated from the volume size distribution [18,19,20].

2.9.4 Rate of Emulsification

The efficiency of self-emulsification of SMEDDS was assessed using a United States Pharmacopoeia (USP) II dissolution apparatus. The specified quantity of the formulation (5ml) was added to 250ml of water at $37 \pm 0.5^\circ\text{C}$ where paddle rotating at 50 rpm to provide the required agitation. After being equilibrated, the time required for the efficiency of self-emulsification was noted [21,22].

2.9.5 Robustness to dilution

It was performed by diluting (100 times) a specified quantity of formulation with various media like water, 0.1N HCl and pH 7.4 phosphate buffer. The diluted microemulsions were stored for 24 hours at room temperature and observed for phase separation or drug precipitation [23].

2.9.6 Cloud point

SMEDDS were diluted with water in the ratio of 1:100, and the sample was placed in a water bath with the temperature increasing gradually. Cloud point was measured as the temperature at which there was a sudden appearance of cloudiness visually [24].

2.9.7 In vitro drug release study

An in vitro drug release study was conducted by the dialysis bag method. Different dissolution media, such as phosphate buffer pH 6.8, acetate buffer pH 4.5 and hydrochloride buffer pH 1.2, were used, and the outcomes were compared with conventional marketed tablets. A 5-mL sample of medium was removed during the release studies and quantified using UV spectrophotometric analysis. Each time, 5 mL of new medium was added to replace the volume that had been withdrawn. To investigate how pH affects drug release, dissolution studies were conducted in different media (buffer pH 4.5 and 7.2) [25].

3. Stability Studies

Stability tests were conducted at 40°C/75% RH after the SMEDDS formulations were placed into empty hard gelatin capsules (size 0). Samples were placed in temperature- and humidity-controlled stability chambers (Thermolab, Mumbai, India). SMEDDS system was further evaluated for rate of emulsification, percentage transparency and drug precipitation [26].

4. RESULTS AND DISCUSSION

4.1 Determination of wavelength of maximum absorption ($\lambda_{\max}$)

Scanning of the qualitative solution of Metolazone in methanol over the range of 200-400 nm produced an absorption spectrum with a distinct absorption maximum at 236 nm (Figure 1), which was accordingly selected as the analytical wavelength for all subsequent spectrophotometric estimations.

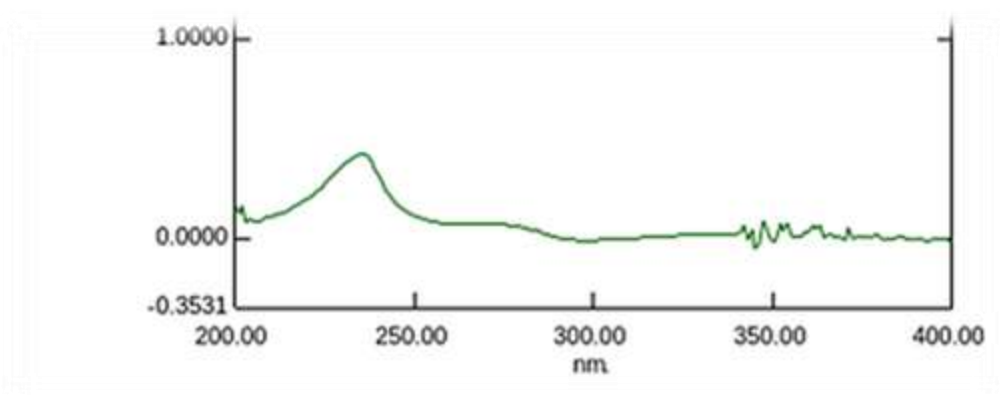


Figure 1: UV absorption spectrum of Metolazone (4 µg/mL) in methanol showing $\lambda_{\max}$ at 236 nm

4.2 FTIR spectral analysis

The FTIR spectrum of the procured Metolazone (Figure 2) displayed characteristic absorption bands corresponding to the principal functional groups reported for the drug. The observed peak positions were in close agreement with the corresponding standard reference frequencies, confirming the identity and purity of the procured drug sample. FTIR analysis revealed absorption bands at 3373, 3341, 3063, 1660, 1644, 1515, 1222, and 759 cm^{-1} , corresponding to O-H, N-H, C-H, C=O, C=N, C=C, C-N, and C-Cl stretching vibrations, respectively, all of which fell within their respective standard reference ranges, thereby confirming the presence of these functional groups in the synthesized compound.

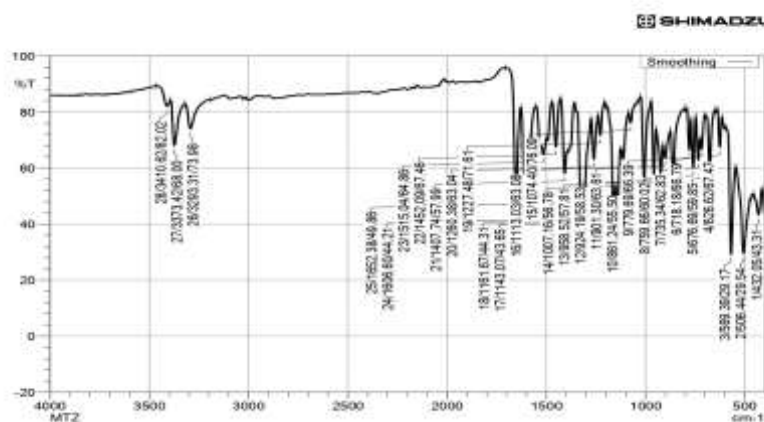


Figure 2: FTIR spectrum of Metolazone

4.3 Melting point determination

The melting point of the procured Metolazone was found to be $256^{\circ}\text{C} \pm 0.816$, which closely matched the reported standard melting point range of $255\text{--}257^{\circ}\text{C}$, further confirming the identity and purity of the drug.

4.4 Solubility studies

The equilibrium solubility of Metolazone in the screened oils, surfactants and co-surfactants is summarized in Figure 3. Among the oils tested, oleic acid (10 ± 1.20 mg/mL) and Capmul MCM PG-8-NF (8 ± 0.92 mg/mL) showed the highest solubilizing capacity for Metolazone, whereas Miglyol 812 N, Captex 355 and Captex 200 P showed comparatively low solubility (2 ± 0.71 mg/mL each). Among the surfactants, Tween 80 exhibited markedly superior solubilization (104 mg/mL) compared with the other surfactants screened, while among the co-surfactants, Transcutol P showed the highest solubility (94 ± 0.98 mg/mL), followed by Acconon MC 8 (29 ± 0.87 mg/mL) and Acrysol EL135 (24 ± 0.85 mg/mL).

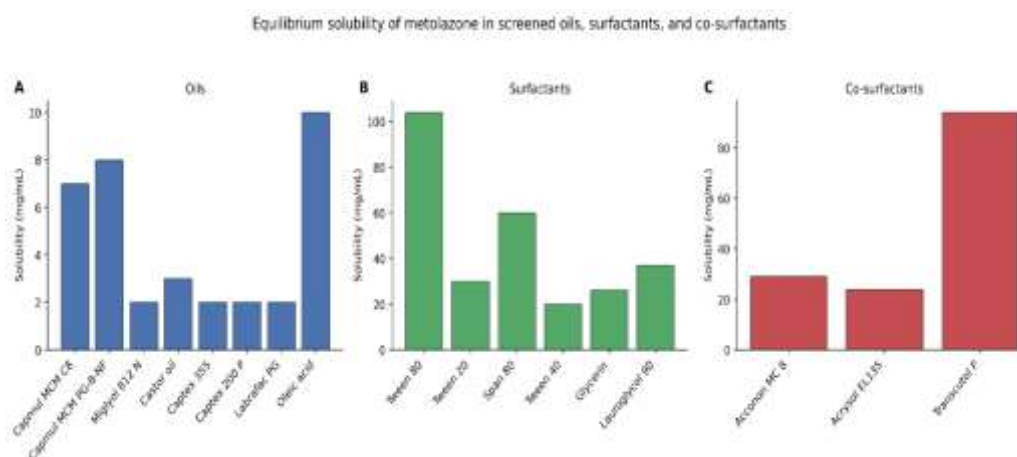


Figure 3: (A) Solubility of Metolazone in various oils (B) surfactants (C) co-surfactants

4.5 Preliminary screening for selection of oil, surfactant and co-surfactant combination

Based on the solubility data, oleic acid and Capmul MCM PG-8-NF (oils), Span 80 and Tween 80 (surfactants), and Transcutol P and Acconon MC 8 (co-surfactants) were shortlisted and combined into eight preliminary batches (MTZ1-MTZ8) for evaluation of self-emulsification efficiency, percentage transmittance and drug precipitation (Table 3). Batches containing Capmul MCM PG-8-NF as the oil phase (MTZ5-MTZ8) consistently showed higher transmittance and grade 'A' emulsification with no visible drug precipitation, compared with batches formulated with oleic acid. Among all combinations, batch MTZ7 (Capmul MCM PG-8-NF, Tween 80 and Transcutol P) showed the highest transmittance ($96.8 \pm 0.2\%$), grade 'A' rapid self-

emulsification and no drug precipitation, and this oil-surfactant-co-surfactant combination was accordingly selected for construction of the pseudo-ternary phase diagram.

Table 3: Preliminary trial batches for selection of oil-surfactant-co-surfactant combination

Batch	Oil	Surfactant	Co-surfactant	Grade	% T	Drug precipitation
MTZ 1	Oleic acid	Tween 80	Acconon MC8	B	55.6±0.1	Yes
MTZ 2	Oleic acid	Span 80	Acconon MC8	B	48.2±0.3	Yes
MTZ 3	Oleic acid	Tween 80	Transcutol P	B	68.3±0.5	Yes
MTZ 4	Oleic acid	Span 80	Transcutol P	B	72.7±0.4	Yes
MTZ 5	Capmul MCM PG-8-NF	Tween 80	Acconon MC8	A	88.6±0.7	Yes
MTZ 6	Capmul MCM PG-8-NF	Span 80	Acconon MC8	A	91.2±0.65	No
MTZ 7	Capmul MCM PG-8-NF	Tween 80	Transcutol P	A	96.8±0.2	No
MTZ 8	Capmul MCM PG-8-NF	Span 80	Transcutol P	A	93.6±1.2	No

Note: Emulsion grading - A: forms rapidly (<1 min) with clear or slightly bluish appearance; B: forms rapidly (<1 min) with less clear, bluish-white appearance; C: forms within 1-2 min with bright white appearance; D: takes more than 2 min, dull greyish, oily appearance; E: poor emulsification with large oil droplets on the surface.

4.6 Construction of pseudo-ternary phase diagram

The selected combination of Capmul MCM PG-8-NF (oil), Tween 80 (surfactant) and Transcutol P (co-surfactant) was used to construct the pseudo-ternary phase diagram. Twenty-eight batches (MTZ9-MTZ28) covering different proportions of oil, surfactant and co-surfactant were prepared and evaluated for emulsification efficiency, percentage transmittance and rate of emulsification (Table 9). Batches with a comparatively higher proportion of surfactant and co-surfactant relative to oil (e.g., MTZ15, MTZ21, MTZ26 and MTZ28) consistently achieved grade 'A' emulsification, high transmittance (>90%) and rapid emulsification (<25 s), whereas oil-rich compositions (e.g., MTZ9, MTZ11 and MTZ13) exhibited grade 'B' emulsification with lower transmittance and slower emulsification times. Plotting these boundaries using Chemix School software delineated a self-microemulsification zone (Figure 4) located predominantly in the region of low-to-moderate oil concentration and higher surfactant/co-surfactant concentration, which was subsequently used to define the component ranges for the simplex lattice mixture design.

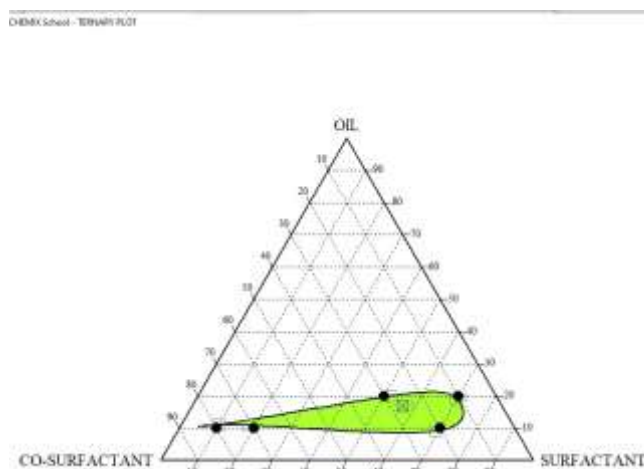


Figure 4: Pseudo-ternary phase diagram showing the self-microemulsifying (microemulsion) zone for the Capmul MCM PG-8-NF-Tween 80-Transcutol P system

4.7 Optimization of formulation using Simplex lattice mixture design

Based on the boundaries of the self-microemulsification zone, the concentration ranges of the three independent mixture components-A: concentration of Capmul MCM PG-8-NF (oil), B: concentration of Transcutol P (co-surfactant) and C: concentration of Tween 80 (surfactant)-were fixed for the Simplex lattice mixture design, with droplet size (R1), equilibrium solubility (R2), self-emulsification time (R3) and percentage transmittance (R4) as the dependent responses (Table 10). Fourteen experimental runs (MTZ 29-MTZ 42) were generated by the software and evaluated (Table 4).

Table 4: Simplex lattice mixture design matrix and observed responses

Batch	A: Oil	B: Surfactant	C: Co-S	R1 Droplet size (nm)	R2 Eqb. solubility (mg/mL)	R3 Emulsification time (s)	R4 %T
MTZ 29	10	40	50	68	62	44	92
MTZ 30	30	40	30	152	48	98	76
MTZ 31	10	60	30	48	88	22	98
MTZ 32	30	40	30	148	50	100	74
MTZ 33	23.33	43.33	33.33	118	60	70	84
MTZ 34	20	40	40	132	58	74	82
MTZ 35	13.33	43.33	43.33	88	72	56	88
MTZ 36	20	50	30	92	70	50	90
MTZ 37	10	50	40	55	78	30	94
MTZ 38	10	40	50	65	65	42	93
MTZ 39	13.33	53.33	33.33	82	82	32	96
MTZ 40	10	60	30	45	90	20	99
MTZ 41	16.66	46.66	36.66	65	68	54	89
MTZ 42	20	50	30	70	72	48	91

Note: A = concentration of oil (Capmul MCM PG-8-NF, %); B = concentration of surfactant (Tween 80, %); C = concentration of co-surfactant (Transcutol P, %).

The four responses were fitted to the mixture models available in Design-Expert software; in each case, a quadratic mixture model provided the best fit to the experimental data, and the analysis of variance (ANOVA) for the fitted models is summarized below.

4.7.1 Droplet size (R1)

The Model F-value of 20.06 ($p = 0.0002$) implies the model is significant, with only a 0.02% chance that an F-value this large could occur due to noise. A, B and C were significant model terms ($p < 0.05$). The lack-of-fit F-value of 3.82 ($p = 0.1113$) was not significant relative to the pure error, indicating that the model fits the data well. The regression equation for droplet size in terms of the mixture components was: $R1 \text{ (droplet size)} = +652.07A - 181.66B - 77.66C - 1907.70AB + 1783.65AC - 223.483BC$. Model summary statistics indicated $R^2 = 0.9261$, adjusted $R^2 = 0.8800$, predicted $R^2 = 0.8351$ and an adequate precision ratio of 24.234, indicating an adequate signal-to-noise ratio (>4) and confirming that the model can be used to navigate the design space.

4.7.2 Equilibrium solubility (R2)

The Model F-value of 92.46 ($p < 0.0001$) indicates that the model is significant, and the lack-of-fit was not significant ($p = 0.2015$) relative to the pure error. The fitted regression equation was: R_2 (equilibrium solubility) = $+48.80A + 89.09B + 64.00C + 7.96AB + 9.18AC + 10.89BC$. The model showed $R^2 = 0.9830$, adjusted $R^2 = 0.9724$, predicted $R^2 = 0.9514$ and an adequate precision of 28.845, confirming a good fit and adequate signal for navigating the design space.

4.7.3 Self-emulsification time (R3)

The Model F-value of 62.26 ($p < 0.0001$) confirmed model significance, with a non-significant lack-of-fit ($p = 0.7443$). The regression equation obtained was: R_3 (emulsification time) = $+98.2A + 20.6B + 43.8C - 42.8AB + 19.2AC + 1.5BC$. The model exhibited $R^2 = 0.9908$, adjusted $R^2 = 0.9851$, predicted $R^2 = 0.9694$ and the highest adequate precision among the four responses (38.569), reflecting a very strong signal-to-noise ratio.

4.7.4 Percentage transmittance (R4)

The Model F-value of 122.44 ($p < 0.0001$) indicated a highly significant model, with A, B, C, and the AB interaction identified as significant terms, and a non-significant lack-of-fit ($p = 0.2953$). The regression equation was: R_4 (% transmittance) = $-75.1A + 98.6B + 92.1C + 16.2AB - 6.78AC - 5.6BC$. Model summary statistics gave $R^2 = 0.9871$, adjusted $R^2 = 0.9790$, predicted $R^2 = 0.9685$ and adequate precision of 32.589, confirming a well-fitting and highly discriminating model.

For all four responses, the difference between the predicted R^2 and adjusted R^2 was less than 0.2, indicating reasonable agreement, and the adequate precision ratios (all >4) confirmed an adequate signal-to-noise ratio, indicating that each model could be reliably used to navigate the design space.

4.7.5 Contour and response surface analysis

Contour plots and the corresponding three-dimensional response surface plots for the four responses (Figure 5-8) illustrated the combined influence of oil, surfactant and co-surfactant concentrations. Droplet size increased with increasing oil concentration and was minimized at low oil and high surfactant/co-surfactant proportions (Figure 5). Equilibrium solubility and percentage transmittance both increased with increasing surfactant and co-surfactant concentration and decreasing oil concentration (Figures 6 and 7), while self-emulsification time decreased (i.e., emulsification became faster) as the oil concentration decreased and surfactant/co-surfactant concentration increased (Figure 8). These trends were consistent with the coefficients of the fitted regression equations and confirmed that a formulation with low oil and comparatively higher surfactant and co-surfactant content would be expected to yield a smaller droplet size, higher solubility, faster emulsification and higher transmittance.

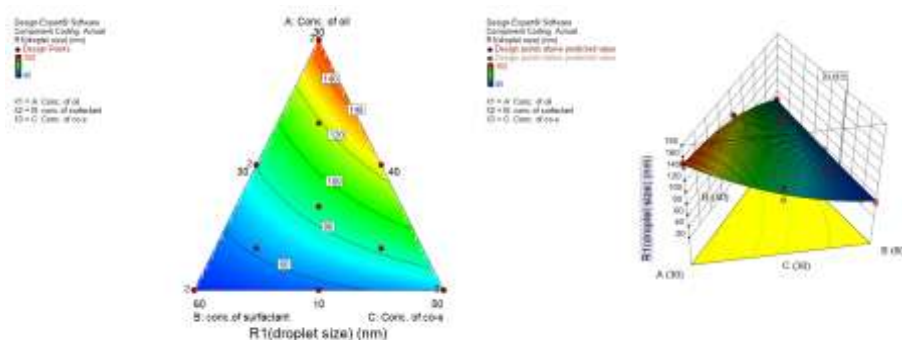


Figure 5: Contour plot and 3D response surface plot for droplet size (R1)

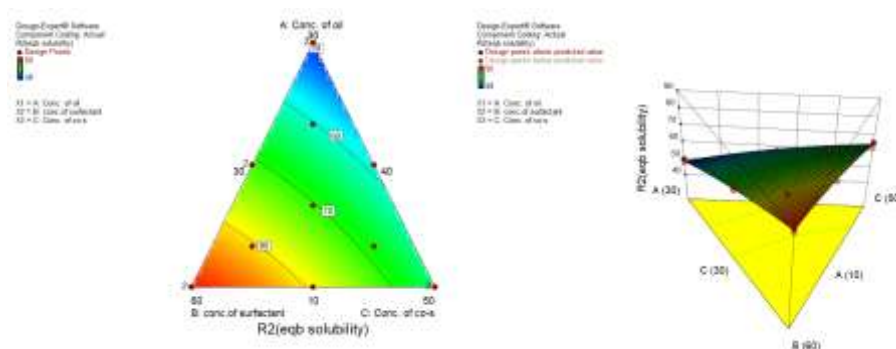


Figure 6: Contour plot and 3D response surface plot for equilibrium solubility (R2)

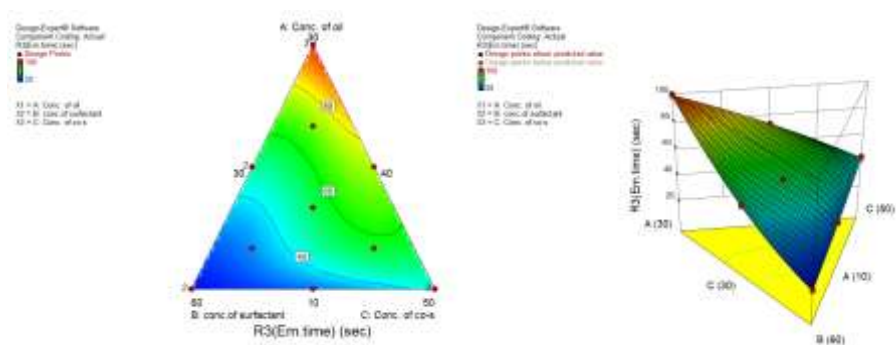


Figure 7: Contour plot and 3D response surface plot for self-emulsification time (R3)

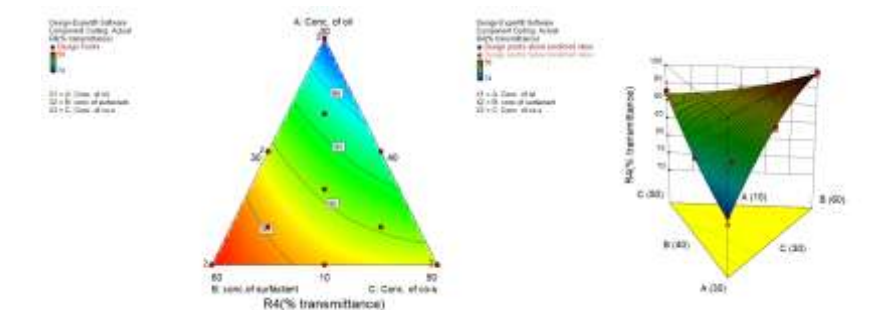


Figure 8: Contour plot and 3D response surface plot for percentage transmittance (R4)

4.8 Validation of the design: checkpoint batch analysis

Two checkpoint batches (MTZ43 and MTZ44) were prepared at compositions within the design space to validate the predictive ability of the fitted models (Table 5). The actual (experimental) values of all four responses for both checkpoint batches were in close agreement with the values predicted by the models, with only small deviations observed, confirming the validity and predictive reliability of the developed mixture models.

Table 5: Composition and predicted versus actual responses of checkpoint batches

Checkpoint batch	Oil (%)	Surfactant (%)	Co-surfactant (%)
MTZ43	10.27	46.49	43.24
MTZ44	15.95	47.03	37.03

Table 6: Predicted versus actual results for checkpoint batches

Batch	R1 - Droplet size (nm)		R2 - Eqb. solubility (mg/mL)		R3 - Emulsification time (s)		R4 - % Transmittance	
	Predicted	Actual	Predicted	Actual	Predicted	Actual	Predicted	Actual
MTZ43	93.46	94.73±0.10	62.38	63.10±0.20	42.30	41±0.34	97.77	98±0.24
MTZ44	97.82	98.1±0.23	74.22	75.60±0.78	38.82	39±0.45	95.61	97.04±0.2

4.9 Selection of the optimized batch

Numerical optimization using the desirability function approach, with minimization of droplet size and emulsification time and maximization of equilibrium solubility and percentage transmittance, identified an optimal composition (MTZ45) comprising 17.62% oil, 52.37% surfactant and 30% co-surfactant, with an overall desirability of 0.724 (Table 6). The optimized batch was prepared and evaluated, and the observed responses were in close agreement with the model-predicted values (Table 7): droplet size of 99.86 ± 0.34 nm, equilibrium solubility of 74.2 ± 0.87 mg/mL, self-emulsification time of 42 ± 0.76 s, and percentage transmittance of $95.31 \pm 0.31\%$, confirming successful development of an optimized liquid SMEDDS of Metolazone.

Table 7: Composition of the desirability-optimized batch

Formulation code	% Oil	% Surfactant	% Co-surfactant	Desirability
MTZ45	17.623	52.373	30	0.724

Table 8: Predicted versus actual results for the optimized batch (MTZ45)

Batch	R1 - Droplet size (nm)		R2 - Equilibrium solubility (mg/mL)		R3 - Emulsification time (s)		R4 - % Transmittance	
	Predicted	Actual	Predicted	Actual	Predicted	Actual	Predicted	Actual
MTZ45	100.9	99.86±0.34	75.61	74.2±0.87	40.3	42±0.76	94.9	95.31±0.31

The physicochemical and functional performance of the optimised SMEDDS of Metolazone are studied. The formulation showed a percentage transmittance of $95.31 \pm 0.31\%$, indicating formation of an optically clear, isotropic and homogeneous microemulsion upon aqueous dilution. Transmittance values near 100% are generally considered confirmatory evidence for nanoemulsification, as such values imply low light scattering and the lack of coarse or turbid dispersed-phase droplets.

No phase separation was observed after dilution and subsequent storage, indicating the selected oil-surfactant-co-surfactant combination had formed a thermodynamically compatible system which was able to self-emulsify spontaneously without droplet coalescence or drug precipitation. This was further confirmed by studies of thermodynamic stability (heating-cooling cycles, centrifugation stress and freeze-thaw cycling), where the formulation was found to be stable throughout, confirming the robustness of the microemulsion against phase inversion and Ostwald ripening - an important criterion for regulatory and shelf-life acceptability of lipid-based formulations.

The mean globule size of the reconstituted microemulsion was found to be 46.05 nm, which falls within the acceptable nanometric range reported for self-microemulsifying systems. In particular, a droplet size below 100 nm is desirable because it provides a much increased interfacial surface area for drug dissolution and partitioning, thus enhancing the rate and extent of gastrointestinal absorption relative to conventional oral dosage forms. The zeta potential of the formulation was found to be -17.72 mV, which indicates the surface charge of the globules and serves as an indicator of electrostatic repulsion between droplets; a sufficiently negative zeta potential magnitude is generally associated with reduced risk of droplet aggregation and improved long-term physical stability of the dispersed system.

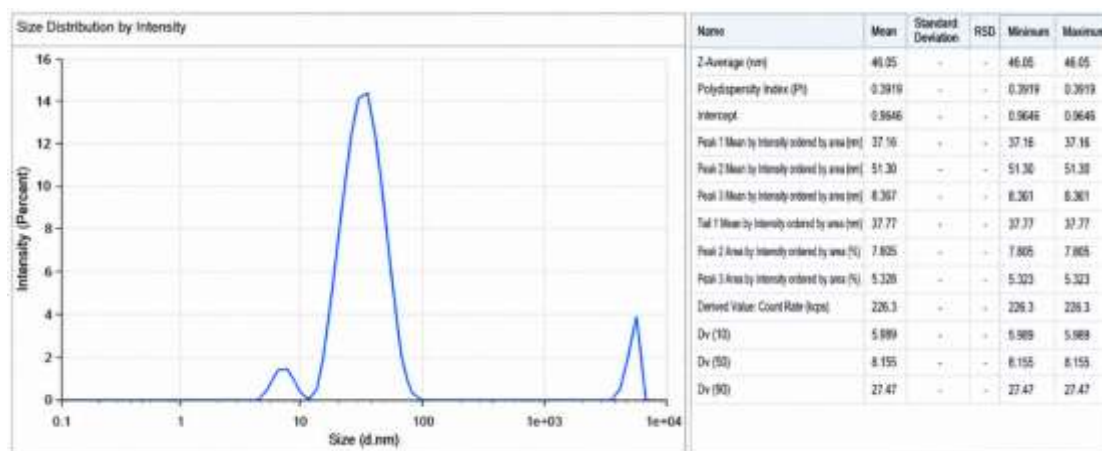


Figure 9: Droplet size distribution by intensity of the optimized MTZ-loaded SMEDDS.

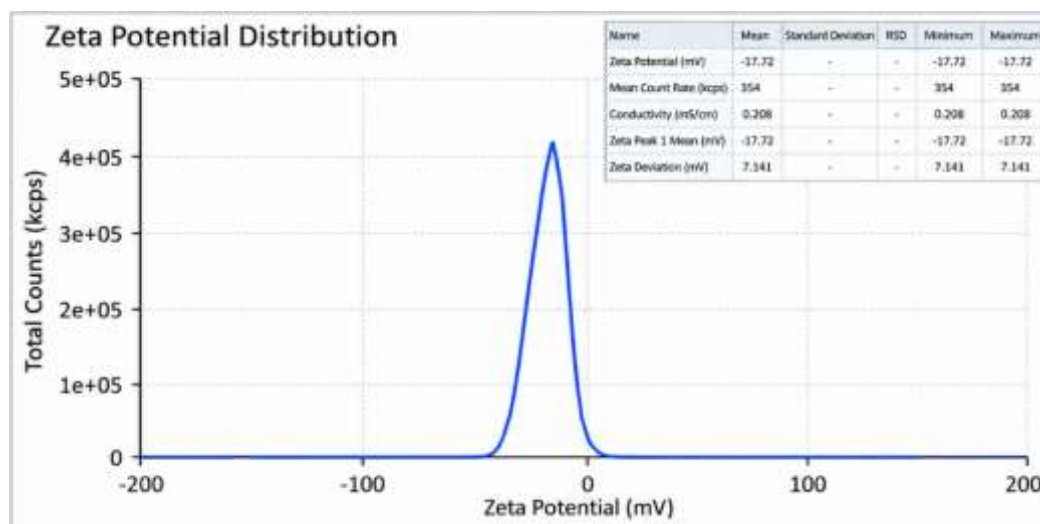


Figure 10: Zeta potential Distribution plot

The equilibrium solubility of Metolazone in the optimised formulation was found to be 74.2 ± 0.87 mg/mL, which is a significant increase in drug solubility as compared to its intrinsic aqueous solubility. This significant increase is attributed to the solubilising ability of the selected oil, surfactant and co-surfactant, which collectively provide a favourable microenvironment to accommodate the lipophilic drug molecule, thus supporting the potential of the formulation to overcome the dissolution-rate-limited oral bioavailability normally associated with poorly water-soluble drugs like Metolazone.

Finally, the cloud point of the formulation was determined to be 68.5 ± 0.11 °C, which is much higher than physiological body temperature (37 °C). This further confirms that the formulation will retain a clear, single-phase microemulsion state under physiological conditions, without any risk of phase separation or precipitation of surfactant components after oral administration, thereby ensuring consistent in vivo performance and reproducible drug release.

4.10 In vitro drug release

It was discovered that the drug release of SMEDDS was significantly greater than that of a traditional tablet. A microemulsion with a small droplet size may have spontaneously formed as a result of the SMEDDS formulation, allowing for a significantly faster rate of drug release into the aqueous phase than that of a traditional tablet. Higher absorption and oral bioavailability may result from the increased availability of dissolved Metolazone from the SMEDDS formulation. Additionally, it was shown that the drug release from both the conventional tablet and the SMEDDS formulation was

unaffected by variations in the dissolution medium (buffer pH 1.2, 4.5, and 7.2) (Figure 11). This observation indicates that Metolazone shows pH-independent solubility and dissolution.

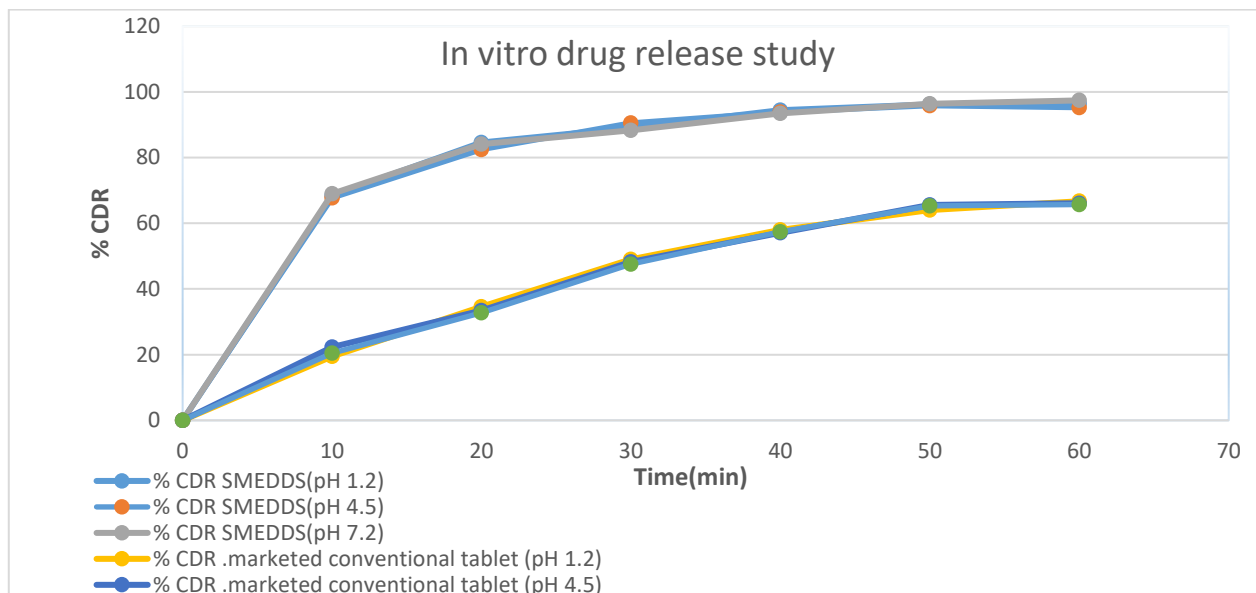


Figure 11: In vitro drug release studies

Stability Studies

It was discovered that the formulation remained stable for 6 months under accelerated conditions. There were no significant alterations in drug content, percentage transparency or rate of emulsification. Since there was no indication of capsule shell deformation, it was also observed that the formulation was compatible with the hard gelatin capsule shells. Additionally, the microemulsifying property and appearance did not significantly change, and there was no phase separation.

CONCLUSION

The present study successfully developed and optimized a liquid SMEDDS of Metolazone, a BCS class II drug whose oral bioavailability is limited by poor aqueous solubility and dissolution rate-limited absorption. Systematic solubility screening identified Capmul MCM PG-8-NF, Tween 80 and Transcutol P as the oil, surfactant and co-surfactant offering the greatest solubilizing capacity and self-emulsification efficiency, and the pseudo-ternary phase diagram constructed for this combination defined a well-resolved microemulsification zone that guided selection of the component ranges for formulation optimization. A simplex lattice mixture design proved to be an efficient and statistically robust tool for simultaneously modelling and optimizing droplet size, equilibrium solubility, self-emulsification time and percentage transmittance, with all fitted quadratic models showing high significance, strong predictive power ($R^2 > 0.92$) and close agreement between predicted and experimental checkpoint values.

The desirability-optimized formulation (17.62% oil, 52.37% surfactant, 30% co-surfactant) produced a nanometric droplet size (46.05 nm), a substantially enhanced equilibrium solubility (74.2 ± 0.87 mg/mL) relative to the intrinsic aqueous solubility of Metolazone, rapid self-emulsification (42 ± 0.76 s) and high optical clarity ($95.31 \pm 0.31\%$ transmittance), all indicative of successful nanoemulsion formation. The formulation demonstrated thermodynamic stability across freeze-thaw cycling, dilution in physiologically relevant media and centrifugation stress, and a cloud point well above body temperature, supporting its stability under physiological conditions. In vitro release studies showed markedly faster and pH-independent drug release from the SMEDDS compared with a marketed conventional tablet, consistent with the pH-independent solubility profile of Metolazone, and the optimized formulation retained its physicochemical and functional integrity after 6 months of accelerated stability testing.

Taken together, these results establish that a simplex lattice mixture design-based liquid SMEDDS is a viable and robust platform for overcoming the solubility- and dissolution-limited oral bioavailability of Metolazone. The liquid SMEDDS

developed in this study provides a strong foundation for future conversion into a solid SMEDDS dosage form (e.g., via adsorption onto solid carriers or spray drying) to improve manufacturability and patient compliance and warrants further evaluation through in vivo pharmacokinetic and bioavailability studies to confirm the anticipated clinical benefit over conventional Metolazone tablets.

Funding:

The authors received no specific financial support or funding from any public, commercial, or not-for-profit organization for the research, authorship, or publication of this article.

Acknowledgement:

The authors would like to acknowledge the support and facilities provided by **K. B. Raval College of Pharmacy, Gujarat Technological University, Gujarat, India**, for carrying out the present research work.

Author Contributions:

Amrutha MOHANDAS: Conceptualization, methodology, investigation, experimental work, data collection, data analysis, visualization, and preparation of the original manuscript.

Kunal N. PATEL: Conceptualization, supervision, methodology, critical review and editing of the manuscript, interpretation of results, and overall guidance of the research work. Both authors reviewed and approved the final version of the manuscript.

Conflict of Interest:

The authors declare that they have **no conflict of interest** related to the publication of this article.

Ethics Statement:

This study involved the formulation and in-vitro evaluation of a pharmaceutical drug delivery system and did not involve human participants, human biological samples, or experimental animals. Therefore, **ethical approval was not required** for this study.

Data Availability:

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

ABBREVIATIONS

1. Metolazone-MZT-
2. Biopharmaceutical Classification System- BCS
3. Self Micro-Emulsifying Drug Delivery System- SMEDDS
4. Fourier Transform Infrared Spectroscopy- FTIR
5. Ultraviolet- UV
6. Analysis of variance-ANOVA
7. United States Pharmacopoeia- USP

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