

QUALITY BY DESIGN-DRIVEN DEVELOPMENT AND OPTIMIZATION OF ACETAZOLAMIDE-LOADED SELF-MICROEMULSIFYING DRUG DELIVERY SYSTEM FOR ENHANCED DISSOLUTION

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ABSTRACT:

Background: Acetazolamide is a poorly soluble drug with permeability-related limitations that can restrict its oral dissolution and absorption. Self-microemulsifying drug delivery systems (SMEDDS) offer a lipid-based approach for improving drug solubilization and promoting rapid dispersion in gastrointestinal fluids. The present study aimed to develop and optimize an acetazolamide-loaded liquid SMEDDS (L-SMEDDS) using a Quality by Design (QbD) approach for enhanced dissolution.

Methods: Acetazolamide was screened for solubility in different oils, surfactants, and co-surfactants. Capryol® 90, Cremophor® RH 40, and Transcutol® HP were selected based on their drug-solubilizing capacity. Pseudo-ternary phase diagrams were constructed using different oil:Smix ratios to identify the microemulsion region. A Box–Behnken design was employed to investigate the effects of oil concentration, Smix concentration, and drug loading on globule size, polydispersity index (PDI), and percentage transmittance. The optimized formulation was further evaluated for physicochemical characteristics, thermodynamic stability, self-emulsification performance, drug content, dilution robustness, droplet morphology, and *in-vitro* drug release.

Results: The optimized L-SMEDDS exhibited a mean globule size of 78.4 nm, PDI of 0.221, and percentage transmittance of 96.8%, indicating the formation of a fine and optically clear dispersion. TEM analysis demonstrated predominantly spherical and well-defined droplets with a size range of 54.8–101.6 nm. The formulation showed satisfactory self-emulsification, dilution robustness, and drug content. In-vitro dissolution demonstrated a marked improvement in acetazolamide release, reaching 48.3% at 10 min and 96.8% at 60 min. The optimized formulation also maintained satisfactory physicochemical characteristics during the short-term stability study under both long-term and accelerated storage conditions.

Conclusion: The QbD-based approach successfully facilitated the development and optimization of acetazolamide-loaded L-SMEDDS. The optimized formulation produced nanosized droplets, rapid self-emulsification, good physical stability, and substantially enhanced in-vitro drug dissolution. Thus, L-SMEDDS represents a promising lipid-based strategy for improving the dissolution performance of poorly soluble acetazolamide.

Keywords: Acetazolamide; Self-microemulsifying drug delivery system; SMEDDS; Quality by Design; Box–Behnken design; Capryol 90; Cremophor RH 40; Transcutol HP; Droplet size; Dissolution enhancement.

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INTRODUCTION:

Oral administration remains one of the most widely preferred routes for systemic drug delivery because of its convenience, non-invasiveness, ease of administration, and high patient acceptability. However, successful oral absorption requires a drug to undergo dissolution in gastrointestinal fluids followed by permeation across the intestinal barrier. Consequently, inadequate aqueous solubility,¹ slow dissolution, poor intestinal permeability, gastrointestinal degradation, and presystemic metabolism can substantially limit systemic drug exposure. Among these factors, poor

aqueous solubility and dissolution are particularly important formulation challenges because a drug must generally be present in a dissolved state before efficient absorption can occur.

The Biopharmaceutics Classification System (BCS) provides a useful framework for relating aqueous solubility and intestinal permeability to the oral absorption behavior of drug substances. Drugs exhibiting limitations in both characteristics present a particularly challenging formulation problem because conventional approaches may not adequately address dissolution and absorption

simultaneously. Various formulation strategies, including particle-size reduction, amorphization, solid dispersion, complexation, nanotechnology-based systems, and lipid-based drug delivery systems, have therefore been investigated to improve the oral performance of poorly soluble drugs.²

Acetazolamide (ACZ) is a carbonic anhydrase inhibitor widely used in the management of conditions such as glaucoma, edema, altitude sickness, and certain seizure disorders. From a formulation perspective, acetazolamide presents challenges associated with its physicochemical characteristics and oral dissolution behavior. Although acetazolamide is frequently described in formulation literature as a BCS Class IV drug, its definitive BCS classification warrants caution because the available experimental information regarding its solubility, intestinal absorption, and permeability has not been considered sufficiently conclusive for an unequivocal classification. Therefore, improving the dissolution and maintaining acetazolamide in a readily dispersible and solubilized state represents a rational formulation objective.³⁻⁵

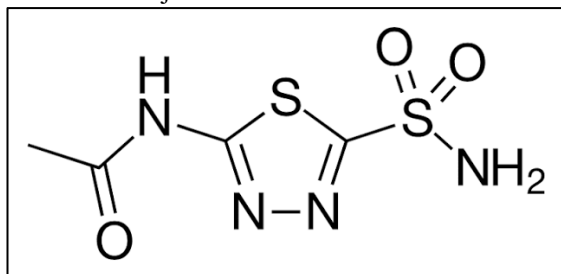


Figure 1: Structure of Acetazolamide

Lipid-based drug delivery systems have received considerable attention for addressing the formulation limitations of poorly water-soluble drugs. These systems incorporate the drug into lipidic vehicles containing combinations of oils, surfactants, and co-surfactants or co-solvents. Following oral administration, appropriate lipid-based formulations can undergo spontaneous dispersion in gastrointestinal fluids, producing fine droplets that increase the interfacial area available for drug transfer into the aqueous gastrointestinal environment. Such systems can consequently improve drug solubilization and reduce the dependence of drug release on dissolution of the crystalline drug substance.⁶⁻⁷

Among lipid-based approaches, self-emulsifying drug delivery systems (SEDDS) and self-microemulsifying drug delivery systems (SMEDDS) have emerged as attractive platforms for enhancing the dissolution performance of poorly water-soluble drugs. SMEDDS are generally isotropic mixtures comprising an oil phase, surfactant, and co-surfactant or co-solvent, with the drug incorporated within the formulation. Upon dilution with an aqueous medium and gentle

agitation, the system spontaneously forms a fine oil-in-water microemulsion. The resulting small droplets provide a large interfacial area and facilitate maintenance of the drug in a solubilized or dispersed state, thereby promoting more rapid drug release compared with conventional drug suspensions.⁸⁻⁹

The performance of SMEDDS is strongly dependent on the qualitative and quantitative selection of formulation components. The oil phase contributes to drug solubilization and determines, together with the surfactant system, the characteristics of the dispersed droplets. Surfactants reduce interfacial tension and facilitate spontaneous emulsification, whereas co-surfactants or co-solvents can improve interfacial flexibility and enhance drug solubilization. The relative proportions of these components influence important formulation characteristics such as globule size, polydispersity, optical clarity, self-emulsification efficiency, drug loading, and physical stability.

Despite their potential, development of SMEDDS is not simply a matter of selecting suitable excipients. The multidimensional interactions among formulation variables can produce substantial changes in the critical quality attributes of the final system. Conventional trial-and-error formulation development may therefore require a large number of experiments and may not adequately identify interactions between critical formulation variables. A Quality by Design (QbD) approach provides a systematic alternative by establishing the quality target product profile (QTPP), identifying critical quality attributes (CQAs), assessing formulation risks, selecting critical material and process variables, and applying statistically designed experiments for formulation optimization. Design of Experiments (DoE), particularly response-surface designs such as the Box-Behnken design (BBD), can efficiently evaluate the influence of multiple variables and their interactions while reducing the number of experimental trials.⁷⁻¹⁰

The application of QbD to SMEDDS is particularly relevant because formulation variables such as oil concentration, surfactant/co-surfactant composition, and drug loading can simultaneously affect several performance attributes. An optimized formulation should therefore provide small and uniformly distributed droplets, low polydispersity, adequate optical clarity, efficient self-emulsification, satisfactory drug loading, and improved drug dissolution. Establishing quantitative relationships between formulation variables and these responses can facilitate rational selection of the optimized composition rather than relying solely on empirical formulation trials.

Although SMEDDS technology has been extensively investigated for poorly water-soluble

drugs, formulation-specific investigation of acetazolamide using a systematic QbD-driven optimization strategy remains comparatively limited. In particular, a formulation approach integrating excipient screening, pseudo-ternary phase-diagram analysis, risk assessment, Box–Behnken experimental design, and comprehensive characterization can provide a structured basis for developing an acetazolamide-loaded SMEDDS. Recent literature continues to identify self-emulsifying systems as promising platforms for addressing solubility- and dissolution-related limitations of challenging oral drug candidates.⁶⁻¹⁰ Therefore, the present study was designed to develop and optimize an acetazolamide-loaded liquid self-microemulsifying drug delivery system (L-SMEDDS) using a Quality by Design approach. Systematic screening of lipid, surfactant, and co-surfactant/co-solvent components was followed by pseudo-ternary phase-diagram construction to identify a suitable self-emulsifying region. A risk-based formulation strategy and Box–Behnken design were subsequently employed to investigate the influence of critical formulation variables on globule size, polydispersity index, and percentage transmittance. The optimized formulation was further characterized for its physical and physicochemical properties, morphology, dilution robustness, self-emulsification behavior, drug content, and in-vitro dissolution performance. The study was ultimately aimed at establishing a rationally optimized L-SMEDDS platform capable of improving the dissolution performance of acetazolamide.

MATERIALS AND METHODS:

MATERIALS:

Acetazolamide was selected as the model drug for development and optimization of the self-microemulsifying drug delivery system (SMEDDS) owing to its limited aqueous solubility and reported permeability-related limitations, which may restrict its dissolution and oral absorption. Acetazolamide has been widely reported as a BCS Class IV drug, although its definitive BCS classification has been subject to some uncertainty (Granero et al., 2008). Acetazolamide was procured from A.G. Traders, Pune, Maharashtra, India. Capryol® 90, Labrafil® M 2125 CS and Transcutol® HP were obtained from Gattefossé, France, while oleic acid, Tween® 80, Kolliphor® EL, Cremophor® RH 40, polyethylene glycol 400 and propylene glycol were procured from Loba Chemie Pvt. Ltd., Mumbai, India. All chemicals and excipients were of pharmaceutical or analytical grade and were used as received.

Preformulation Studies:¹¹⁻¹⁴

Organoleptic Properties

Acetazolamide was examined visually for colour, odour, texture, and physical state under suitable illumination. The observed characteristics were

documented and compared with reported specifications.

Melting Point

The melting point of acetazolamide was determined by the capillary tube method using a calibrated melting-point apparatus. Finely powdered drug was filled into a dry capillary tube and heated gradually at a controlled rate. The temperatures corresponding to the onset and completion of melting were recorded. The determination was performed in triplicate.

Solubility Study

The equilibrium solubility of acetazolamide was determined by the shake-flask method. An excess amount of drug was added to selected solvents and aqueous media and equilibrated under controlled shaking conditions. The samples were filtered to remove undissolved drug, suitably diluted, and analysed using the established UV-visible spectrophotometric method. The study was conducted in triplicate.

Partition Coefficient

The partition coefficient of acetazolamide was determined using the shake-flask method with n-octanol and an aqueous phase. After equilibration, the two phases were separated and analysed for drug concentration. The partition coefficient was calculated from the concentration ratio between the organic and aqueous phases, and the corresponding log P value was determined. Measurements were performed in triplicate.

Determination of pKa

The pKa of acetazolamide was estimated by a UV-visible spectrophotometric approach using drug solutions adjusted to different pH conditions. The absorbance response across the selected pH range was evaluated to establish the ionization profile and estimate the pKa value.

Determination of λ_{max}

A suitably diluted acetazolamide solution was scanned from 200–400 nm against the corresponding solvent blank using a UV-visible spectrophotometer. The wavelength exhibiting maximum absorbance was recorded as λ_{max} and used for subsequent spectrophotometric estimation.

Preparation of Calibration Curve

Standard acetazolamide solutions in methanol and phosphate buffer pH 6.8 were prepared over the concentration range of 2–12 µg/mL. Absorbance was measured at the predetermined λ_{max} , and calibration plots were constructed by plotting absorbance against drug concentration. Linear regression was performed to obtain the regression equation and coefficient of determination (R^2).

Drug–Excipient Compatibility Studies

Fourier Transform Infrared Spectroscopy

FTIR spectroscopy was employed to evaluate the compatibility of acetazolamide with the selected SMEDDS components. Spectra of pure acetazolamide and its physical mixtures with

Capryol® 90, Cremophor® RH 40, and Transcutol® HP were recorded over the appropriate infrared region. The characteristic absorption bands of the drug were compared to identify any notable changes indicative of possible drug–excipient interactions.

Differential Scanning Calorimetry

The thermal behaviour and compatibility of acetazolamide with the selected excipients were investigated using DSC. Approximately 5 mg of each sample was sealed in an aluminium pan and heated from 40–300°C at 10°C/min under nitrogen purge. The thermograms were evaluated by comparing the characteristic thermal transition of acetazolamide with those of the drug–excipient mixtures.

Screening of SMEDDS Components:¹⁵⁻¹⁶

The solubility of acetazolamide was screened in selected oils, surfactants, and co-surfactants. An excess amount of drug was added to 2 mL of each vehicle and equilibrated at $25 \pm 2^\circ\text{C}$ for 48 h with shaking at 250 rpm. Following centrifugation at 3000 rpm for 15 min, the supernatant was filtered through a 0.45- μm membrane filter, suitably diluted with methanol, and analysed spectrophotometrically at 287 nm. Based on drug solubilization and formulation suitability, the most appropriate oil, surfactant, and co-surfactant were selected for further development.

Construction of Pseudo-Ternary Phase Diagrams

Pseudo-ternary phase diagrams were constructed to identify the microemulsion region and optimize the composition of the SMEDDS. Surfactant and co-surfactant were combined as Smix at 1:1, 1:2, and 2:1 ratios. Capryol® 90 and Smix were subsequently mixed at oil-to-Smix ratios ranging from 1:9 to 9:1. Distilled water was added gradually under gentle stirring, and the resulting systems were visually assessed for clarity, homogeneity, and phase separation. Clear and isotropic systems were considered suitable microemulsion regions. The phase diagrams were constructed using CHEMIX ternary plot software, and the Smix ratio providing the most favourable microemulsion region was selected for further formulation development.

Risk Assessment for SMEDDS Formulation Development¹⁷⁻¹⁹

A systematic risk assessment was performed to identify formulation and process variables that could influence the critical quality attributes (CQAs) of acetazolamide-loaded SMEDDS. The CQAs considered included globule size, polydispersity index (PDI), zeta potential, self-emulsification time, drug content, and in-vitro drug release. Potentially influential variables, including oil concentration, surfactant/Smix concentration, drug loading, mixing conditions, and temperature, were evaluated using a risk estimation matrix

(REM) and categorized as low, medium, or high risk. The high-risk variables identified through the assessment were subsequently considered for systematic optimization using Design of Experiments (DoE).

Quality Target Product Profile and Critical Quality Attributes

The Quality Target Product Profile (QTPP) was established to define the desired quality characteristics of the acetazolamide SMEDDS, with emphasis on rapid self-emulsification, small globule size, narrow size distribution, high optical clarity, adequate drug loading, and enhanced dissolution. Based on the QTPP, the CQAs were identified and their desired targets were established. For statistical optimization, globule size, PDI, and percentage transmittance were selected as the principal responses, with globule size and PDI targeted for minimization and percentage transmittance targeted for maximization.

Box–Behnken Design for Optimization of SMEDDS

A three-factor, three-level Box–Behnken design (BBD) was employed using Design-Expert® software (Version 11, Stat-Ease Inc., Minneapolis, USA) to optimize the SMEDDS formulation. Based on preliminary screening and pseudo-ternary phase-diagram studies, oil concentration (A), Smix concentration (B), and drug loading (C) were selected as independent variables. Each factor was investigated at low (–1), medium (0), and high (+1) levels. Globule size (R1), PDI (R2), and percentage transmittance (R3) were selected as dependent responses. A total of 13 experimental formulations were generated according to the BBD. The experimental data were fitted to linear, two-factor interaction, and quadratic models, and model adequacy was assessed by analysis of variance (ANOVA). The optimized formulation was selected using a desirability function by targeting minimum globule size and PDI and maximum transmittance.¹⁸

The quadratic polynomial model used for response prediction was expressed as:

$$Y = a_0 + a_1A + a_2B + a_3C + a_{12}AB + a_{13}AC + a_{23}BC + a_{11}A^2 + a_{22}B^2 + a_{33}C^2 \dots\dots\dots (Eq. 1)$$

where Y represents the measured response, a_0 is the intercept, a_1 – a_3 are the linear coefficients, a_{12} – a_{23} are the interaction coefficients, and a_{11} – a_{33} are the quadratic coefficients. A, B, and C represent oil concentration, Smix concentration, and drug loading, respectively. Response-surface and contour plots were generated to evaluate the influence of formulation variables on the selected responses.

Preparation of Acetazolamide-Loaded SMEDDS

The liquid SMEDDS was prepared using Capryol® 90 as the oil, Cremophor® RH 40 as the surfactant, and Transcutol® HP as the co-surfactant.

Cremophor® RH 40 and Transcutol® HP were mixed at the selected 2:1 Smix ratio. Acetazolamide was incorporated into Capryol® 90 with gentle heating at 40–45°C and continuous stirring until adequately solubilized. The Smix was then added gradually under magnetic stirring, and the mixture was stirred for approximately 20 min until a clear, homogeneous and isotropic formulation was obtained. The prepared formulations were visually examined for phase separation and stored in tightly closed glass vials at room temperature until further evaluation.¹⁸⁻²²

Characterization of Acetazolamide-Loaded L-SMEDDS²³⁻³⁰

Physical Appearance

The prepared acetazolamide-loaded SMEDDS formulations were visually inspected for colour, clarity, homogeneity, fluidity, turbidity, precipitation, and phase separation. Each formulation was observed in a transparent glass vial under suitable illumination, and the physical appearance was recorded.

Globule Size, PDI and Zeta Potential

The optimized SMEDDS was diluted 1:100 (v/v) with deionized water and gently stirred for approximately 5 min. The mean globule size and polydispersity index (PDI) were determined by dynamic light scattering using a particle-size analyser. Zeta potential was measured using the corresponding electrophoretic light-scattering technique. All measurements were performed in triplicate and expressed as mean values.

Thermodynamic Stability

The thermodynamic stability of the prepared SMEDDS formulations was evaluated by sequential stress testing. Formulations were first centrifuged at 3500 rpm for 30 min and examined for phase separation. Stable formulations were subjected to heating–cooling cycles between 4 and 45°C, followed by freeze–thaw cycles between –21 and +25°C, with samples maintained at each temperature for 48 h. Formulations showing no visible phase separation, precipitation, creaming, or cracking were considered stable.

Self-Emulsification Time

Self-emulsification performance was assessed using a USP Type II dissolution apparatus. One millilitre of SMEDDS was introduced into 500 mL distilled water maintained at $37 \pm 0.5^\circ\text{C}$ and 50 rpm. The time required for complete dispersion and formation of a homogeneous emulsion was recorded as the self-emulsification time. The resulting dispersion was visually assessed for clarity, homogeneity, and phase separation. The experiment was performed in triplicate.

Cloud Point

The cloud point of the optimized SMEDDS was determined after 1:100 (v/v) dilution with distilled water. The diluted formulation was gradually heated in a temperature-controlled water bath, and

the temperature at which the clear dispersion became visibly turbid was recorded as the cloud-point temperature. The measurement was performed in triplicate.

Drug Content

An accurately measured quantity of SMEDDS equivalent to the intended acetazolamide dose was diluted with methanol and sonicated for approximately 10 min to ensure complete drug extraction. The solution was suitably diluted and analysed spectrophotometrically at 287 nm using methanol as the blank. Drug content was calculated from the established calibration curve and expressed as a percentage of the theoretical drug content. The analysis was performed in triplicate.

Robustness to Dilution

The dilution robustness of the SMEDDS was evaluated using distilled water, 0.1 N HCl, and phosphate buffer pH 6.8 at selected dilution ratios of 1:50, 1:100, and 1:250. The resulting dispersions were examined immediately and after standing for visible precipitation, turbidity, flocculation, and phase separation. Formulations maintaining their physical characteristics after dilution were considered dilution-robust.

Percentage Transmittance (%T)

The optical clarity of the optimized SMEDDS was assessed by measuring percentage transmittance at 650 nm using a UV-visible spectrophotometer. The formulation was appropriately diluted with distilled water, which was used as the blank. Higher transmittance values were considered indicative of greater optical clarity of the resulting dispersion.

Morphological Characterization

The morphology of the microemulsion generated from the optimized ACZ-loaded L-SMEDDS was examined using transmission electron microscopy (TEM). The L-SMEDDS formulation was diluted suitably with distilled water and gently mixed to obtain a uniform dispersion. A drop of the diluted formulation was placed on a carbon-coated copper grid and allowed to adsorb. Excess sample was removed, and the grid was subjected to appropriate staining and drying according to the standard TEM procedure. The prepared grid was examined at suitable magnification, and representative micrographs were recorded to evaluate the shape, surface characteristics, droplet size distribution, and possible aggregation of the microemulsion droplets (Sharma et al., 2023).

In-vitro Drug Release Study

The *in-vitro* drug release of the optimized ACZ-loaded L-SMEDDS was evaluated using a USP Type II paddle dissolution apparatus. The study was performed in 900 mL phosphate buffer (pH 6.8) maintained at $37 \pm 0.5^\circ\text{C}$ and stirred at 50 rpm. An accurately weighed quantity of L-SMEDDS equivalent to 20 mg of Acetazolamide was filled into a hard gelatin capsule and introduced into the dissolution medium. A suspension containing an

equivalent quantity of pure Acetazolamide was evaluated under the same conditions as the reference preparation.

Aliquots of 10 mL were withdrawn at predetermined intervals of 10, 20, 30, 40, 50, and 60 min, and the withdrawn volume was immediately replaced with an equal volume of pre-warmed dissolution medium. The samples were passed through a 0.45 µm membrane filter, suitably diluted with phosphate buffer, and analyzed using a UV-visible spectrophotometer at 287 nm. The cumulative percentage of Acetazolamide released was calculated and plotted against time to assess the dissolution enhancement achieved by the L-SMEDDS formulation.

Stability study³¹⁻³³

The stability of the optimized Acetazolamide-loaded L-SMEDDS was evaluated under long-term and accelerated storage conditions to assess its physical and chemical stability. The formulation was filled into tightly closed glass vials and stored at $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{ RH}$ and $40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$ for 3 months. Samples were withdrawn at predetermined intervals and evaluated for visual appearance, globule size, PDI, and drug content. The stored formulations were also examined for any visible changes such as precipitation, turbidity, phase separation, creaming, or cracking. The formulation was considered physically stable when no significant changes in its appearance or globule characteristics were observed during storage. Changes in drug content were used to assess the chemical stability of Acetazolamide in the L-SMEDDS formulation. The study was conducted with reference to the general principles described in ICH Q1A (R2) for stability evaluation.

RESULTS AND DISCUSSION:

Pre-formulation Studies

Description and Organoleptic Properties of Acetazolamide

Acetazolamide was obtained as a white to pale yellowish-white, odourless crystalline powder with a uniform texture. No visible foreign particles or agglomeration were observed. The observed characteristics were consistent with the expected physical appearance of Acetazolamide, indicating satisfactory identity and suitability for further pre-formulation investigations.

Table 1: Organoleptic characteristics of Acetazolamide

Sr. No.	Parameter	Observation
1	Colour	White to pale yellowish-white
2	Odour	Odourless
3	Texture	Fine crystalline powder

4	Physical state	Solid
5	Appearance	Crystalline powder
6	Foreign matter	Absent
7	Agglomeration	Absent

Determination of Melting Point

Acetazolamide exhibited a narrow melting transition, with a mean observed melting point of $258.10 \pm 0.01^\circ\text{C}$. The close agreement among the three determinations indicated good reproducibility of the measurement. The observed melting behaviour was consistent with the reported thermal characteristics of Acetazolamide, supporting its identity and crystalline nature.

Table 2: Melting point determination of Acetazolamide

Trial	Melting onset ($^\circ\text{C}$)	Complete melting ($^\circ\text{C}$)	Observed melting point ($^\circ\text{C}$)
1	257.6	258.4	258.0
2	257.8	258.6	258.2
3	257.7	258.5	258.1
Mean $\pm$ SD	257.70 ± 0.04	258.50 ± 0.07	258.10 ± 0.01

Determination of Solubility

Acetazolamide showed marked variation in solubility among the investigated solvents. The highest solubility was observed in methanol ($8.47 \pm 0.05 \text{ mg/mL}$), whereas the drug exhibited considerably lower solubility in distilled water ($0.73 \pm 0.01 \text{ mg/mL}$). The limited aqueous solubility confirmed the dissolution-related challenge associated with Acetazolamide and supported the selection of a lipid-based formulation approach for improving its solubilization and dissolution performance.

Table 3: Solubility of Acetazolamide in selected solvents

Sr. No.	Solvent	Trial 1 (mg/mL)	Trial 2 (mg/mL)	Trial 3 (mg/mL)	Mean $\pm$ SD (mg/mL)
1	Distilled water	0.72	0.74	0.73	0.73 ± 0.01
2	Methanol	8.42	8.51	8.47	8.47 ± 0.05
3	Ethanol	2.31	2.38	2.35	2.35 ± 0.04
4	0.1 N	1.24	1.29	1.27	$1.27 \pm$

	HCl				0.03
5	Phosphoric buffer pH 6.8	1.56	1.62	1.59	1.59 ± 0.03

Determination of Partition Coefficient

The experimental n-octanol/water partition coefficient of Acetazolamide was 0.546 ± 0.002 , corresponding to a mean log P of -0.262 ± 0.001 . The relatively low log P indicated a greater distribution of the drug toward the aqueous phase than the organic phase. This physicochemical characteristic was considered during formulation development because the drug's partitioning behaviour can influence its interaction with the lipid components of the SMEDDS.

Determination of pKa

The UV-visible spectrophotometric evaluation demonstrated a measurable change in absorbance with variation in pH, confirming the ionization behaviour of Acetazolamide. The mean pKa was determined to be 7.20 ± 0.02 , with a low %RSD of 0.21%, indicating good repeatability. The obtained value was considered relevant for formulation development because the ionization state of Acetazolamide can influence its aqueous solubility and dissolution characteristics.

The pre-formulation results established that Acetazolamide possessed a crystalline nature, high melting transition, limited aqueous solubility, and relatively low lipophilicity. These characteristics indicated a potential dissolution limitation and provided a scientific basis for investigating L-SMEDDS as a lipid-based formulation strategy. The obtained physicochemical profile was subsequently considered during excipient screening and formulation optimization.

Determination of λ_{max} and Calibration Curve of Acetazolamide

The UV absorption spectrum of Acetazolamide recorded between 200–400 nm showed a distinct maximum at 287 nm. Therefore, 287 nm was selected as the analytical wavelength for subsequent spectrophotometric estimation of Acetazolamide.

Calibration curves were prepared in methanol and phosphate buffer pH 6.8 over the concentration range of 2–12 µg/mL. A concentration-dependent increase in absorbance was observed in both media. The calibration curve in methanol showed the regression equation $y = 0.05325x + 0.01121$ with $R^2 = 0.9991$, whereas phosphate buffer pH 6.8 showed $y = 0.08466x + 0.00418$ with $R^2 = 0.9992$. The R^2 values (>0.999) confirmed excellent linearity and demonstrated the suitability of the method for quantitative estimation of Acetazolamide during formulation and *in-vitro* dissolution studies.

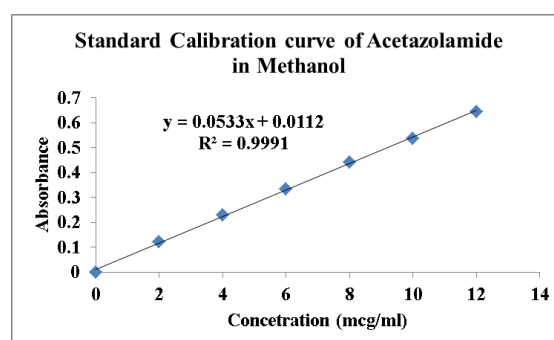


Figure 2: Calibration curve of Acetazolamide in methanol.

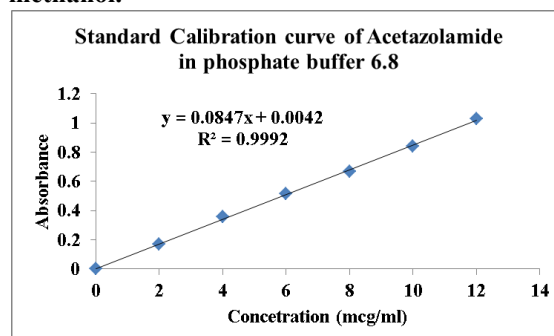


Figure 3: Calibration curve of Acetazolamide in phosphate buffer pH 6.8

FTIR Studies

The FTIR spectrum of pure Acetazolamide exhibited characteristic absorption bands at 3451.70, 1685.90, 1259.86, 1317.20, and 1507.25 cm^{-1} , corresponding to N–H, C=O, S=O, C–N, and aromatic C=C stretching vibrations, respectively. The presence of these characteristic bands confirmed the functional-group characteristics of the drug.

The FTIR spectrum of the Acetazolamide–Capryol® 90–Cremophor® RH 40–Transcutol® HP physical mixture retained the principal characteristic bands of Acetazolamide at 3451.70, 1685.90, 1259.86, 1317.20, and 1507.25 cm^{-1} . Additional absorption bands attributable to the selected excipients were also observed. Importantly, no major disappearance or substantial shifting of the characteristic Acetazolamide bands was evident. These findings indicated the absence of any prominent chemical interaction between Acetazolamide and the selected excipients, supporting their compatibility for L-SMEDDS formulation development.

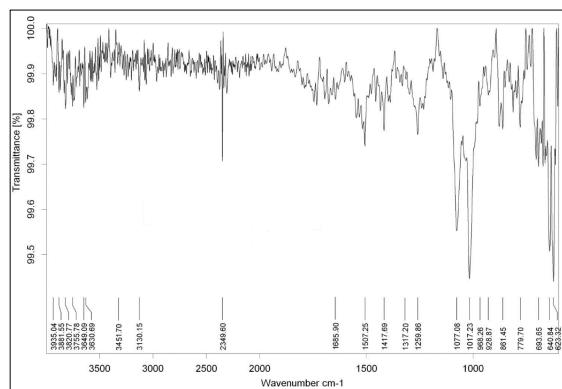


Figure 4: FTIR Spectra of Acetazolamide

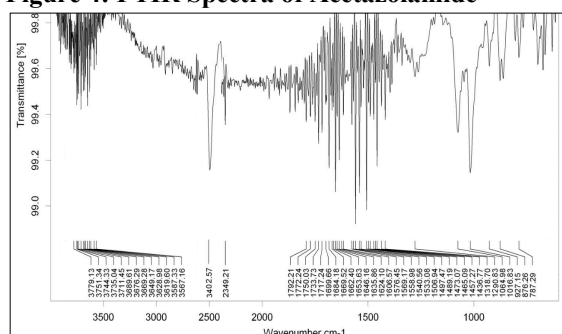


Figure 5: FT-IR Spectra of physical mixture of Acetazolamide, Capryol 90, Cremophor® RH 40 and Transcutol® HP

Differential Scanning Calorimetry (DSC) Study

The DSC thermogram of pure acetazolamide exhibited a sharp endothermic peak at 268.52°C, with an enthalpy change (ΔH) of 319.9478 J/g. The well-defined thermal transition represents the characteristic thermal behaviour of acetazolamide and indicates a consistent thermal event in the drug sample. The sharp endothermic transition further supports the presence of a defined crystalline drug structure.

The DSC thermograms of acetazolamide and the drug–excipient mixture showed endothermic transitions within the approximate range of 267–270°C. Acetazolamide exhibited an endothermic peak at 269.58°C with a ΔH of 218.7643 J/g, whereas the acetazolamide–Capryol® 90–Cremophor® RH 40–Transcutol® HP mixture showed a peak at 267.85°C with a ΔH of 210.5486 J/g. The slight reduction in peak temperature and enthalpy may be associated with changes in the physical environment of acetazolamide following incorporation of the formulation components. Importantly, the characteristic thermal transition of acetazolamide was retained in the drug–excipient mixture, without any prominent new thermal event. These observations indicate the absence of major thermal incompatibility between acetazolamide and the selected excipients and support their suitability for further SMEDDS development.

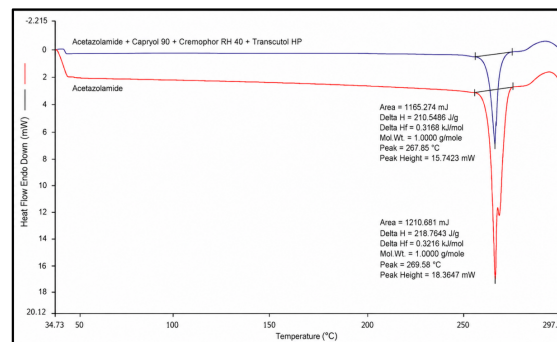


Figure 6: DSC Thermogram of Acetazolamide with excipients

SMEDDS Formulation Development of Acetazolamide

Screening of Components

Selection of suitable formulation components is an important step in SMEDDS development because the solubilization capacity and interfacial properties of the oil, surfactant, and co-surfactant influence drug loading, emulsification behaviour, formulation stability, and drug release. Therefore, acetazolamide solubility was comparatively evaluated in the selected oils, surfactants, and co-surfactants. The results are presented in Figure 6.11A–C.

Among the tested oils, Capryol® 90 showed the highest solubilization capacity for acetazolamide, followed by Labrafil® M 2125 CS and oleic acid. Capryol® 90 was therefore selected as the oil phase. Among the surfactants, Cremophor® RH 40 exhibited the highest drug solubilization compared with Tween® 80 and Kolliphor® EL and was selected as the surfactant. For the co-surfactants, Transcutol® HP demonstrated the highest solubilization capacity, followed by PEG 400 and propylene glycol. Accordingly, Transcutol® HP was selected as the co-surfactant. Thus, Capryol® 90, Cremophor® RH 40, and Transcutol® HP were selected for subsequent SMEDDS development and optimization.

Construction of Pseudo-Ternary Phase Diagram

Pseudo-ternary phase diagrams were constructed to determine the microemulsion region and select the appropriate surfactant-to-co-surfactant ratio for the SMEDDS formulation. Smix ratios of 1:1, 1:2, and 2:1 were investigated using the water titration method. The phase behaviour varied with the composition of Smix. The 1:2 Smix ratio produced a comparatively smaller microemulsion region, indicating relatively limited emulsification efficiency at the tested composition. Increasing the surfactant proportion resulted in expansion of the microemulsion region, with the 2:1 Smix ratio producing the largest region.

The larger microemulsion region obtained with Smix 2:1 indicated more favourable interfacial stabilization and spontaneous dispersion of the oil phase upon aqueous dilution. Therefore, Smix 2:1 (Cremophor® RH 40:Transcutol® HP) was

selected for further formulation optimization. The optimized component combination of Capryol® 90–Cremophor® RH 40–Transcutol® HP demonstrated a favourable microemulsion region and was subsequently used for development of the acetazolamide-loaded SMEDDS.

SMEDDS Formulation Development of Acetazolamide Optimization of Developed SMEDDS Formulation

A 13-run Box–Behnken design (BBD) was applied to optimize the acetazolamide SMEDDS using oil concentration (A), Smix concentration (B), and drug loading (C) as independent variables. Globule size (R1), PDI (R2), and transmittance (R3) were selected as responses, with the objectives of minimizing globule size and PDI and maximizing transmittance.

The formulations showed globule sizes of 78.4–168.5 nm, PDI values of 0.221–0.436, and transmittance values of 68.4–96.8%. Run 13 showed the most desirable characteristics, with a globule size of 78.4 nm, PDI of 0.221, and transmittance of 96.8%. The quadratic models were significant, with Smix concentration showing the strongest influence on all responses, followed by oil concentration, whereas drug loading exhibited a comparatively lower effect.

The developed models demonstrated good statistical adequacy, with R² values of 0.9913, 0.9963, and 0.9920 for globule size, PDI, and transmittance, respectively. Adequate precision values ranged from 18.78 to 27.29, indicating satisfactory model reliability and predictive performance. Response-surface plots further demonstrated that higher Smix concentration and moderate oil concentration favoured smaller globules, lower PDI, and improved transmittance. Based on the desirability approach, the optimized formulation corresponded approximately to 15% oil, 60–65% Smix, and 10 mg drug loading, producing approximately 78 nm globule size, 0.22 PDI, and >96% transmittance.

Table 4: Optimization of Acetazolamide SMEDDS Formulation

R un	A (O il)	B (S _m ix)	C (Dr ug)	Glob ule Size (nm) (R1)	PD I (R 2)	Transmit tance (%) (R3)
1	-1	-1	0	145.2	0.412	72.3
2	+1	-1	0	168.5	0.436	68.4
3	-1	+1	0	92.4	0.2	91.5

					56	
4	+1	+1	0	110.3	0.298	88.2
5	-1	0	-1	130.6	0.365	78.6
6	+1	0	-1	152.1	0.389	74.2
7	-1	0	+1	138.4	0.372	76.8
8	+1	0	+1	160.7	0.401	72.5
9	0	-1	-1	140.8	0.395	75.6
10	0	+1	-1	98.6	0.268	90.3
11	0	-1	+1	148.3	0.408	73.1
12	0	+1	+1	105.2	0.281	89.4
13	0	0	0	78.4	0.221	96.8

Final Optimized Formulation Composition

The optimized acetazolamide SMEDDS consisted of Capryol® 90, Cremophor® RH 40, and Transcutol® HP as the oil, surfactant, and co-surfactant, respectively. Acetazolamide (10 mg) was incorporated into Capryol® 90, followed by Cremophor® RH 40 and Transcutol® HP at a 2:1 Smix ratio. BHT (10 mg) was included as an antioxidant. The components were mixed thoroughly to obtain a homogeneous liquid SMEDDS concentrate. The optimized formulation exhibited approximately 78 nm globule size, 0.22 PDI, and >96% transmittance, indicating satisfactory formulation characteristics.

Table 5: Optimized Formulation Composition (For 10 mL)

Sr. No	Ingredient	Function	Quantit y
1	Acetazolamide	Active pharmaceutical ingredient (API)	10 mg
2	Capryol 90	Oil phase	1.5 mL

		(solubilizer)	
3	Cremophor RH40	Surfactant (emulsifying agent)	4.3 mL
4	Transcutol HP	Co-surfactant (co-emulsifier)	2.2 mL
5	Butylated Hydroxytoluene (BHT)	Antioxidant	10 mg
6	Purified Water*	Diluent (for evaluation)	q.s.

Characterization of SMEDDS Formulation

Thermodynamic Stability Study

The developed formulations were evaluated under centrifugation, heating-cooling, and freeze-thaw conditions. The pure drug showed sedimentation and precipitation, indicating poor physical stability. In contrast, L-SMEDDS showed no phase separation or precipitation under the tested conditions. L-SMEDDS remained clear and transparent.

Table 6: Thermodynamic stability of developed formulations

Formulation	Centrifugation	Heating–Cooling	Freeze–Thaw	Appearance	Overall stability
Pure drug	Sedimentation	Instability	Precipitation	Turbid	Unstable
L-SMEDDS	No separation	Stable	No precipitation	Clear & transparent	Stable

Self-Emulsification

The developed SMEDDS formulations showed rapid and spontaneous emulsification, with self-emulsification times ranging from 24.1 to 48.3 s. Higher Smix concentration generally promoted faster emulsification, whereas higher oil concentration increased the emulsification time. The optimized formulation F13 showed a self-emulsification time of 20.3 ± 0.7 s and produced a clear, homogeneous dispersion without precipitation or phase separation.

Cloud-Point Determination

The optimized acetazolamide SMEDDS showed a cloud point of $81.7 \pm 0.6^\circ\text{C}$. The formulation remained clear at temperatures below the cloud point and became cloudy only at elevated temperature, indicating satisfactory thermal stability of the nonionic surfactant-based system.

Drug Content Determination

The drug content of the developed formulations ranged from 96.8–99.2%, indicating satisfactory drug incorporation and uniformity. The optimized F13 formulation showed a drug content of $98.6 \pm 0.7\%$, confirming efficient incorporation of acetazolamide into the SMEDDS system.

Dilution Robustness

The optimized SMEDDS showed good dilution robustness in distilled water, 0.1 N HCl, and phosphate buffer pH 6.8 at 1:50, 1:100, and 1:250 dilution ratios. No precipitation, flocculation, or phase separation was observed. The formulation remained clear to slightly opalescent, indicating good maintenance of the drug in the dispersed state after aqueous dilution.

Table 7: Dilution robustness of optimized acetazolamide SMEDDS

Medium	Dilution ratio	Clarity	Precipitation	Phase separation	Observation
Distilled water	1:50	Clear	Absent	Absent	Stable
Distilled water	1:100	Clear	Absent	Absent	Stable
Distilled water	1:250	Slightly opalescent	Absent	Absent	Stable
0.1 N HCl	1:50	Clear	Absent	Absent	Stable
0.1 N HCl	1:100	Clear	Absent	Absent	Stable
0.1 N HCl	1:250	Slightly opalescent	Absent	Absent	Stable
Phosphate	1:50	Clear	Absent	Absent	Stable

buffer pH 6.8					
Phosphate buffer pH 6.8	1:100	Clear	Absent	Absent	Stable
Phosphate buffer pH 6.8	1:250	Slightly opalescent	Absent	Absent	Stable

Transmittance (%T)

The optimized SMEDDS exhibited a high transmittance of $96.8 \pm 0.5\%$, consistent with its low globule size (78.4 nm) and PDI (0.221). The high %T indicated the formation of a clear and finely dispersed system and supported the successful optimization of the acetazolamide SMEDDS.

Overall, the optimized formulation demonstrated rapid self-emulsification, good thermodynamic stability, high drug content, satisfactory dilution robustness, and high optical clarity, confirming its suitability for further evaluation.

Morphological Characterization

TEM analysis of the optimized acetazolamide-loaded SMEDDS revealed predominantly spherical, well-defined, and uniformly distributed droplets. The observed droplet size ranged from 54.8–101.6 nm, with a mean size of 78.4 ± 5.8 nm.

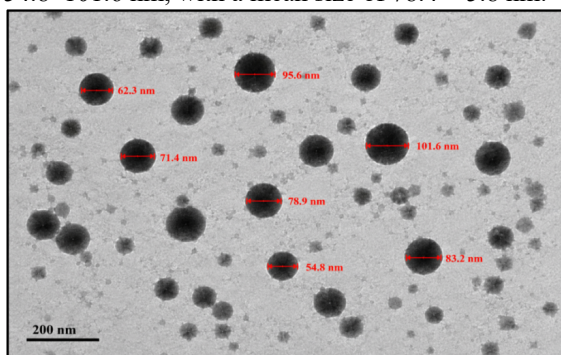


Figure 7: Transmission electron microscopic image of optimized acetazolamide-loaded SMEDDS

The droplets appeared as discrete rounded structures with clear boundaries and no prominent aggregation. The mean size was consistent with the DLS measurement (78.4 nm), supporting the formation of a fine nanosized dispersion. Overall, the TEM findings confirmed the spherical

morphology, uniform distribution, and nanoscale characteristics of the optimized SMEDDS.

In-vitro Drug Release from Developed L-SMEDDS

The *in-vitro* drug release profile of the optimized L-SMEDDS was evaluated in phosphate buffer pH 6.8. The formulation exhibited a rapid and progressive release of acetazolamide, increasing from 48.3% at 10 min to 96.8% at 60 min. The rapid release may be attributed to spontaneous formation of fine droplets upon dilution, resulting in improved drug solubilization and increased interfacial surface area. The results demonstrate the ability of L-SMEDDS to enhance the dissolution of acetazolamide compared with the pure drug.

Table 8: *In-vitro* drug release profile of optimized L-SMEDDS

Time (min)	Concentration released ($\mu\text{g/mL}$)	Drug release (%)
10	9.6	48.3
20	13.5	67.9
30	16.4	82.5
40	18.3	91.6
50	19.2	94.2
60	19.8	96.8

Overall, the optimized L-SMEDDS showed rapid and enhanced acetazolamide release, reaching 96.8% within 60 min, indicating improved dissolution performance of the developed lipid-based formulation.

Stability Study

The optimized L-SMEDDS was evaluated for stability under long-term ($25 \pm 2^\circ\text{C}/60 \pm 5\%$ RH) and accelerated ($40 \pm 2^\circ\text{C}/75 \pm 5\%$ RH) conditions for 3 months. The formulation showed only minor changes in globule size, PDI, and drug content during storage. Under long-term conditions, globule size increased from 78.4 to 80.6 nm, PDI from 0.221 to 0.231, while drug content remained high at 97.8% after 3 months. Under accelerated conditions, globule size increased to 83.1 nm, PDI to 0.241, and drug content remained 97.2%. These changes were relatively small, indicating satisfactory stability of the optimized L-SMEDDS during the study period.

Table 9: Stability study of optimized L-SMEDDS at $25 \pm 2^\circ\text{C}/60 \pm 5\%$ RH

Storage period	Globule size (nm)	PDI	Drug content (%)
Initial	78.4 ± 1.2	0.221 ± 0.008	98.6 ± 0.7
1 month	79.1 ± 1.3	$0.224 \pm$	98.3 ± 0.6

		0.009	
2 months	79.8 ± 1.4	0.227 ± 0.010	98.1 ± 0.7
3 months	80.6 ± 1.5	0.231 ± 0.011	97.8 ± 0.6

Table 10: Stability study of optimized L-SMEDDS at 40 ± 2°C/75 ± 5% RH

Storage period	Globule size (nm)	PDI	Drug content (%)
Initial	78.4 ± 1.2	0.221 ± 0.008	98.6 ± 0.7
1 month	80.2 ± 1.4	0.226 ± 0.009	98.0 ± 0.6
2 months	81.7 ± 1.5	0.233 ± 0.010	97.6 ± 0.7
3 months	83.1 ± 1.7	0.241 ± 0.012	97.2 ± 0.6

Overall, the optimized L-SMEDDS maintained its nanosized characteristics and high drug content throughout the 3-month study, with only slight changes under both storage conditions. The formulation therefore demonstrated satisfactory short-term stability.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

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