RESEARCH PAPER

QbD-Guided Formulation and Evaluation of Clove Oil-Based Glibenclamide Microemulsion for Mucoadhesive Buccal Patch Delivery

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ABSTRACT

Type II diabetes requires long-term drug therapy; however, oral glibenclamide is limited by poor solubility, erratic absorption, and high first-pass metabolism, affecting bioavailability and adherence. This study developed a QbD-guided clove oil-based glibenclamide microemulsion incorporated into a mucoadhesive buccal patch for sustained delivery and better compliance. Glibenclamide was quantified using a validated UV–visible method (R^2=0.9989/0.9993, accuracy 98–100%, precision %RSD<2, LOD 6.40 µg/mL, LOQ 19.17 µg/mL). FT-IR confirmed drug–excipient compatibility. Solubility screening identified 50 mg/mL clove oil as optimal. Pseudo-ternary phase diagrams showed the largest microemulsion region at a S_mix of 2:1. A factorial design evaluated oil and surfactant concentrations against particle size, drug load, and transmittance; analysis revealed a quadratic design space (oil 5–7%, Smix 60–70%). The optimized microemulsion was clear, stable, highly transmittant (~98.45%), with nanometric droplets (78–96 nm; PDI ~0.03), zeta potential −1.23 mV, high drug loading (~95%) and entrapment (91.35%), pH ~6.4, and low viscosity (~25 cP). The patch (Polyvinyl Alcohol and Polyvinylpyrrolidone (PVA:PVP 1:1 with Carbopol); solvent casting) was uniform (88.25–88.33 mg; 0.162–0.214 mm), flexible (250-fold endurance), buccal-compatible (pH ~6.7), and drug-uniform (~95%). It released the drug over 8 h (~74% in-vitro vs. ~88% marketed tablet), followed Korsmeyer–Peppas kinetics, had good ex vivo mucoadhesion, and ~60% permeation at 8 h without permeation enhancers, and remained stable for 2 months at 40°C/75% RH. Overall, QbD optimization produced a robust microemulsion-based buccal patch that may bypass first-pass metabolism, enable prolonged delivery, and improve type II diabetes treatment.

Keywords: Quality by Design (QbD); Microemulsion; Buccal patches; Glibenclamide; Mucoadhesive

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INTRODUCTION:

Diabetes mellitus (DM) is a chronic metabolic disorder with increasing global prevalence, burdening healthcare. It causes persistent high blood sugar due to insulin issues, disrupting carbohydrate, fat, and protein metabolism[1]. If unmanaged, it can lead to microvascular complications like retinopathy, nephropathy, and neuropathy, and macrovascular issues including coronary artery disease, stroke, and peripheral artery disease. These complications impair quality of life and increase mortality risk, emphasising the importance of blood

sugar control. Epidemiological data show DM has become epidemic[2]. The International Diabetes Federation reported about 463 million adults with diabetes in 2019, expected to reach 700 million by 2045. India, recognised as the "diabetes capital," recorded 77 million cases in 2019, with projections indicating an increase to nearly 101 million by 2030 [3]. Type II diabetes mellitus (T2DM) accounts for approximately 90–95% of all diagnosed cases and is strongly associated with obesity, sedentary behavior, and genetic predisposition. The chronic nature of T2DM demands lifelong pharmacotherapy along with lifestyle modifications [4]. Glibenclamide (GLB), also known as glyburide, belongs to the second generation of the oral anti-diabetic class of sulfonylureas. The chemical name of glibenclamide is 5-Chloro-N-(2-ethyl)-2-methoxybenzamide, and its chemical formula is C_{23} H_{28} ClN_3 O_5[5], which is more potent than the first-generation sulphonylureas [6]. Glibenclamide (glyburide) is a potent second-generation sulfonylurea that is extensively used in the management of T2DM. It lowers blood glucose by binding to sulfonylurea receptor-1 (SUR1) on pancreatic β-cells, closing ATP-sensitive potassium channels, leading to cell depolarization, opening of voltage-gated calcium channels, and subsequent insulin release[7]. Glibenclamide is more potent and lasts longer than first-generation sulfonylureas but has poor solubility, erratic absorption, and extensive first-pass metabolism, leading to low, variable bioavailability. Conventional doses often need multiple daily doses, which can reduce adherence[8]. Buccal drug delivery systems (BDDS) provide a promising alternative to oral administration for drugs vulnerable to enzymatic degradation or first-pass metabolism. The highly vascularized buccal mucosa allows rapid absorption and bypasses the gastrointestinal tract[9]. Benefits include easy, non-invasive administration, controlled release potential, and easy removal. The mucosa's lower enzymatic activity reduces drug degradation, making BDDS suitable for various therapies[10.] Microemulsion systems enhance the solubility and permeation of poorly water-soluble drugs like Glibenclamide. These thermodynamically stable, isotropic mixtures of oil, water, surfactants, and co-surfactants have droplet sizes of 10-100 nm. Their small droplets increase interfacial area, boosting drug solubilization and absorption. They also protect against degradation, improve dosing, and enable controlled release[11]. When Integrated into a mucoadhesive buccal patch, microemulsions enhance solubility and prolong residence time at the absorption site. The mucoadhesive polymer matrix ensures close contact with the mucosa, enabling sustained release and improved bioavailability. This system offers the convenience of once-daily dosing and potentially better patient compliance than traditional oral therapy[12]. Ding *et al*.(2022) developed a baicalin–clove oil microemulsion (BC-MEs) incorporated into a thermosensitive poloxamer gel (Gel-BC-MEs) for in situ periodontal therapy. The system provided sustained drug release, suitable gelation, and effective pH, whereas *in-vivo* studies in rats showed enhanced collagen regeneration and alveolar bone repair through reduced osteoclast activity. This study presents a promising nanogel reservoir strategy for periodontitis treatment [13]. Shaikh S. *et al*.(2021) developed and optimized Glibenclamide mucoadhesive buccal films (MBF) using a quality-by-design approach. Screening and factorial design identified critical factors influencing drug release, mucoadhesion, and permeability. The optimized formulation demonstrated desirable performance and aligned well with the Quality Target Product Profile (QTPP), ensuring accuracy and robustness [14]. Microemulsion-based formulations have been explored for drugs, but few studies combine microemulsions with mucoadhesive buccal patches for antidiabetic agents like glibenclamide. Conventional oral glibenclamide has limitations, prompting the need for new delivery systems that improve solubility, bypass first-pass metabolism, and sustain plasma levels. This study developed and characterised a microemulsion-loaded buccal patch of glibenclamide to enhance solubility, bioavailability, and therapeutic duration. Using a 3^2 factorial design, the formulation was optimised and evaluated through physicochemical, *in-vitro*, *ex-vivo*, and stability tests to assess clinical potential.

MATERIAL AND METHODS

Materials

The materials used in the present study included glibenclamide (procured from Swapnroop Drugs and Pharmaceuticals, Aurangabad), polyvinyl alcohol and polyvinyl pyrrolidone (PVP), Tween 20 (Loba Chem Pvt. Ltd., Mumbai), and propylene glycol, methanol, clove oil, sodium hydroxide, and sodium bicarbonate (Research-Lab Fine Chem Industries, Mumbai).

UV–Visible Spectrophotometric Analysis of Glibenclamide

Method Development

Qualitative and quantitative analyses of glibenclamide were performed in both methanol and phosphate buffer (PBS, pH 6.8) using a UV-visible spectrophotometer (Jasco model. V630, Japan). Standard stock solutions (100 µg/mL) were prepared by accurately weighing 10 mg of glibenclamide, dissolving it in the respective solvent, and making up the volume to 100 mL in a calibrated volumetric flask. Aliquots of 2–10 mL were diluted to 10 mL from these stock solutions to obtain calibration standards in the concentration range of 20–100 µg/mL. Each solution was scanned between 200 and 400 nm using the respective solvent as a blank. The λmax was 239 nm in methanol and 299 nm in PBS (pH 6.8). The absorbance values at the λmax were used to construct calibration curves, which demonstrated good linearity.

Method Validation: UV–visible spectrophotometry (Jasco model. V630, Japan) method was validated according to the ICH Q2 (R1) guidelines[15].

Linearity and Range: Calibration curves were obtained at 20, 40, 60, 80, and 100 µg/mL. Five aliquots of each concentration were analyzed, and linear regression analysis was performed[16]. Linearity was confirmed across the range, and regression statistics (slope, intercept, and R^2) were calculated.

Accuracy: Accuracy was assessed through recovery studies at three concentrations (20, 60, and 100 µg/mL) in triplicate[17]. Standard deviation (SD) and relative standard deviation (%RSD) were calculated. Accuracy was expressed as % predicted using Eq. (1):

$$\text{Accuracy} = \frac{\text{Predicted concentration} - \text{Actial Concentration}}{Actual\ Concentration} \text{X100} \ldots\ldots (1)$$

Precision: Precision was assessed at three concentrations (20, 60, and 100 µg/mL) in triplicate. Intraday precision measured repeatability on the same day; interday on three consecutive days[18]. Precision is expressed as % RSD using Eq. (2):

$$\%\ \text{RSD} = \frac{Standard\ Deviation}{Mean} \text{x100} \ldots\ldots (2)$$

Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD and LOQ were determined from the standard deviation of the Y-intercept (SD) and slope (S) of the calibration curve[19] Eq. (3) and (4):

$$LOD = 3.3\frac{SD}{S} \ldots (3)$$

$$LOQ = 10\frac{SD}{S} \ldots (4)$$

Ruggedness and Robustness: Ruggedness was evaluated by two analysts. The %RSD of the absorbance values was calculated[20]. The robustness was assessed by varying the detection wavelength (299 ± 0.5 nm). The %RSD was calculated to confirm the method reliability.

Drug–Excipient Compatibility Studies

Infrared (IR) spectroscopy was used to assess the compatibility between glibenclamide and the excipients. IR spectra of pure glibenclamide, excipients, and their mixtures were compared with a reference spectrum [21]. Characteristic peaks of glibenclamide (e.g., N–H, C=O, and S=O stretches) were analyzed for shifts, disappearance, or new peaks, indicating drug–excipient interactions. No significant spectral changes confirmed compatibility.

FORMULATION STRATEGY

Identification of Critical Material Attributes (CMAs) for Microemulsion Formulation

As per the Quality by Design (QbD) approach, the identification of suitable excipients is essential for defining critical material attributes (CMAs) that directly influence the critical quality attributes (CQAs) of the microemulsion[22]. Excipients for the glibenclamide-loaded microemulsion for buccal delivery were selected for their capacity to maximize drug solubility, improve permeability, and ensure safety (non-irritant).

Screening of the oil phase: Because the oil phase is a CMA that controls solubilization capacity, droplet size, and drug permeability, different oils were tested for glibenclamide solubility, including olive oil, castor oil, isopropyl myristate, clove oil, and oleic acid[23].

Procedure (risk-assessment driven): Approximately 1 mL of oil was accurately weighed, and 10 mg of glibenclamide was added, followed by ultrasonication to dissolve the drug. Additional aliquots of 10 mg were added until saturation was reached. The saturated solutions were filtered, and the drug content was measured spectrophotometrically at 299 nm. As shown in **Table 1**, clove oil demonstrated the highest

solubilizing capacity (50 mg/mL) and was thus designated as the optimal CMA for the oil phase in further formulation studies. Approximately 1 mL of oil was weighed again, and 10 mg of glibenclamide was added, followed by ultrasonication to facilitate dissolution. Once the drug was fully dissolved, an additional 10 mg of glibenclamide was added, and ultrasonication was continued. This process was repeated until a saturated solution was obtained. The total amount of dissolved drug was quantified using a UV-visible spectrophotometer (Jasco model. V630, Japan) at 299 nm. Among the tested oils, clove oil exhibited the highest solubilizing capacity for glibenclamide and was therefore selected as the oil phase for the microemulsion formulation[24].

Table 1. Solubility of Glibenclamide in Various Oils

Oils (mg/mL)	Drug solubility (10mg/mL) of oil	Surfactant (Saturated solution)
Olive oil	40	Tween 20
Castor oil	35	Tween 80
Isopropyl Myristate	40	Span 20
Clove oil	50	Labrasol
Oleic acid	30	Cremophor

Screening of Surfactant and Co-Surfactant

Surfactants and co-surfactants are additional CMAs that affect CQAs, such as droplet size distribution, stability, and drug release. The solubility of glibenclamide in different surfactants (Tween 20, Tween 80, Span 20, Labrasol, and Cremophor) and co-surfactants (PEG 400, propylene glycol, and isopropyl alcohol) was determined using the same method described for oils. Surfactants and co-surfactants with the highest solubilizing potential were selected as critical excipients for microemulsion optimization.

Construction of the pseudo-ternary phase diagram:

To determine the best core composition for the microemulsion formulation, oils, surfactants, and cosurfactants were evaluated and grouped into three different combinations. The surfactant and co-surfactant (Smix) were mixed at various volume ratios (1:1, 2:1, and 3:1). These ratios were selected to represent varying proportions of the co-surfactant relative to the surfactant, thereby enabling a systematic assessment of their influence on microemulsion formation. Pseudo-ternary phase diagrams were created using aqueous-phase titration. In this process, mixtures of oil and Smix were prepared in different volume ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1) and stirred for 5 min until they were homogeneous. Each mixture was gradually titrated with water while being stirred gently. The endpoint was marked by the appearance of turbidity or bluish opalescence, indicating the boundary between the microemulsion and non-microemulsion phases. Transparent systems with low viscosity were considered to be in the microemulsion region. Pseudo-ternary phase diagrams were plotted using the CHEMIX ternary plot software, and the microemulsion zones were identified for subsequent formulation development[25 26].

Optimization of Glibenclamide Microemulsion Using 3^2 Full Factorial Design

A 3^2 full factorial design (FFD) with two factors at three levels, requiring nine experiments, was used to optimize the glibenclamide microemulsion (ME) formulation. The study employed Design-Expert software (version 7.0; Stat-Ease Inc., Minneapolis, MN, USA)27. Two key material attributes (CMAs) were chosen as independent variables: oil concentration (X1) and Smix concentration (X2) [28.] Both variables were studied at three levels (low, medium, and high), as shown in **Table 2.**

Table 2. Independent Variables in 3^2 Factorial Design and Their Levels

Independent Variables	Low (-1)	Medium (0)	High (+1)
Oil Concentration (%) X1	5	7	9
Smix Concentration (%) X2	60	65	70

The formulation performance was evaluated using three critical quality attributes (CQAs).

i) Particle size (R1), ii) Drug loading (R2) iii) % Transmittance (R3)

These responses were selected for their critical roles in ensuring microemulsion stability, solubilization efficiency and optical clarity. The design constraints for the responses are summarized in **Table 3.**

Table 3. Responses in 3^2 factorial design and their constraints

Responses	Constraints
Particle size (R1)	Minimize
Drug loading (R2)	Maximize
% Transmittance (R3)	Maximize

The rationale for adopting a three-level factorial design was to:

Capture curvature effects in the response surface and assess potential quadratic relationships between formulation variables and responses. Offer a systematic approach for multi-response optimization consistent with the QbD principles. Based on the design matrix, nine experimental batches of Glibenclamide ME were prepared and evaluated for the chosen responses. The collected data were statistically analyzed and used for model fitting with the Design-Expert software to determine the optimal formulation conditions[29].

Formulation of Glibenclamide Buccal Patch

A microemulsion-loaded glibenclamide mucoadhesive buccal patch was fabricated using the solvent casting method. A trial-and-error process, guided by the QbD framework, was employed to select the optimal polymer blend and excipient ratio, ensuring clarity, flexibility, and strong mucoadhesion. Polyvinyl alcohol (PVA) was chosen as a hydrophilic polymer to provide elasticity and a smooth texture. Polyvinyl pyrrolidone (PVP) served as the film-forming agent to improve mechanical strength[30]. Carbopol was included as a mucoadhesive polymer to enable prolonged contact with the buccal mucosa31. A consistent volume (5 mL) of glibenclamide-loaded microemulsion was incorporated into the polymer solution for drug loading. The formulations were cast into films, dried, and evaluated for transparency, uniformity, and physical stability. The details of the formulations and the results from the trial batches are summarized in **Table 4.**

Table 4. Trial Batches for Buccal Patch Formulation

Formulation Code	PVA (%)	PVP (gm)	Carbopol (mg)	ME (mL)	Solvent	Result
F1	2.0	1.5	50	5	Water	Fail
F2	2.0	1.2	50	5	Water	Fail
F3	1.5	1.2	50	5	Water	Fail
F4	1.5	1.0	40	5	Water	Fail
F5	**1.0**	**1.0**	**20**	**5**	**Water**	**Pass**

The key finding was that higher PVA concentrations caused turbid and brittle patches, showing poor polymer compatibility at elevated ratios. An optimal PVA to PVP ratio of 1:1, combined with 20 mg of Carbopol and 5 mL of microemulsion, resulted in a transparent, flexible, and stable patch (F5). This formulation was chosen for further testing and analysis.

CHARACTERIZATION PARAMETERS OF GLIBENCLAMIDE MICROEMULSION

Clarity: Clarity was observed visually.

Centrifugation: The microemulsion system was centrifuged at 3000 rpm for 15 minutes.

Measurement of % Transmittance: Percent transmittance indicates the clarity and transparency of the formulation and was measured as % transmittance. The microemulsion was accurately diluted with 10 mL of double-distilled water. Using water as a blank, the sample's transmission was tested with a UV-visible spectrophotometer (Jasco model. V630, Japan) at 299 nm.

Particle Size and Polydispersity index (PDI) and Zeta potential

Particle size and polydispersity index (PDI) of the enhanced glibenclamide microemulsion were measured using a particle size analyzer (NANOPHOX, Sympatec, Germany)[32]. The measurements used double-distilled water as the dispersion medium at a 90° scattering angle and maintained at 25 °C. 27 The zeta potential of the samples was determined using a Zetasizer (Delsa Nano C, Micromeritics, USA). For these measurements, the samples were placed in clear disposable zeta cells, and the results were recorded[33].

Viscosity Measurement

Viscosity is an essential parameter for assessing the rheological properties of a microemulsion. The viscosity of the optimized formulation was measured using a Brookfield viscometer with spindle no. 21 at 25 °C, in accordance with the manufacturer's standard procedures[34].

pH

The pH of the microemulsions was measured at 25 °C using a digital pH meter (Elico India). All measurements were performed in triplicate.

Drug loading

The drug loading of the microemulsion was determined using a UV-visible spectrophotometer (Jasco model V630, Japan) at 299 nm; 1 mL of ME formulation was diluted up to 10 mL with pH buffer 6.8 and centrifuged at 15000 rpm at 2 5 ± 0. 0 1°C for 20 min using a centrifuge. The supernatant was separated and filtered, and 1 mL of the supernatant was taken from the solution and diluted with 10 mL of phosphate buffer (pH 6.8)[5]. Drug loading was calculated using the formula:

$$\text{Drug Loading } (\%) = \frac{WA - WF}{(WA - WF) + W0} \text{X100}$$

WA, amount of drug loaded in the ME; Wo, amount of oil added in the ME; Wf, amount of free drug

EVALUATION OF MICROEMULSION-BASED BUCCAL PATCHES

Physical appearance

All the prepared buccal patches were visually inspected for color, transparency, smoothness, flexibility, and overall uniformity.

Weight uniformity

Four patches from each formulation batch were weighed individually using a calibrated digital balance. The average weight and standard deviation were calculated for each batch to assess weight uniformity[35].

Thickness measurement

The thickness of each buccal patch was determined using a digital vernier caliper (Multicomp PRO MP012475). Measurements were taken at five locations: the four corners and center of the patch. The average thickness and standard deviation were then calculated[36].

Folding endurance

Folding endurance was measured by manually repeatedly folding a 2 mm × 2 mm section of the patch at the same spot until it tore. The total number of folds before breaking was noted as the folding endurance value[35].

Surface pH determination

Surface pH was determined by allowing the patches to swell on the surface of agar gel (2% w/v prepared in phosphate buffer pH 6.8) for 2 h at room temperature. A calibrated digital pH meter (Elico India) was used to measure the pH of the swollen patch surface, and the readings were recorded. The procedure was performed three times[37].

Drug content uniformity

A 2 × 2 cm² section of each patch (without backing membrane) was placed in 100 mL of simulated saliva solution (pH 6.8) and kept under occasional shaking for 12 h to allow complete dissolution. The solution was filtered and analyzed spectrophotometrically at 299 nm to determine drug content[36].

Swelling index

Each patch was initially weighed (W1) and placed in a Petri dish containing phosphate buffer (pH 6.8). After predetermined time intervals, the patch was removed, the surface water was blotted with filter paper, and the swollen patch was reweighed (W2) [38]. The swelling index (SI) was calculated using the following formula:

$$Swelling\ index = \frac{W2 - W1}{W1}$$

***In-vitro* drug release**

An in vitro release study was performed using a USP Type II dissolution apparatus (paddle method). The buccal patch, with its backing layer attached to a glass slide, was fixed to the paddle using an adhesive. The dissolution medium consisted of 250 mL phosphate buffer pH 6.8), maintained at 37 ± 0.5 °C, and stirred at 50 rpm. At predetermined intervals, 1 mL aliquots were withdrawn and replaced with fresh medium. The samples were filtered and analysed at 299 nm using a UV-visible spectrophotometer (Jasco model. V630, Japan) [39].

***Ex-vivo* mucoadhesive strength**

Mucoadhesive strength was evaluated using a modified two-arm balance method. Fresh porcine buccal mucosa was fixed to the base of a Petri dish with the mucosal side exposed and moistened with simulated saliva (pH 6.2). A glass disc with a buccal patch attached was brought into contact with the mucosal surface for 5 min under a 5 g preload. Weights were gradually added to the opposite pan until detachment occurred [40]. 34 Mucoadhesive strength was calculated as the weight required for detachment, and the mucoadhesive force was determined using the following equation:

$$Mucoadhesive\ force\ (kg/m/s) = \frac{Mucoadhesive\ strength\ X\ g}{1000}$$

***Ex-vivo* permeation study**

Ex-vivo drug permeation was studied using a modified Franz diffusion cell with freshly excised porcine buccal mucosa mounted between the donor and receptor compartments. The buccal patch was placed on the mucosal surface in the donor compartment, which was moistened with 1 mL of simulated saliva (pH 6.2). The receptor compartment contained phosphate buffer pH 6.8), maintained at 37 ± 0.2 °C, and stirred at 50 rpm. At predetermined intervals, samples were withdrawn from the receptor compartment, replaced with fresh buffer, and analyzed spectrophotometrically at 299 nm[41].

Stability studies :

The optimized formulation was subjected to stability testing in accordance with ICH guidelines. Patches were stored at 40 ± 2 °C and 75 ± 5% relative humidity in a stability chamber. Samples were withdrawn at one-month intervals for up to 2 months and evaluated for physical appearance, drug content, and drug release profile [42].

RESULT AND DISCUSSION

Development of UV–Visible Spectrophotometric Method

The UV absorption spectrum of Glibenclamide (GLB) exhibited a distinct maximum at 229.5 nm. This wavelength was chosen for all future analyses. The method was validated following ICH guidelines, assessing parameters such as linearity, accuracy, precision, LOD, LOQ, ruggedness, and robustness.

Calibration curve in methanol and Phosphate buffer

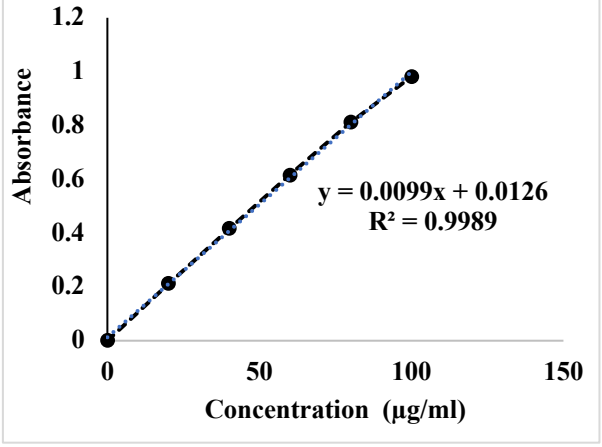
A linear calibration curve was obtained for GLB in methanol within the concentration range of 0–100 µg/mL, showing the regression equation y = 0.0099x + 0.0126 with a correlation coefficient (R^2 = 0.9989), indicating excellent linearity. Similarly, in phosphate-buffered saline (PBS, pH 6.8), GLB also exhibited good linearity over the same concentration range with the regression equation y = 0.0099x + 0.0005 and a correlation coefficient (R^2 = 0.9993). The calibration data are presented in **Table 5,** with the corresponding calibration curves depicted in **Figure 1a** and **1b.**

Table 5. Calibration Data of Glibenclamide

Concentration (µg/mL)	Methanol Absorbance (nm)	Phosphate buffer Absorbance (nm)
0	0.000 ± 0.000	0.000 ± 0.000
20	0.210 ± 0.002	0.198 ± 0.002
40	0.409 ± 0.003	0.389 ± 0.003
60	0.606 ± 0.004	0.585 ± 0.003
80	0.801 ± 0.003	0.781 ± 0.004
100	0.999 ± 0.002	0.979 ± 0.002

Mean ± S.D (n=3)

a



b

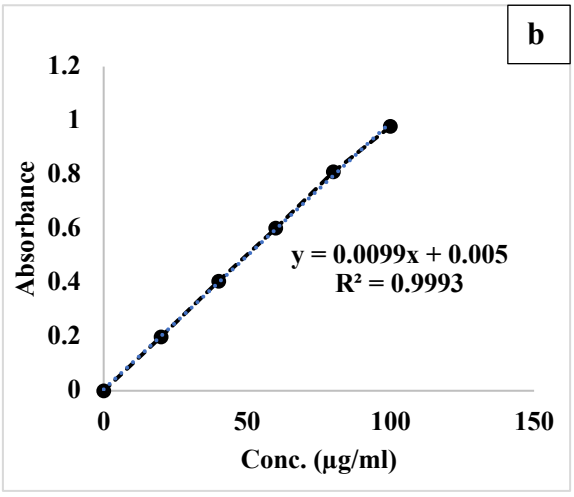


Figure 1. Calibration curve of Glibenclamide in a) Methanol, b) Phosphate buffer

a) Accuracy

Accuracy was assessed at three concentrations (20, 60, and 100 µg/mL). The percentage recovery was found to be between 98–100%, confirming the reliability of the method.

b) Precision:

Precision was assessed based on intra-day and inter-day variations. In both studies, the %RSD values were below 2% (**Table 6** and **7),** indicating the method's high reproducibility.

Time	Actual concentration		
	LQC (20 µg/ mL)	MQC (60 µg/ mL)	HQC (100 µg/ mL)
	Predicted concentration (µg/ml)		
10.00 am	27.19 ± 0.008	66.55 ± 0.03	96.60 ± 0.01
12.00 am	27.28 ± 0.04	66.54 ± 0.02	95.11 ± 0.05
2.00 am	26.50 ± 0.5	67.53 ± 0.08	98.06 ± 0.1
Mean	26.99	66.87	97.25
S.D	0.42	0.36	1.8
% RSD	1.58	0.85	1.9
% Accuracy	98.98	99.04	99.52

Table 6. Intraday Precision (%RSD) and Accuracy Results for Three Determinations for Estimation of Glibenclamide in a Day.

Mean ± S.D (n=3)

Table 7. Inter-Day Precision (%RSD) and Accuracy Results for Three Determinations for Estimation of Glibenclamide on Different Days.

Day	Actual concentration		
	LQC (20µg/mL)	MQC (60µg/mL)	HQC 100µg/mL)
	Predicted concentration (µg/mL)		
1	25.80 ± 0.001	67.76 ± 0.08	98.14 ± 0.05
2	26.14 ± 0.07	67.65 ± 0.03	98.61 ± 0.1
3	25.90 ± 0.02	67.50 ± 0.09	98.50 ± 0.1
Mean	25.94	67.63	98.41
S.D	0.17	0.13	0.24
% RSD	0.67	0.19	0.249
% Accuracy	99.31	99.81	99.80

Mean ± S.D (n=3)

e) Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ, derived from the calibration curve slope and standard deviation, were determined to be 6.40 µg/mL and 19.17 µg/mL, respectively. These results demonstrate the effectiveness of the method for detecting and measuring GLB at low levels.

f)Ruggedness and Robustness

Ruggedness was validated through analyses conducted by different analysts, while robustness was assessed at slightly different detection wavelengths (297, 299, and 301 nm). In both scenarios, the %RSD was less than 2% (**Tables 8** and **9**), indicating that small operational variations did not substantially affect the results.

Table 8. Ruggedness Method for Glibenclamide

Ruggedness					
Analyst	**Conc.**	**Absorbance**	**Mean**	**S.D**	**% RSD**
1	60	0.6745	0.674		
	60	0.6729			
	60	0.6772			
2	60	0.6734	0.674		
	60	0.6763			
	60	0.6743			

Table 9. Robustness Method for Glibenclamide

	301 nm		
Absorbance	0.678		
	0.6716		
	0.6695		
Mean	0.673033	0.676833	0.662367
S.D	0.004428	0.00179	0.001818
% RSD	0.657852	0.264435	0.2739

The UV-Vis spectrophotometric method developed for Glibenclamide was found to be simple, accurate, precise, and robust, fulfilling all ICH validation parameters. Linearity with high correlation coefficients ($R^2 > 0.998$), recovery values close to 100%, low %RSD values in precision studies, and acceptable LOD/LOQ demonstrate the reliability of the method. These findings confirm that the method is suitable for the routine analysis of GLB in bulk drugs and formulations.

Drug-Excipient Compatibilities Studies

Research is needed to determine which drugs interact with excipients. Because vibrational shifts serve as a probe for potential intermolecular interactions among the components, FT-IR can be used as a compatibility screening method. The FT-IR spectra in **Figure 2** and **3** show no difference in the functional group peaks of the drugs in the physical mixtures, as they were unaltered, indicating chemical compatibility. Furthermore, **Table 10** lists the characteristics of the functional groups.

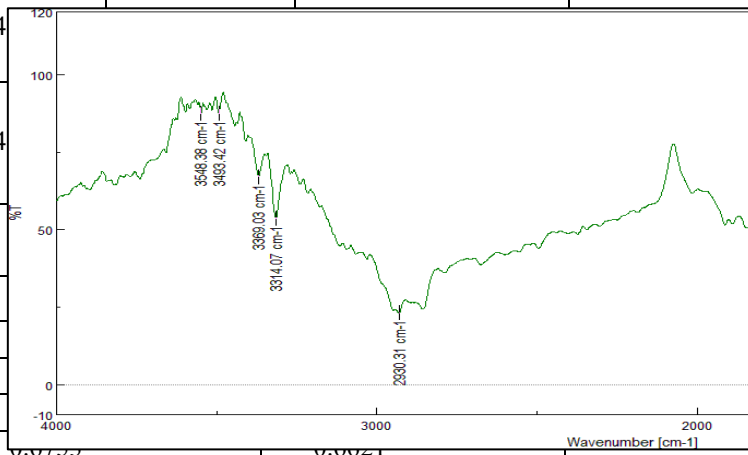


Figure 2. FT-IR spectra of Physical mixture (Glibenclamide + PVA)

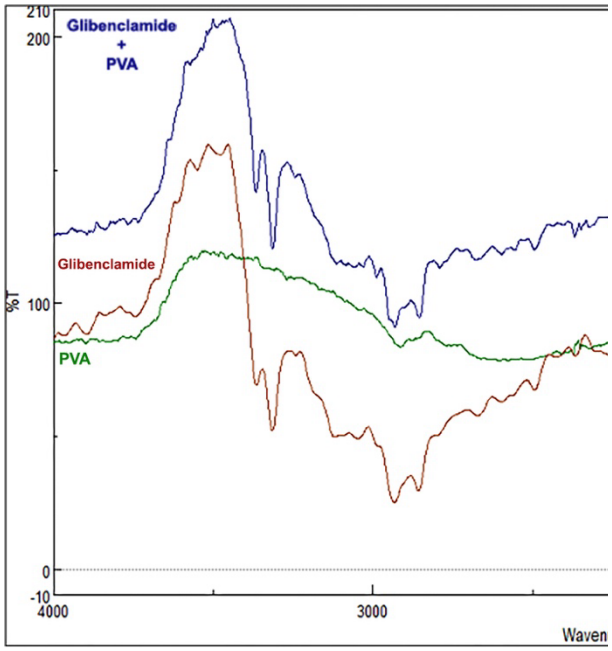


Figure 3. FT-IR Glibenclamide + PVA, (green) PVA, (red) Glibenclamide

Table 10. List of Functional Groups and Frequency

r. No	Functional group (GLI)	Frequency (cm^{-1})	Functional group(PVA)			
1	N-H	3314	O-H			
2	C-H	2936	CH2			
3	O-H	2857	C=O	1618	C=O	1694
4	N=O	1552	C-O	1304	C-N	1291
5	C-N	1248	C-O-C	1027	C-C	1021
6	C-O	1023	C-C	820	N-C=O	

Formulation Development

Pseudo-Ternary Phase Diagram Studies

Pseudo-ternary phase diagrams were successfully constructed for three Smix ratios (1:1, 2:1, and 3:1) using clove oil as the oil phase, Tween 80 as the surfactant, and PEG 400 as the co-surfactant. The diagrams reveal clear differences in the size of the microemulsion region depending on the S_mix ratio. The largest isotropic microemulsion region was obtained with an S_mix ratio of 2:1, indicating an optimal balance between the surfactant and co-surfactant for efficient interfacial film formation and stabilization. The S_mix ratio of 1:1 exhibited a smaller microemulsion area, likely due to the insufficient surfactant concentration to effectively reduce the interfacial tension. Conversely, at a 3:1 ratio, the microemulsion region also decreased, which may be attributed to the reduced fluidity of the interfacial film caused by the lower co-surfactant content. For all three ratios, transparency and flowability were maintained until the aqueous titration point, where the system became turbid or bluish, marking the boundary between the microemulsion and biphasic regions. The phase behavior trends obtained are consistent with previous studies, confirming that an appropriate surfactant–co-surfactant balance is critical for maximizing the microemulsion region, as shown in **Figure 4.**

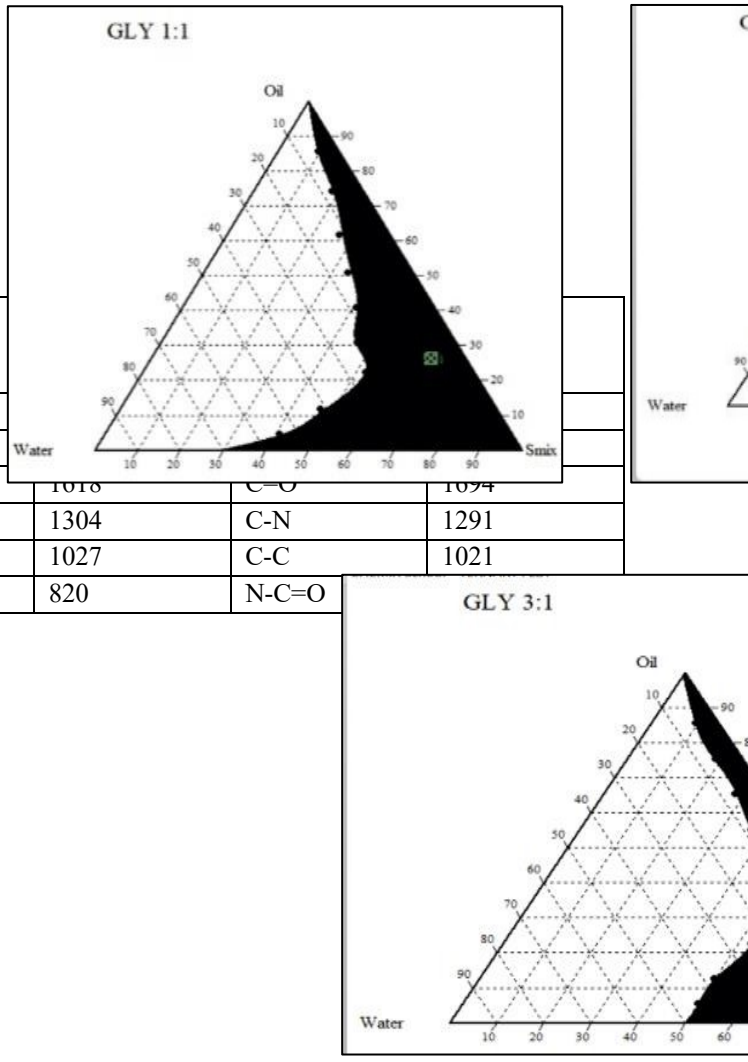


Figure 4. Pseudo-ternary phase diagrams of the oil-S mix-water system at K_m varied as 1:1, 2:1 and 3:1

Optimization of Glibenclamide Microemulsion

Design Expert software (version 7.0) was used to study the effect of independent variables on

responses. The experimental design layout developed for the nine possible combinations of Glibenclamide micro emulsions is shown in **Table 11-13**. Quadratic and cubic were fitted to the data, and the model that fit well was suggested by software and tested for analysis of variance (ANOVA). Regression polynomials were calculated for the individual dependent variables and contour plots and 3D surface graphs were obtained for each dependent variable. Mathematical models were generated for each individual dependent variable or response (R) and are expressed as Equations (3.3)-(3.5). The main effects (X1 and X2) represent the average results of changing one factor at a time from its low to high value. The interaction terms (X1 and X2) indicate how the response changes when two factors are changed simultaneously. A positive coefficient indicates an antagonistic effect on the response. Using the ANOVA provision available in the software, polynomial equations involving the main effects and interaction factors were determined based on the estimation of various statistical parameters. Finally, the software suggested quantities of oil and Smix for the preparation of ME were found 5-7%, 60-70% respectively. Using the ANOVA provision available in the software, the polynomial equations involving the main effects and interaction factors were determined based on the estimation of various statistical parameters. Accordingly, the F- values for R1, R2, and R3 were 9.26, 15.57, and 39.20, respectively, which implied that the quadratic model selected was significant for all responses. Moreover, values of "prob > F" < 0.05 indicate that the model terms are significant. Response surface analysis plots in a three-dimensional model graph were constructed using the software.

Table 11. Experimental Design Layout of Glibenclamide Microemulsion

Run	Batch Code	Factor X1 = Clove oil X2= Tween 20 & PEG 400		Response 1	Response 2	Response 3
		In Percentage		Particle Size (nm)	Drug Loading (%)	ercentage ransmittance (%)
1	B1	9	65	104.00	91.73	85.29
2	B2	7	60	90.00	93.45	95.33
3	B3	7	65	95.87	93.45	96.22
4	B4	9	70	95.80	91.73	88.46
5	B5	5	70	88.00	95.23	98.67
6	B6	5	60	78.00	95.23	86.99
7	B7	7	70	92.00	93.41	98.85
8	B8	5	65	95.85		
9	B9	9	60	95.20		

Table 12. Model Summary Statistics

Models	Response (R1)	Response
R^2	0.9392	1
Adjusted R^2	0.8377	0.999
Predicted R^2	0.4320	0.996
SD	2.88	0.015
Remark suggested	Quadratic	Quadratic

Table 13. ANOVA for Response Surface Quadratic Model

Model Term	R1		R2	
	F value	P value	F value	
	9.26	0.0482	15.50	0
A	22.05	0.0183	77.51	0
B	3.19	0.1723	1.12	0
AB	2.66	0.2014	0.00	1
A^2	8.24	0.9334	15.84	0
B^2	18.40	0.0233	0.38	0

($p < 0.0500$ indicates that the model terms are significant)

Effect of Formulation Variables on Particle Size

The surface plot of particle size indicated that an increase in the ratio of the (oil phase) resulted in a proportional increase in particle size because of the simultaneous decrease in the Smix proportion. Applying factorial design, the quadratic model was suggested by the software and found to be significant, with a model F value of 9.26 and p-value $F < 0.05$ for each term, indicating that every model term was significant. In this case, X1 and X2 are significant model terms, and the model for the response Y1 (particle size) is as follows: Particle size: **$Y1=+98.45+5.53 * A+2.10 * B-2.35 * A * B+0.18 * A^2-8.74 * B^2$**

A positive sign before a factor in polynomial equations indicates that the response increases with the factor, whereas a negative sign indicates that the response and factor have a reciprocal relationship. Independent factors, such as A: oil and B: Smix, significantly affect the particle size. From the above equation, it can be concluded that the particle size in nm (R1) had a positive effect on conc. of oil and the concentration of Smix, as shown in **Figure 5.**

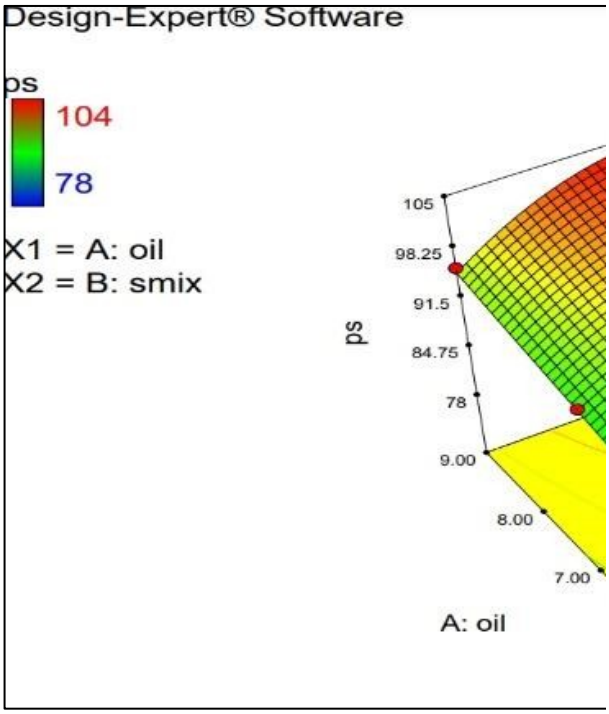


Figure 5. Response surface plot three-dimension showing effect of (a) Clove oil (oil) (A) and Tween 20 : PEG 400 (S_{mix}) (B) b) S_{mix} (B) & (c) A) oil on particle size (R1)

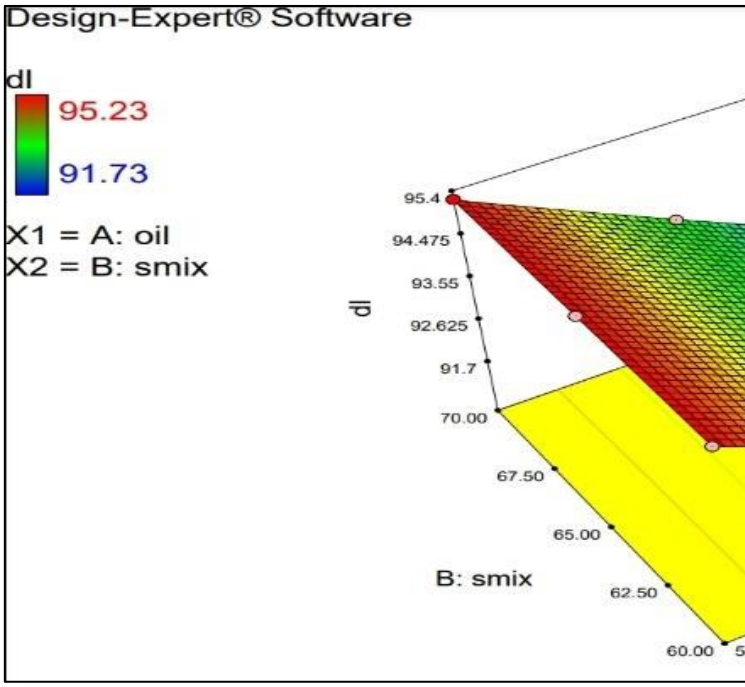


Figure 6. Response Surface Plot Three-Dimension showing effect of (a) Clove oil (oil) (A) and Tween 20 : PEG 400 (S_{mix}) (B) b) S_{mix} (B) & (c) A) oil on drug loading (R2)

b) Effect of Formulation Variables on Drug Loading

The drug loading of the formulation affects its solubility and uniformity. The counterplot of drug loading showed that an increase in the concentration of the oil phase (clove oil) resulted in a proportional increase in drug loading and no effect of Smix. The results of drug loading are shown in **Figure 6.** Applying factorial design, the software suggested a quadratic model, which was found to be significant with a model F value of 15.50. Values of "prob >F" less than 0.05 for each term were obtained, which indicates that every model was significant. In this case, X1 and X2 were significant model terms, and the model for response Y2 (drug loading) was as follows:Drug loading:**$Y2=+93.44-1.75 * A-0.033* B+0.000 * A * B+0.043 * A^2-2.666 * B^2$**

c) Effect of Formulation Variables on % Transmittance

The absorbance of the Microemulsion was measured against distilled water using a UV-visible spectrophotometer (Jasco model V630, Japan) at 650 nm. A formulation with a higher %transmittance was selected, which is indicative of an isotropic and clear formulation. The counterplot of % T showed that increasing the concentration of the oil phase (clove oil) resulted in a proportional decrease in %T because of the simultaneous decrease in the Smix proportion. The results for % T are shown in **Figure 7**. By applying factorial design, the quadratic model was suggested by the software and found to be significant, with a model F value of 39.20, p value< 0.00562, and R^2 value of 0.9849, which implies that the model was significant. Values of "probe >F" less than 0.05 for each term were obtained, which indicates that every model term was significant. The model for response Y3 (% Transmittance) is as follow:

% Transmittance: **Y3=+97.43 -7.32 * A +2.78 * B +2.45 * A * B -5.90 * A2 -0.95 * B2**

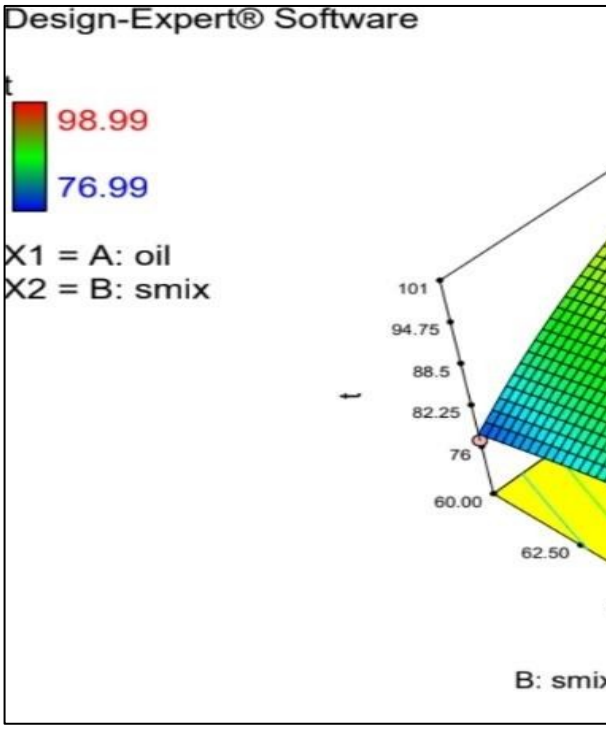


Figure 7. Response Surface Plot Three-dimension showing effect of (a) Clove oil (oil) (A) and Tween 20 : PEG 400 (S_{mix}) (B) b) S_{mix} (B) & (c) A) oil on% Transmittance (R3)

Selection of Optimized Batch

Three solutions were suggested by the software based on desirability, which was close to one, to obtain the best optimized batch. The total desirability was defined as the geometric mean of the individual desirability for particle size, % drug loading, and % T. The desirability lies between 0 and 1 and represents the closeness of a response to its ideal value. This value indicated that the predicted responses were obtained according to the expected results, as shown in **Table 14.**

Table 14. Formulation and its Overall Desirability

Drug (mg)	Oil (%) (A)	Smix (%) (B)	Water (%) (C)	Particle Size (nm) (R1)	%Drug loading (R2)	% T (R3)	Desirability
5	5	70	25	86.47	95.22	98.4556	0.869
5	7	70	23	98.81	93.42	100.372	0.610
5	9	60	31	93.32	91.73	78.2622	0.031

Mean ± SD, n=3

Validation of Model

The reliability of the developed model was evaluated by experimentally determining the responses for the optimized trials, along with several random trials covering the entire range of the experimental domain. The variables of checkpoints, predicted and experimental values of all response variables, and % prediction error in prognosis are listed in the **Table 15**. The % prediction error was found to be < 4%. The observed values of R1, R2, and R3 were in close agreement with the predicted values. Thus, the validity of the optimization procedure was proven. This indicates that the model used was reliable. The optimized batch, as shown in **Table 16,** was selected based on desirability, which should be close to 1.

Table 15. Comparative Levels of Predicted and Observed Responses

Factors		Predicted values			Experimental va	
A	B	R1	R2	R3	Y1	Y2
5	70	86.47	95.22	98.45	89.5	95.23
7	70	98.81	93.42	100.3	95.76	93.44
9	60	93.32	91.73	78.26	95.79	91.73

$$\% \text{ Prediction error} = \frac{\text{Predicted value} - \text{Experimental value}}{Predicted\ value} \text{x} 100$$

Table 16. Optimized Batch of Glibenclamide Microemulsion

Sr No.	Excipients	Quantity
1	Glibenclamide (Drug)	5.00 mg
2	Clove oil (Oil)	0.35 gm
3	Tween 20 (Surfactant)	1.75 gm
4	PEG 400 (Cosurfactant)	1.75 gm
5	Water	1.15 mL

CHARACTERIZATION

Evaluation of Optimized Glibenclamide Microemulsion:

The optimized microemulsion was clear and transparent, showing no signs of creaming or phase separation during storage or after centrifugation, which confirms its physical stability and isotropic nature. It demonstrated a high percent transmittance of 98.45 ± 0.00%, indicating excellent optical clarity and suggesting that nanometric droplets were evenly dispersed throughout the continuous phase. Dynamic light scattering revealed an average globule size of 95.76 ± 2.50 nm, within the typical microemulsion droplet size range, supporting improved dissolution and permeation. The polydispersity index was extremely low at 0.03 ± 0.00, indicating a narrow size distribution and a homogeneous droplet population, which is beneficial for consistent drug release and stability. The system's low viscosity of 25 ± 0.00 cP allows easy pouring, spreading, and buccal application while maintaining structural integrity. The pH was 6.4 ± 0.00, close to physiological buccal pH, thereby reducing mucosal irritation risk. The formulation had a high drug loading of 95.23 ± 0.00%, confirming efficient solubilization of the active ingredient without drug precipitation. A slightly negative zeta potential of -1.23 mV, as depicted in **Figure 8,** indicates that the droplets have a net negative surface charge; combined with non-ionic surfactants, this suggests that steric stabilization is sufficient to ensure colloidal stability of the microemulsion.

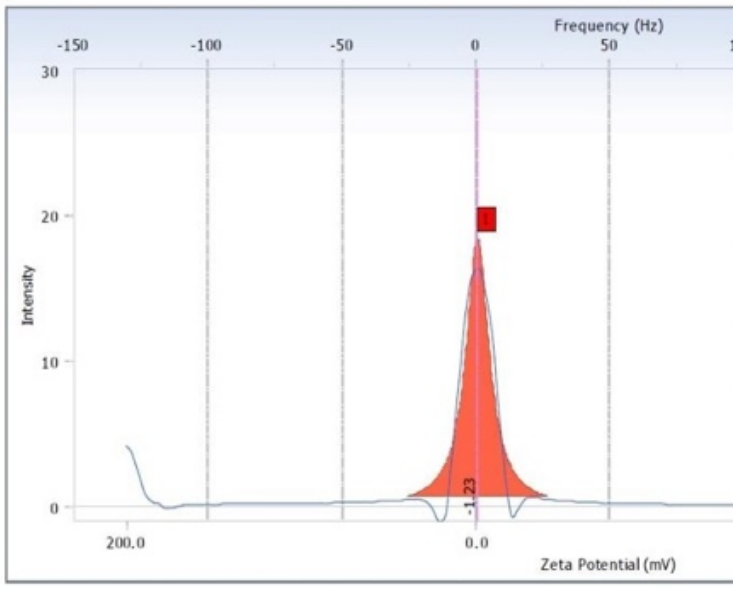


Figure 8. Zeta Potential of Optimized Batch

Microemulsion-Based Buccal Patch

The prepared buccal films displayed a smooth, uniform surface, indicating effective film-forming ability and even distribution of formulation components. The narrow weight range (88.25–88.33 mg) and thickness range (0.162–0.214 mm) confirmed consistent casting, which is crucial for accurate dosing, reliable mechanical properties, and controlled drug release. The films exhibited excellent flexibility, with a folding endurance of 250 ± 0.00, indicating that they can be handled routinely without cracking. A surface pH of 6.7 ± 0.00, close to the natural pH of the buccal mucosa, suggests a low risk of irritation and good compatibility. Additionally, a drug content of 95.10 ± 0.00% indicates effective drug loading and uniform distribution within the polymer matrix. Swelling tests showed that the PVA–PVP buccal patches absorbed water slowly, with formulation F5 achieving the highest swelling index at 30 min, likely due to the specific combination of 1% PVA and 1 g PVP, which facilitated proper hydration and improved drug permeation through the buccal mucosa, as detailed in **Table 17.**

Table 17. % Swelling Index of GLI buccal patch

Sr. No	Time (Min)	% Sw
1	5	2.27
2	10	4.54

3	15	7.95
4	20	10.22
5	25	13.63
6	30	15.90

***In-vitro* drug release**

The release behavior of the prepared buccal patch was assessed and compared with that of a marketed tablet (**Figure 9**). The marketed tablet showed a quicker release, reaching about 88% of the drug release after 8 hours. Meanwhile, the patch displayed a slower, more controlled release, with roughly 74% released in the same period. In the initial 0–2 h, both systems released the drug similarly; however, after 3 h, the tablet continued to release the drug faster than the patch. The sustained release from the buccal patch indicates its potential for prolonged drug delivery, which may help reduce dosing frequency and enhance patient compliance compared to traditional tablets.

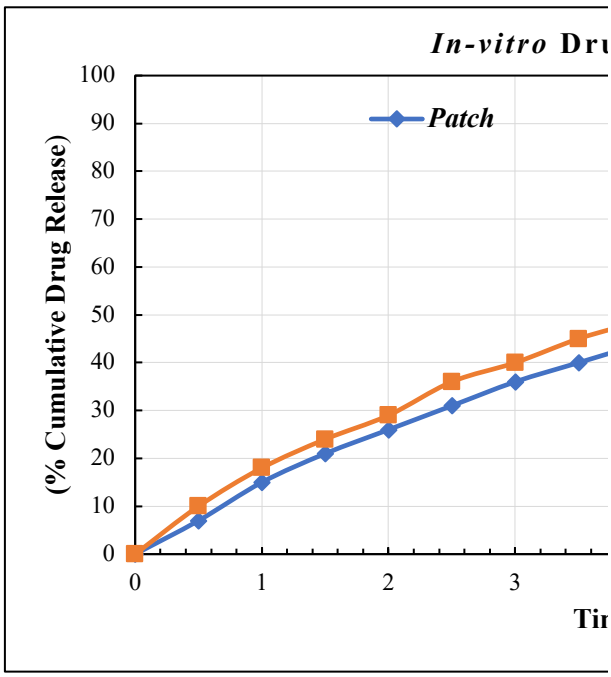


Figure 9. *In-vitro* release kinetics of Glibenclamide from buccal patches.

The values of the regression coefficients for the different models for the glibenclamide buccal patch are listed in **Table 18.** The regression coefficient value was found to be the highest for the Korsmeyer–Peppas model in the case of the glibenclamide patch; hence, the release mechanism was found to be the Korsmeyer–Peppas model, as depicted in **Figure 10.**

Table 18. Data for Kinetic Models for GLI Buccal Patch

Sr No.	Kinetic model
1	Zero order
2	First order
3	Higuchi
4	Korsmayer peppas

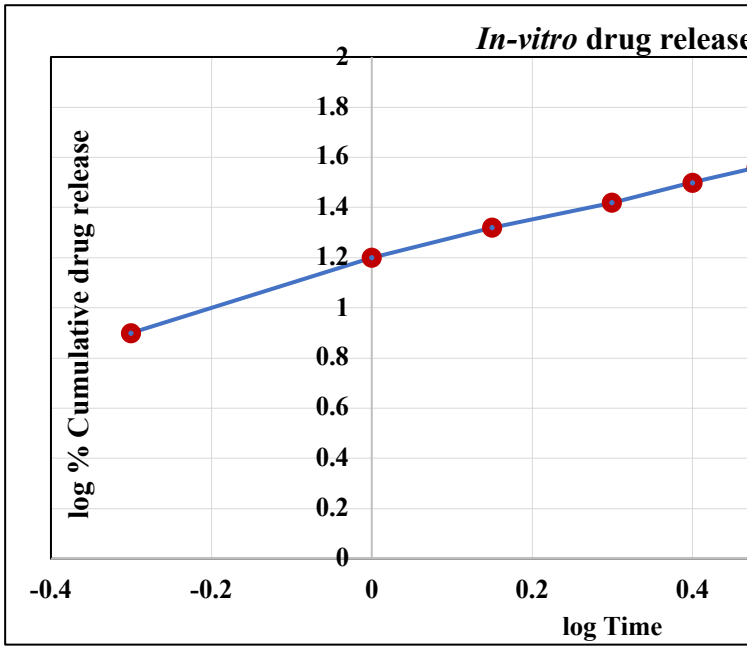


Figure 10. *In-vitro* Drug Release of Glibenclamide Buccal Patch by Korsmeyer Peppas Model

***Ex-vivo* Mucoadhesive strength**

Table 19 displays the weight (g) needed to detach the patch from the mucosal surface, serving as an indicator of mucoadhesive strength. All values are shown as mean ± SD, with a sample size of n=3.

Table 19. *Ex-vivo* mucoadhesion Characteristics of Buccal Patches

Formulation	M
Glibenclamide buccal patch	20

***Ex-vivo* permeation study**

The permeation characteristics of the optimized glibenclamide buccal patch indicated good mucoadhesive strength. Hence, due to its

mucoadhesive strength and residence time, the microemulsion-loaded glibenclamide buccal patch showed that 60% drug release at 8 h without any permeation enhancer, as shown in **Figure 11.**

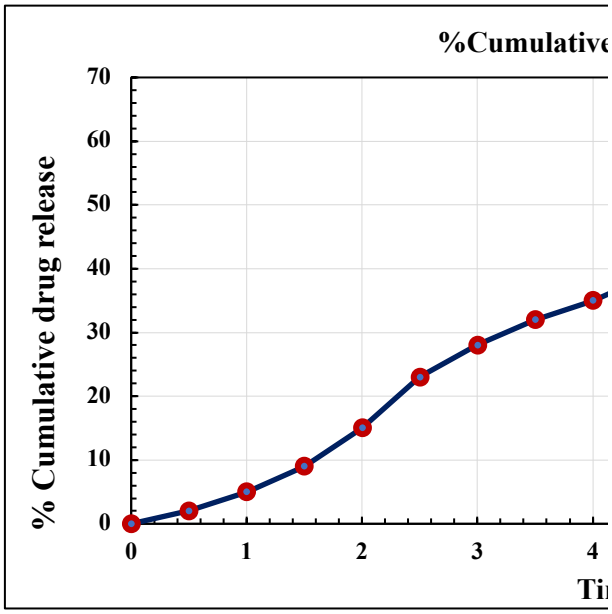


Figure 22. *Ex-vivo* Permeation of Microemulsion-Loaded GLI Buccal Patch

Stability Study

After two months of study, no visible change was observed in the appearance of the patch. There was also a negligible change in the drug content, folding endurance, and percentage drug release. The stability study results for the Glibenclamide patch are presented in **Table 20.**

Table 20. Evaluation of Glibenclamide Patch During Stability Studies

Time in days	Drug Content	olding ndurance	Physical appearance	Drug release h 8 Hrs
0	95.10 %	250	No change	90.10
30	96.10%	255	No change	91.60
60	94.60%	255	No change	93.30

CONCLUSION

This study shows how applying Quality by Design (QbD) principles developed a microemulsion-loaded mucoadhesive buccal patch with clove oil and Glibenclamide for better type II diabetes management. Systematic risk assessment and validated UV-visible spectrophotometry ensured reliable drug quantification, with FT-IR confirming drug-excipient compatibility. Solubility tests showed clove oil's superior capacity, and phase diagrams identified optimal Smix ratio for microemulsion formation. A 3^2 factorial design optimized formulation factors, yielding a stable nano-sized system with high transmittance, drug loading, entrapment efficiency, and low viscosity. The PVA:PVP (1:1) patch with Carbopol had excellent physical properties, uniform drug release over 8 hours, and strong mucoadhesion and permeation without enhancers. Stability testing confirmed integrity and a consistent release profile, aligning with ICH guidelines. This approach addresses Glibenclamide solubility and absorption issues via the buccal route, improving bioavailability and patient adherence, especially amid India's rising diabetes cases. Limitations include a lack of *in-vivo* data and long-term stability; future work should validate efficacy, incorporate enhancers, and explore PK modelling. Overall, this prototype demonstrates how QbD can enable scalable, non-invasive diabetes therapy, reducing health burdens.

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Conflict of interest: None

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