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Original Article

Bile Acid-Based Lipid Nanoparticles to Ameliorate Oral Bioavailability of Gliclazide: A Response Surface Methodology Approach

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ABSTRACT

Background/Objectives: Gliclazide is one of the commonly used oral anti-diabetic agents. It suffers limited and variable bioavailability due to poor solubility and intra- and inter-subject variability. The prime objective of this work was to upgrade its oral bioavailability via enhancing the solubility and dissolution rate by making lipid-based bile nanoparticles. **Materials and Methods:** Gliclazide loaded nanoparticles made up of lipids and bile salts were developed by emulsion solvent evaporation technique followed by ultrasonication. The response surface methodology was employed to optimize various formulation factors towards achieving maximum solubility of the drug with greater entrapment efficiency. Design of experiments analysis was performed to elucidate the impact of the factors on the responses. The optimized nanoparticles were characterized for particle size, dissolution rate and permeability. **Results:** All the formulation factors exhibited significant impact on both solubility and drug entrapment at $p<0.05$. The optimized nanoparticles exhibited an improvement of 30.7 times in solubility against pure drug. The optimized formulation enhanced the solubility of gliclazide to 1.32 mg/mL. The particle size of the nanoparticles from the optimized formulation resulted was 197.4 nm with PDI of 0.239. **Conclusion:** These results concluded that the oral bioavailability would be improved through improved solubility and dissolution rate from the lipid-based nanoparticles of gliclazide.

Keywords: Gliclazide, Solubility, Bioavailability, Lipid-based nanoparticles, Response surface methodology.

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INTRODUCTION

Gliclazide (GCZ), a sulfonylurea class drug, is one of the commonly used oral antidiabetic drugs. It comes under class II of the Biopharmaceutic Classification System (BCS) owing to its practically insoluble nature in water with high permeability.[1] GCZ undergoes intestinal metabolism owing to its sensitivity to gut microflora and majorly undergoes hydroxylation and hydrolysis.[2] Besides, GCZ suffers significant first-pass metabolism.[3] These limitations cause high intra- and inter-subject variability, which in turn make oral bioavailability of this drug a challenging task. These limitations necessitate the importance of enhancing the solubility and dissolution rate of GCZ to improve its bioavailability.

Many researchers made attempts to ameliorate the oral bioavailability of GCZ through several techniques, including complexation,[4] solid dispersions,[5] chitosan nanoparticles,[6] sustained release formulations with Eudragits,[7] nanocrystals,[8] and nanosuspensions.[9] From these techniques, several mechanisms were described in enhancing the solubility and dissolution rate such as improving wettability, decreasing particle size, and conversion to an amorphous state. All these techniques have addressed remedies for enhancing only solubility and dissolution, yet with some drawbacks like scale-up challenges, expensive excipients, and lower drug loading. No single technique addressed the issue of inter- and intra-subject variability due to gut metabolism and first-pass metabolism. This literature review suggested that a formulation strategy that can address both poor solubility and inter-/intra-subject variable bioavailability would be a potential alternative to the existing techniques in enhancing the oral bioavailability of GCZ.

Bile salts are proved to enhance oral bioavailability of drugs with a wide range of physicochemical properties. They can enhance both solubility/dissolution rate and permeability to augment the bioavailability of BCS class II and III drugs. In addition, the bile salts aid in drug absorption via including but not limited to carrier-mediated transport, lymphatic absorption, minimizing enzymatic decomposition, and decreasing first-pass

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metabolism.[10,11] These characteristics make the drug absorb rapidly and safely, thus minimizing the metabolism by the gut microflora. Due to their amphiphilic nature, they form mixed micelles with surfactants and enhance the water solubility of poorly soluble drugs like GCZ.[12] These characteristics make bile acids an essential addition in the formulation of low oral bioavailable drugs. Solid lipid nanoparticles can become the best approach by incorporating bile acids and surfactants to achieve the best results in enhancement of oral bioavailability.[13-15] These bile acid-Based Lipid Nanoparticles (BLNs) can improve the oral bioavailability of GCZ via multiple mechanisms, like i) improved dissolution owing to micellar solubilization and reduced particle size, ii) improved permeation via carrier mediated and lymphatic absorption, and iii) improved stability against gut microflora. With this hypothesis, the current research was planned to developed GCZ loaded BLNs (GBLNs) to improve solubility, dissolution and thus the oral bioavailability of GCZ. Response surface methodology via Central Composite Design (CCD) was implemented to optimize lipids, bile salt and surfactant to achieve maximum improvement in solubility of GCZ and maximum drug loading into the GBLNs. Later, the optimized GBLNs formulation was characterized for size, permeability and dissolution rate to prove the improved oral bioavailability against pure GCZ so as to justify the developed hypothesis.

MATERIALS AND METHODS

Materials

GCZ was gained as a gift sample form Hetero Drugs Pvt. Ltd. TS, glyceryl monostearate (GMS), Ursodeoxycholic Acid (UDC), Compritol 888 ATO (COMP) and Poloxamer-188 (PLX) were procured from Merck Ltd.

Compatibility Studies of MTF and Excipients

Compatibility of GCZ with the excipients employed in this study was verified by Fourier Transform Infrared Spectroscopy (FTIR)[16] and Differential Scanning Calorimetry (DSC).[17] The FTIR analysis was performed through the KBr pellet method for the pure GCZ and its physical mixtures with the excipients. The spectra for each sample were recorded and taken as an average of 16 scans over a wavenumber range of 400 to 4000 cm^{-1}. The DSC spectra were also recorded for the pure GCZ and the physical mixtures. In each of both the cases, the spectrum of pure GCZ was compared with those of the physical mixtures to verify the compatibility.

Development of GBLNs

Response surface methodology

The Response Surface Methodology (RSM)[18] was carried out using Design Expert v13.0. The aim in developing the GBLNs was to ameliorate oral bioavailability of GCZ. Hence, enhancing the solubility together with high drug loading were taken as objectives of the formulation development. Summarizing the extensive literature in this context, four formulation factors were chosen viz. A: Type of lipid (COMP and GMS), B: Concentration of lipid (200, 300 and 400 mg), C: Concentration of the bile acid UDC (50, 100, 150 mg), and D: Type of surfactant at 2% w/v (Tween 80 and PLX). Drug Entrapment Efficiency (EE) and solubility of GCZ and were taken as the responses R1 and R2 respectively. The response surface methodology was adopted to optimize the GBLNs with maximum solubility and highest EE. As the number of numeric factors were only two, the Box-Behnken design could not be applied. Considering the levels and types of the factors, the CCD was selected to investigate the impacts of these factors on the responses, and to develop regression equations towards optimization. The mixtures of the factors with levels as per the CCD for preparing the GBLNs are presented in Table 1.

Preparation of GBLNs

The GBLNs were prepared by emulsification followed by sonication.[19,20] Required amount of the lipid (COMP / GMS) as per the quantities mentioned in Table 1 was taken in a glass vial and melted. Into this molten lipid, 100 mg of GCZ was added and mixed to dissolve/disperse. In a stoppered test tube, 3 mL of ethanol was transferred and heated to the temperature of the molten lipid mass. Required amount of the UDC as per the quantities mentioned in Table 1, was added into the test tube and mixed to dissolve. The hot molten lipid-drug mixture was transferred into the test tube containing UDC to form the oil phase. In a beaker, 7 mL of distilled water was added, and 200 mg of the surfactant (Tween 80 / PLX) was dissolved in it to make the aqueous phase. Added the oily phase to the aqueous phase under high-speed homogenization (10,000 rpm) and continued the mixing until ethanol evaporated completely. Later, the dispersion was subjected to ultrasonication with 10s-2s start-stop cycles for 10 min. to produce the GBLNs.

Characterization of the GBLNs

Yield

$$Yield\ (\%) = \frac{Weigth\ of\ the\ GBLNs\ obtained}{Weight\ of\ all\ the\ non-volatile\ solids\ taken} x100$$

Entrapment Efficiency (EE)

The GBLNs were centrifuged for 30 min at 8,000 rpm following their preparation. The solid pellet and supernatant were parted. The pellet was precisely cleaned with water using a filtration medium to eliminate the free GCZ. Following the amalgamation of the washings with the supernatant, spectrophotometric measurement was conducted to quantify the unentrapped amount of GCZ.[21] The following formula was utilised to determine drug entrapment efficiency.

$$EE\ (\%) = \frac{Quantity\ of\ GCZ\ taken - Quantity\ of\ GCZ\ in\ the\ supernatant}{Quantity\ of\ GCZ\ taken} x100$$

Solubility

This assessment was carried out with the shake-flask method[22] for both pure GCZ and the synthesised GBLNs. GCZ was added to 2 mL of water in a sealed test tube and stirred for 48 hr. An excessive quantity of the GCZ was introduced until saturation occurred. The saturation was confirmed by observing undissolved GCZ. After the specified duration, the slurry was filtered, and the obtained filtrate was gathered for further analysis. A spectrophotometric examination was performed on the material to determine its solubility at the peak wavelength of 230 nm. The identical approach was executed for all formulations of GBLNs.

Design of experiments analysis, Design validation and Optimization

As part of the Design of Experiments (DoE) analysis in RSM, sequential model Sum of Squares (SMS) analysis was carried out separately for each response to identify the suitable regression model to demonstrate the influences of the factors on the responses. The found regression model from the SMS analysis was validated by Analysis of Variance (ANOVA) at an α level of 0.05 and predicted vs. actual plots.[23] Finally, optimization was carried out by setting the desirability of maximizing the EE with a lower limit of 75% and also maximizing the solubility with a lower limit of 1.28 mg/mL (to get the dose-to-solubility ratio not more than 250 mL at the maximum daily dose of GCZ i.e. 320 mg).

Characterization of the optimized GBLNs

Particle size and Zeta potential

The optimised GBLNs were analyzed by dynamic light scattering method (NanoPlus, Particulate Systems) to quantify particle size and zeta potential. The GBLNs were suitably diluted with distilled water prior to measurement at 25°C temperature and 90° scattering angle. Each sample underwent three measurements, and the average values were recorded.

Surface morphology

The surface morphology and configuration of the optimised GBLNs were examined by Transmission Electron Microscopy (TEM). A tiny aliquot of the GBLNs dispersion, after suitable dilution, was deposited onto the carbon-coated copper grid and allowed to dry. The specimen was later examined under a microscope, and micrograph images were recorded.

Permeability

This test was carried out by the *ex vivo* method.[24] Duodenal part of the small intestine of sheep from slaughterhouse was collected on the day of the experiment, washed with Ringer's solution and kept in formalin solution. The internal diameter of the duodenum was measured to be 1.6 cm. The optimized GBLNs solution, equivalent to a GCZ concentration of 0.04 mg/mL, was prepared in water. A 5 cm length portion of the duodenum was taken; one end of it was tied, 5 mL of the previously prepared GBLNs solution was placed in it, and the other end of it was tied. It was ensured that a length of 3 cm was maintained between the knots for drug permeation. This duodenal setup was immersed in 100 mL of phosphate buffer at pH 7.4 in a beaker with the help of a burette stand. This beaker was kept on a magnetic stirrer that was set to rotate at 100 rpm. A sample of 5 mL was withdrawn at every 5 min. and was replaced with fresh buffer. These samples were subjected to quantification spectrophotometrically. The sample collection and quantification were continued till the permeation of not more than 10% of the initial GCZ taken. The data obtained only to this level should be considered to ensure sink conditions in calculating the drug permeability *ex vivo*. The apparent Permeability (P_{app}) of GCZ was calculated by using the below formula.[25]

$$P_{app} = \frac{\Delta Q}{\Delta t.\,60.\,A.\,C_0}$$

Where, P_{app} is permeability in cm/sec; ΔQ/Δt is the permeability rate in mg/min. (it was taken till 10% of GCZ permeated); A is the surface area of the membrane {2Πr (r + h)} in cm^2; C_0 is the initial concentration of GCZ solution taken. The experiment was repeated with the pure GCZ also in the same manner.

Dissolution

The optimized GBLNs was subjected to dissolution testing in comparison with pure GCZ. Pure GCZ was taken a weight of 80 mg, and GBLNs equivalent to 80 mg of GCZ were taken. Phosphate buffer pH 7.4 was taken as the dissolution medium at a volume of 900 mL.[26] The paddle apparatus at 100 rpm was used to carry out the dissolution testing and the test was carried out for 60 min. Samples were removed at different time points, quantified spectrophotometrically and the obtained data was analyzed.

RESULTS

GCZ-Excipients Compatibility Studies

FTIR and DSC analysis were carried out to verify the compatibility of the lipids, bile acid and surfactants used in this study with GCZ. Figure 1(a) illustrated the stack of FTIR spectra of pure GCZ and its physical mixtures with the additives. The observed thermograms from the DSC study for the pure GCZ and the physical mixtures are presented in Figure 1(b).

Characterization of the GBLNs

The GBLNs obtained from all the formulation combinations were first checked for the yield in addition to the responses EE and solubility. The findings are presented in Table 2. The product yield was obtained in the range of 78.39–92.67% across all the formulations. These higher yield values indicated that proper alignment of the experimental conditions to the different formulation compositions of the GBLNs. Besides, these results also signified the suitability of the developed method for the

Table 1: Mixtures of the factors and their levels as different formulations for preparing the GBLNs as per the CCD.

Sl. No.	Formulation code	Levels of the Factors			
		Factor A (mg)	Factor B (mg)	Factor C	Factor D
1	GBLN1	300	29.29	COMP	Tween 80
2	GBLN2	200	50	COMP	Tween 80
3	GBLN3	400	50	COMP	Tween 80
4	GBLN4	158.58	100	COMP	Tween 80
5	GBLN5	300	100	COMP	Tween 80
6	GBLN6	441.42	100	COMP	Tween 80
7	GBLN7	200	150	COMP	Tween 80
8	GBLN8	400	150	COMP	Tween 80
9	GBLN9	300	170.71	COMP	Tween 80
10	GBLN10	300	29.29	GMS	Tween 80
11	GBLN11	200	50	GMS	Tween 80
12	GBLN12	400	50	GMS	Tween 80
13	GBLN13	158.58	100	GMS	Tween 80
14	GBLN14	300	100	GMS	Tween 80
15	GBLN15	441.42	100	GMS	Tween 80
16	GBLN16	200	150	GMS	Tween 80
17	GBLN17	400	150	GMS	Tween 80
18	GBLN18	300	170.71	GMS	Tween 80
19	GBLN19	300	29.29	COMP	PLX
20	GBLN20	200	50	COMP	PLX
21	GBLN21	400	50	COMP	PLX
22	GBLN22	158.58	100	COMP	PLX
23	GBLN23	300	100	COMP	PLX
24	GBLN24	441.42	100	COMP	PLX
25	GBLN25	200	150	COMP	PLX
26	GBLN26	400	150	COMP	PLX
27	GBLN27	300	170.711	COMP	PLX
28	GBLN28	300	29.29	GMS	PLX
29	GBLN29	200	50	GMS	PLX
30	GBLN30	400	50	GMS	PLX
31	GBLN31	158.579	100	GMS	PLX
32	GBLN32	300	100	GMS	PLX
33	GBLN33	441.421	100	GMS	PLX
34	GBLN34	200	150	GMS	PLX
35	GBLN35	400	150	GMS	PLX
36	GBLN36	300	170.711	GMS	PLX

preparation of GBLNs. The EE results were obtained in the range of 70.42–92.65% and the solubility results were found to be in the range of 0.38-1.36 mg/mL. These higher variations in the case of the EE and solubility were attributed to the variations in the levels of the four formulation factors. The solubility of GCZ was found to be 0.043 mg/mL. Hence, the results of these two responses were subjected to DoE analysis to find out the impacts of the formulation factors on and the regression models for the two responses.

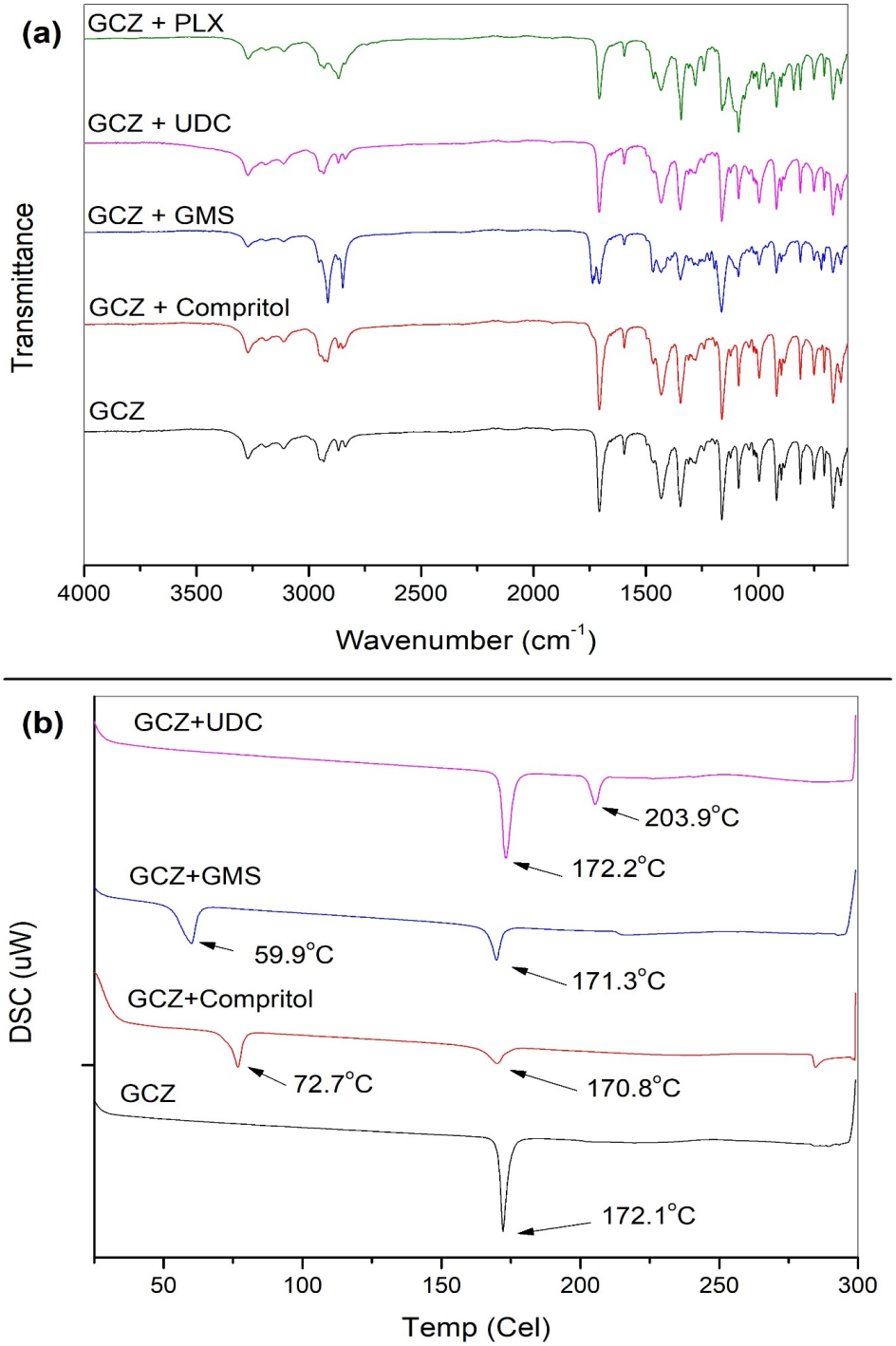

Figure 1: Spectra of pure GCZ and its physical mixtures with lipids and surfactants from (a) FT-IR, (b) DSC.

DoE Analysis and Design Validation

The SMS analysis revealed that both the responses were influenced by the factors through linear regression model. These linear models for both the responses were tested by ANOVA and the results are presented in Table 3. All the impacts of the four factors on both the responses are illustrated by contour plots as presented in Figure 2(a) and 2(b) for the response EE and in Figure 2(c) and 2(d) for the response solubility.

Linear regression equations of the responses in coded factors

EE = 79.89 + 5.65*A + 2.7*B – 1.92*C – 1.35*D

Solubility = 0.8761 – 0.1116*A + 0.2334*B + 0.1394*C + 0.0522*D

Optimization

Optimization was carried out by setting the desirability of maximizing both the EE and solubility. The predicted design

space by the software is presented as an overlay plot, illustrated in Figure 3. The yellow color region in the overlay plot demonstrates the design space with GMS as the lipid and PLX as the surfactant. Other combinations of the lipid and surfactant could not yield the design space. Hence, this combination of GMS and PLX was considered. Any combination of the amounts of the GMS and UDC along with PLX within the region of the design space would produce GBLNs with maximum EE and maximum solubility as per the desirability criteria. One such combination with GMS at 250 mg and UDC at 150 mg for 100 mg of GCZ was chosen by

Table 2: Findings of GBLNs characterization parameters.

Sl. No.	Formulation code	Yield (%)	R1: Entrapment efficiency (%)	R2: Solubility (mg/mL)
1	GBLN1	88.35±2.35	79.48±3.21	0.38±0.03
2	GBLN2	84.15±1.89	77.52±4.62	0.59±0.07
3	GBLN3	81.22±3.55	82.93±3.98	0.41±0.02
4	GBLN4	90.24±4.03	75.81±5.06	0.74±0.11
5	GBLN5	83.16±2.68	83.07±4.28	0.76±0.08
6	GBLN6	87.25±3.11	92.65±1.94	0.51±0.06
7	GBLN7	80.53±1.75	81.72±3.54	0.98±0.16
8	GBLN8	91.37±3.16	90.73±2.68	0.84±0.03
9	GBLN9	85.06±4.35	84.29±4.71	1.01±0.07
10	GBLN10	78.39±1.98	74.51±2.63	0.59±0.04
11	GBLN11	83.46±4.66	71.43±5.26	1.02±0.14
12	GBLN12	84.54±3.25	81.35±1.75	0.61±0.05
13	GBLN13	80.55±3.74	69.62±3.09	0.99±0.08
14	GBLN14	86.37±2.41	81.55±4.11	0.86±0.05
15	GBLN15	82.52±5.16	88.27±2.37	0.77±0.03
16	GBLN16	89.07±4.19	77.39±2.01	1.33±0.17
17	GBLN17	84.32±2.78	89.16±4.33	1.19±0.09
18	GBLN18	80.94±1.59	80.78±3.18	1.25±0.11
19	GBLN19	80.53±3.45	76.03±4.62	0.41±0.05
20	GBLN20	90.68±2.71	75.28±1.89	0.66±0.03
21	GBLN21	83.36±3.26	81.54±4.55	0.47±0.06
22	GBLN22	78.59±1.86	70.42±3.21	0.85±0.11
23	GBLN23	83.44±2.47	80.19±2.44	0.73±0.08
24	GBLN24	79.53±2.95	91.67±1.63	0.58±0.02
25	GBLN25	91.12±3.27	78.43±5.37	1.36±0.15
26	GBLN26	92.67±2.61	88.51±2.05	1.05±0.03
27	GBLN27	82.25±5.17	82.35±1.98	0.93±0.06
28	GBLN28	88.34±2.61	70.94±4.67	0.64±0.07
29	GBLN29	93.16±1.32	68.09±5.13	1.21±0.09
30	GBLN30	90.04±2.75	79.32±2.66	0.69±0.02
31	GBLN31	81.19±4.18	66.58±3.74	1.15±0.08
32	GBLN32	84.53±2.63	78.01±4.21	0.98±0.10
33	GBLN33	86.22±3.52	85.44±3.53	0.83±0.06
34	GBLN34	80.64±4.78	76.15±4.09	1.52±0.13
35	GBLN35	91.15±1.36	86.27±1.58	1.31±0.09
36	GBLN36	87.34±3.08	78.53±3.25	1.34±0.15

* Presented as Mean±Std. Dev. for n=3

Table 3: Findings of ANOVA test for the linear model of both the responses.

Source	Sum of Squares	Degrees of freedom	Mean Square	F value	*p*-Value	Inference (at $p<0.05$)
For the response EE						
Model	1451.48	4	362.87	107.11	< 0.0001	Significant
A	1020.55	1	1020.55	301.26	< 0.0001	Significant
B	232.42	1	232.42	68.61	< 0.0001	Significant
C	133.13	1	133.13	39.30	< 0.0001	Significant
D	65.37	1	65.37	19.30	0.0001	Significant
Residual	105.02	31	3.39			
Cor Total	1556.50	35				
For the response Solubility						
Model	2.94	4	0.7351	63.83	< 0.0001	Significant
A	0.3985	1	0.3985	34.60	< 0.0001	Significant
B	1.74	1	1.74	151.41	< 0.0001	Significant
C	0.7000	1	0.7000	60.79	< 0.0001	Significant
D	0.0982	1	0.0982	8.53	0.0065	Significant
Residual	0.3570	31	0.0115			
Cor Total	3.30	35				

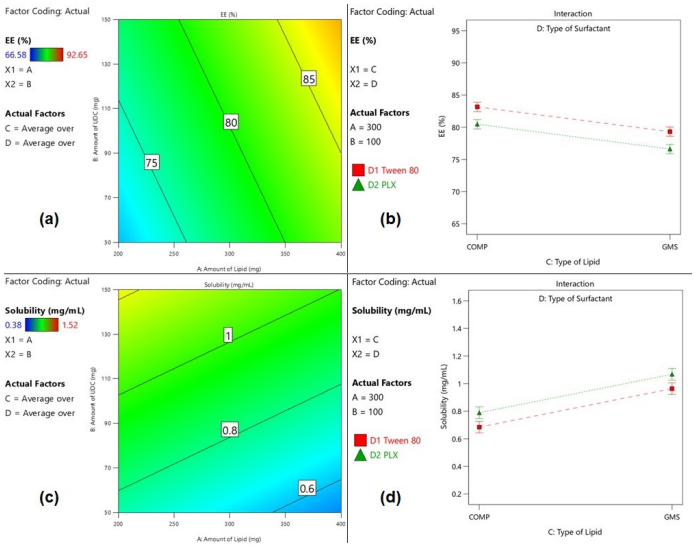

Figure 2: Illustration of the impacts of the factors. (a) Impacts of the factors A and B on EE, (b) Impacts of the factors C and D on EE, (c) Impacts of the factors A and B on solubility, (d) Impacts of the factors C and D on solubility.

the software as the best formulation with maximum desirability. The EE and solubility of the GBLNs at this combination were predicted as 76.47% and 1.36 mg/mL respectively, which are shown in Table 4.

Characterization of the optimized GBLNs

The optimized GBLNs formulation was analyzed for particle size, zeta potential, permeability and dissolution rate. The resultant spectrum of particle size analysis is displayed in Figure 4(a). The resulting particle size was 197.4 nm with a PDI of 0.239. The zeta potential was found to be -32.9 mV. The TEM study for studying the particle shape and surface morphology was carried out, and the resultant micrograph is presented in Figure 4(b). The permeability was carried out *ex vivo* for the pure GCZ and the optimized GBLNs, and the results were found to be 2.09 x 10-5 and 4.37 x 10-5 cm/sec, respectively. The optimized GBLNs were tested for dissolution in comparison with pure GCZ. Pure GCZ was taken at a weight of 80 mg, and the GBLNs equivalent to 80 mg of GCZ were taken. The obtained data is presented, and the dissolution profiles are illustrated in Figure 4(c). The findings of the dissolution test conveyed that the % drug dissolved from the optimized GBLN was increased to 67.12% from just 9.83% in the case of pure GCZ after 60 min.

DISCUSSION

The spectra of FTIR as shown in Figure 1(a), exhibit the characteristic peaks observed in the spectrum of pure GCZ at 3270.7, 1706.7, 1431.3, 1345.6 and 1161.5 cm^{-1}, corresponding to their characteristic functional groups: N-H stretching of amide, C=O stretching of amide, Cyclic C-H bending, C-N stretching, and SO_2 stretching of sulfonyl group respectively.[27] The peaks at similar positions were also appeared in every spectrum of the physical mixtures. These observations demonstrated that GCZ did not show any chemical reactivity and was compatible with all the selected excipients. The DSC spectrum of the pure GCZ, shown in Figure 1(b), exhibited a sharp endotherm at 172.1°C.[28] The endotherm at this temperature might be attributed to the melting point of GCZ. Besides, the shape of the endotherm demonstrated that the GCZ taken was in crystalline form. The

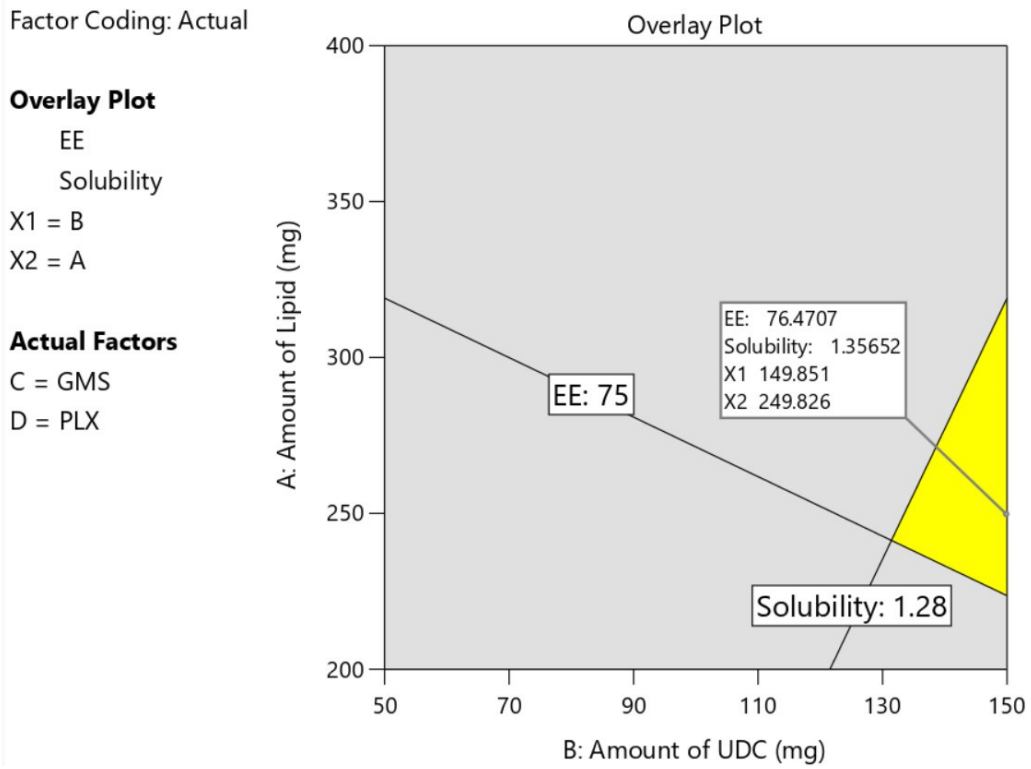

Figure 3: Overlay plot illustrating the design space.

Table 4: Optimized factors' combination for the GBLNs with the predicted and the observed values of the responses.

Factors combination	Responses	Predicted values	95% CI low	95% CI high	Observed values
A: 250 mg B: 150 mg C: GMS D: PLX	R1: Entrapment Efficiency (%)	76.47	75.18	77.80	77.26
	R2: Solubility (mg/mL)	1.36	1.28	1.43	1.32

individual spectrum of every physical mixture shows endotherms at temperatures of 72.7°C, 59.9°C, and 203.9°C which correspond to the melting points of COMP, GMS, and UDC respectively. This observation demonstrated that these additives were in a crystalline state. The spectra of these physical mixtures also exhibited another sharp endotherm at a similar temperature to that of the pure GCZ which is conforming to the melting point of the GCZ. As there was no significant change in the shape and position of the GCZ-corresponding endotherm, this observation signified that the GCZ did not undergo any physical modifications and was compatible with the lipids, bile acid and the surfactant employed in this study.

The SMS analysis revealed that both the responses were influenced by the factors through the linear regression model. These linear models for both the responses were tested by ANOVA and found that the models were significant (Table 3). Besides, the impacts of all four factors on both responses were significant at $p<0.05$. The adjusted and predicted R^2 values were found to be 0.9238 and 0.9083 in the case of EE and 0.8778 and 0.8531 in the case of solubility. The difference between the two R^2 values in each individual case of both the responses was found to be less than 0.2 which confirmed the significance of these regression models. These findings confirmed that these models were significant enough in elucidating the mechanisms behind the effects of the factors on the responses and also to forward to optimization.

Influences of the four factors on EE are illustrated in Figure 2(a) and Figure 2(b). In the case of factor A, upon increasing the concentration of the lipid, the EE was also increased. This observed increase could be attributed to the greater loading of the drug at higher concentrations of the lipids. Besides, the increased lipophilicity at higher lipid concentrations might protect the drug from being leached out to a greater extent. These findings are allied with the reports of Chettupalli AK *et al.*[29] The factor B also found to have a positive effect, as the EE was increased with the concentration of the UDC. UDC is a bile salt which also can act as an emulsifier and stabilizer. At lower concentrations of UDC, poor emulsification in the preparation leads to less drug loading. As its concentration is increased, increased emulsification followed by greater solubilization of gliclazide in the lipid matrix might happen. Also, as it can act as a stabilizer, leakage of the

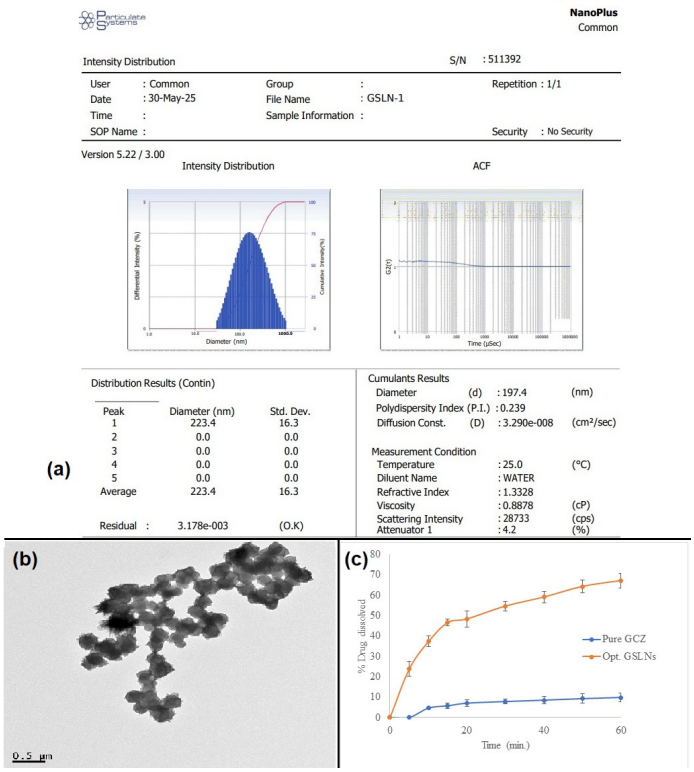

Figure 4: Characterization of the optimized GBLNs. (a) Spectrum of particle size analysis of the optimized GBLNs, (b) TEM micrographs depicting surface morphology, (c) Comparative dissolution profiles of GCZ from pure drug and from the optimized GBLNs (% drug dissolved is presented as mean±standard deviation for *n*=3 with error bars).

drug from the lipid might be reduced, which in turn might result in greater loading of the drug. Similar findings with justification were reported by Narayanan VA *et al.*[30]

The influence of the factor C, the type of lipid on the EE is illustrated in Figure 2(b). The EE was found to be more in the case of COMP than that in the case of GMS. Gliclazide is a highly lipophilic drug, and hence its loading into the SLNs is affected by the extent of lipophilicity of the lipids. COMP is composed of behenic acid, which has a longer C22 chain than the stearic acid C18 chain present in GMS which indicates COMP is more lipophilic than GMS.[31] Greater lipophilicity of the lipid can load a greater amount of the lipophilic drug. Further, COMP is more crystalline (melting point is 72.7°C) than GMS (melting point is 59.9°C). Greater crystallinity of COMP makes it more rigid upon solidification of the GBLNs and could better prevent the leakage of the loaded drug than the less crystalline GMS which might allow some drug to migrate out of the lipid matrix. Hence, the higher lipophilicity and higher crystallinity might account for greater EE of gliclazide in the case of the GBLNs prepared from COMP. these findings are justified by similar higher EE results reported for the lipid-based GCZ nanoparticles by Nazief AM *et al.*[32] The impact of the factor D on the EE was found to be that among the two surfactants, the EE was found to be more in the case of Tween 80 than that in the case of PLX. Tween 80 has a stronger emulsification ability, while PLX has greater micellization potential in water. The lower Hydrophilic-Lipophilic Balance (HLB) of Tween 80 than that of PLX could emulsify hydrophobic drugs like GCZ to a greater extent.[33] was So, the greater emulsification potential of Tween 80 might be responsible for higher EE. On the other hand, PLX might cause partitioning of the drug into water from the lipid matrix due to its greater micellization ability, which in turn leads to lesser EE.

Influences of the four factors on solubility are illustrated in Figure 2(c) and Figure 2(d). In the case of factor A, an increase in the concentration of the lipid resulted in a decrease in the solubility. When compared to the solubility of pure GCZ (0.043 mg/mL), a greater increase in solubility was observed at lower lipid concentrations, whereas the increase in solubility was lesser at higher lipid concentrations. Higher amounts of the lipids might make the GBLNs more lipophilic and hence result in lesser improvement in solubility. In contrast to factor A, a rise in the concentration of the UDC (factor B) resulted in an increase in the solubility. UDC is amphiphilic and has surfactant-like properties, and it can form mixed micelles in water with other surfactants.[34,35] Hence, an increase in the amount of UDC might enhance the solubility of GCZ to a greater extent by enhancing micellar solubilization.

The influence of the type of lipid (factor C) on the solubility is illustrated in Figure 2(d). Among the two lipids, GBLNs prepared from the GMS showed higher improvement in solubility, whereas the GBLNs prepared from COMP exhibited lesser improvement in solubility. COMP is composed of behenic acid, which has a longer C22 chain than the stearic acid C18 chain present in GMS which indicates COMP is more lipophilic than GMS. Further, COMP is more crystalline (melting point is 72.7°C) than GMS (melting point is 59.9°C). These properties of the COMP[36] might pack the drug more tightly in the more rigid and ordered lipid matrix, which resulted in lesser improvement in solubility. On the other hand, a relatively less lipophilic, less ordered and more flexible lipid matrix of GMS resulted in relatively greater enhancement of solubility of GCZ. In the case of factor D, among the two surfactants, the solubility was found to be more in the case of PLX than that in the case of Tween 80. Tween 80 has an HLB value of around 15 whereas PLX has an HLB value nearly 29. Besides, PLX has greater micellization potential in water.[37] These properties of higher hydrophilic nature and greater micellization potential might be responsible for the greater increase in solubility of GCZ from the GSLNs than their counterparts containing Tween 80.

A new formulation of the GBLNs was prepared at the suggested combination in the design space area of the overlay plot resulting from the optimization (shown in Figure 3). The prepared GBLNs were subjected to EE and solubility determinations. The EE and solubility were observed to be 77.36% and 1.32 mg/mL. These observed response values were found to be within the 95% confidence intervals of the predicted values by the software (given

in Table 4). Hence, the optimization process was successful, and the GBLNs at this combination were considered as the optimized formulation. This optimized formulation exhibited a water solubility of 1.32 mg/mL, which was 30.7 times more when compared to the solubility of 0.043 mg/mL of pure GCZ. The dose-to-solubility ratio even for a maximum daily dose of 320 mg at this solubility value, was 242 mL, which is below the maximum limit of 250 mL for any drug to be considered as highly soluble as per the BCS classification. Now the GBLNs form of GCZ could be considered as highly soluble, which might overcome the problem of dissolution-limited bioavailability.

The particle size of the optimized GBLNs resulted as 197.4 nm with a PDI of 0.239. This size was highly acceptable to recognize the product as nanoparticles. And the obtained PDI was found to be below 0.3 and thus inferred an advantageous narrow distribution of the GBLNs.[38] The zeta potential was found to be -32.9 mV, which could be attributed mainly to the characteristic of UDC upon its deprotonation. Also, this high zeta potential could bring good stability to the GBLNs.[39] The TEM micrograph image demonstrated that the GBLNs have a shape almost near to spherical with near-to-smooth texture which can contribute to the stability of the GBLNs. The permeability was carried out *ex vivo* for the pure GCZ and the optimized GBLNs, and the results were found to be 2.09 x 10^{-5} and 4.37 x 10^{-5} cm/sec, respectively. GCZ is a BCS class II drug, and thus intrinsically it has good permeability (i.e., above 1.0 x 10^{-5} cm/sec).[40] However, formulating GCZ into GBLNs resulted in an enhancement of 2.1 times to the original permeability. This can further enhance the rate of permeation of GCZ and thus the absorption and bioavailability of GCZ. Besides, the bile acid UDC present in the UDC could minimize the gut wall metabolism, first-pass metabolism and thus inter- and intra-subject variability in oral bioavailability of GCZ.

The findings of the dissolution test conveyed that the % drug dissolved from the optimized GBLN was increased to 67.12% from just 9.83% in the case of pure GCZ after 60 min. This exhibited an improvement of 6.8 times in the dissolution rate of the GCZ. This great enhancement in dissolution could be due to the nano-size of the GBLNs in addition to the increased solubility. This result confirmed that the dissolution-limited oral bioavailability of GCZ can be greatly improved from the optimized GBLNs formulation.

CONCLUSION

This work was planned with an aim of improving oral bioavailability of GCZ, a BCS class II drug with high variability in oral bioavailability. Bile acid-based lipid nanoparticles were selected as the formulation strategy to reach the aim. Formulation optimization to select the right lipid and right quantities of the lipid and bile acid by response surface methodology. The DoE analysis followed by optimization resulted in a combination of 250 mg of GMS as the lipid and 150 mg of UDC as the bile acid with PLX at 2% w/v of PLX as the surfactant for 100 mg of GCZ as the optimized formulation of GBLNs. The optimized GBLNs exhibited near-to-spherical shape with a size of 197.4 nm. This optimized formulation exhibited an improved solubility of 1.32 mg/mL (against 0.043 mg/mL for pure GCZ) with 67.12% dissolution in 60 min. (against 9.83% in 60 min. for pure GCZ). This result demonstrated that the main objective of enhancing the solubility and dissolution rate of GCZ was achieved. In addition, permeability was also increased by 2.1 times for the GBLNs against pure GCZ. This improved permeability, in addition to the presence of bile acid, would decrease the inter-subject and intra-subject variability in GCZ absorption and bioavailability. These results signified that the aim of enhancing oral bioavailability and minimizing variability of GCZ was achieved successfully through the GBLNs.

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ABBREVIATIONS

PDI: Polydispersity index; **GCZ:** Gliclazide; **BCS:** Biopharmaceutic classification system; **BLNs:** Bile acid-based lipid nanoparticles; **GBLNs:** GCZ loaded BLNs; **CCD:** Central composite design; **GMS:** Glyceryl monostearate; **UDC:** Ursodeoxycholic acid; **COMP:** Compritol 888 ATO; PLX: Poloxamer – 188; **FTIR:** Fourier Transform Infrared Spectroscopy; **DSC:** Differential Scanning Calorimetry; **RSM:** Response surface methodology; **EE:** Entrapment efficiency; TEM: Transmission Electron Microscopy; **ANOVA:** Analysis of variance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUMMARY

Gliclazide is a BCS class II drug with dissolution limited oral bioavailability. Besides, it suffers with high intra- and inter-individual variability in oral bioavailability, gut wall metabolism, first-pass metabolism. To overcome all these challenges and to improve oral bioavailability, only solubility / dissolution enhancement is not sufficient. Hence, BLNs technology was adopted in this work and executed by response surface methodology. Optimized formulation exhibited nearly 31 times improvement in solubility, nearly 7 times improvement in dissolution and also 2-fold improvement in permeation also. These improved dissolution and permeation can increase the rate of absorption and decrease gut wall metabolism. Besides, the presence of bile acid can lower gut wall and first-pass metabolism and aid in absorption via lymphatic absorption by which intra- and inter-individual variability can be minimized.

These characteristics make the GBLNs a potential technology to improve oral bioavailability of GCZ. However, further *in vivo* characterization is required to strengthen the advantages claimed.

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