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Chemometrics Assisted Formulation of Glimepiride Nanosuspension for Solubility Enhancement in Diabetic Therapy—A Systematic Approach

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Glimepiride (GLM), a third-generation sulfonylurea oral hypoglycemic medication, is used to treat type II diabetes. Admittedly, it has a low bioavailability, a relatively short half-life ($t_{1/2}$), and high toxicity. To address the issue, a nanosuspension of poorly soluble GLM is designed using a Quality-by-Design (QbD) method, to enhance its solubility. Prospective risk factors are identified and assessed using Critical Material Attribute (CMAs) and Critical Processing Parameter (CPPs) to evaluate the targets by Taguchi orthogonal array (OA) design and to study the effects of formulation and process variables on dependent variables. The amount of Polyvinylpyrrolidone (PVP) (X2), agitation time (X5), and concentration of Poloxamer (X7) are found as significant parameters ($p < 0.05$), and are further optimized using the Box Behnken Design as response surface methodology model. The optimized GLM nanosuspension 1) has mean particle size (PS), zeta potential, viscosity (VS), and percentage cumulative drug release (CDR%) of 258.17 nm, −25.2 mV, 1.087cPs, and 98.52%, respectively. The pattern of drug release is fitted to Non-Fickian kinetics. The study results in an economic and efficient nano formulation with enhanced solubility.

1. Introduction

In almost every country diabetes mellitus is one of the most prevalent chronic diseases and its prevalence and impact are growing as economic development and urbanization result in changing lifestyles marked by decreased physical activity and increased obesity.

Diabetes may be one of the most discussed diseases in the world, particularly in India, but awareness of the disease can be measured by the fact that India now has more individuals with type II diabetes (more than 50 million) than any other country. Diabetes is creating a huge health problem in our country today, with the country having the greatest number of diabetes patients in the globe.[2]

Glimepiride (GLM) is an oral hypoglycemic sulfonylurea drug that is used to treat type II diabetes, was chosen as a model medicine for nanosuspension.[1] Because of its relatively low solubility at acidic and neutral pH (0.004 mg mL^{-1}) and high permeability through Caco-2 monolayer (30.4 106 cm s^{-1}), GLM is classed in Biopharmaceutics Classification System (BCS) class II,[3] indicating 100% absorption after oral treatment. However, because to its low water solubility and dissolving rate, it has a slower beginning of action, variable bioavailability, and unpredictable drug plasma levels after oral administration, which might result in a lack of therapeutic response and expose the patient to significant hyperglycemia.

GLM has poor water solubility, which is the most significant barrier to clinical applicability for diadetic therapy. Lower solubility complicates drug administration by causing irregular absorption and, as a result, poor oral bioavailability. Micronization, cyclodextrin, and co-solvents are among the traditional methods used to improve the solubility of poorly soluble drugs. However, in many circumstances, the issue of bioavailability remains unresolved. Micronization does not produce enough surface area to increase the dissolving rate of poorly water-soluble drugs. As a result, companies are transitioning from micronization to nanonization (formulation of nanosuspension). Nanomedicines have risen to prominence in scientific study, and their use as medications has become increasingly important due to their higher surface area in comparison to particle size. Increased solubility

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causes a faster rate of drug flooding and hence a faster reach of maximum plasma level.

NS is a submicron colloidal suspension composed of medication particles distributed in water with polymer as a stabilizer or surfactant using top-down or bottom-up procedures. Since the previous decade, NS has emerged as a possible solubility augmentation technology in which poorly soluble pharmaceuticals are suspended in dispersion without any matrix components.[4] NS improves the solubility of poorly soluble compounds in both aqueous and non-aqueous environments. Increased solubility causes a faster rate of drug influx and hence a faster reach of maximum plasma level. Because of the smaller particle size, these medications can be delivered intravenously without obstructing blood capillaries.[5]

Furthermore, no previous research has provided any vision into the fracture mechanism of GLM crystal based on intermolecular interactions in nanosuspension using agitation time.[6] As a result, the current study analyses the use of NS stabilized by several independent factors such as PVP, Poloxamer, and agitation time as a method to increase GLM dissolving rate and optimal viscosity range to ensure sustained drug delivery.

The International Conference on Harmonization (ICHQ8) of technical standards in the formulation of pharmaceutical products recommended the use of QbD principles.[7] Manufacturing variability owing to a lack of awareness of the influence of critical material attributes (CMAs) and critical processing parameters (CPPs), attainment of compact size, and narrow polydispersity index (PDI) are the major obstacles encountered during the development of NS.[8] The toxicities of nanoparticulate drug delivery systems (NDDS) are widely documented due to its rapid onset of action, prolonged release, permeability, enhanced solubility, and bioavailability. As a result, the purpose of this study was to create GLM-NS using a QbD technique in order to determine the influence of CMAs and CPPs on critical quality attributes (CQAs), improve formulation safety and quality, reduce manufacturing variability, and control manufacturing costs.

The use of statistical design of experiments (DOE) is an effective approach for experiment planning.[9] The Taguchi OA of screening experiments and the Box Behnken Design (BBD) of response surface methodology (RSM) are well-established approaches for pharmaceutical formulation optimization and development.[10]

2. Experimental Section

2.1. Materials

Glimepiride was received as gift sample from Lyrus Life Science Pvt. Ltd., Bengaluru, India. PVPk30 and poloxamer 188 were procured from Merck Specialties Pvt. Ltd. Mumbai, India. Acetone and HPMC K100M were procured from Sigma–Aldrich Chemicals Private Limited. Bengaluru, India. Sodium lauryl sulfate was procured from rxchemicals, Mumbai, India. Lyophilizer from Lyophilization Systems India Private Limited, Hyderabad, India. Dissolution tester from Electro lab, Mumbai, India. Cooling ultra sonicator from Athena Technology from Mumbai, India Double distilled water was prepared in the laboratory and all other reagents utilized in the study were of analytical quality.

2.2. Methods

2.2.1. Preparation of Glimepiride Nanosuspension by Solvent Evaporation Method

The solvent evaporation approach was used to create GLM-NS.[11] Poloxamer 188 was weighed out and properly mixed with the 50 mL amount of water (aqueous phase). A 2 mg of GLM drug and calculated PVP K30 were dissolved in acetone (organic phase). Under ultra-sonication, organic phase was introduced to aqueous phase for a given time. This solution was allowed to be agitated by magnetic fields. After cooling the solution, the NS was lyophilized using lyophilizer. The freeze-dried materials were put through characterization tests.

2.2.2. QbD Driven Method Development

Analyzing Critical Attributes (CAAs): Critical analytical attributes were those that had the greatest impact on the quality of the output. CAAs were identified and selected by compiling a complete list of relevant analytical features and connecting them to the method's unique quality outputs. Among the CAAs, particle size (PS), percentage cumulative drug release (CDR %), and viscosity (VS) were chosen for the investigation based on literature and a complete grasp of the existing analytical procedure (**Table 1**).

Factor Screening Studies: The independent variables were identified by chosen CAAs, such as PS, CDR%, and VS, a L8 (2^7) Taguchi design process with seven factors and eight runs were utilized. Further, statistical analysis was conducted by using Design Expert software version 8.0.6, Minneapolis, MN, USA. **Table 2** depicts the seven factors and their levels chosen for screening studies. Ultimately, by properly reading the Pareto charts, the influential factors among them were determined. Taguchi design chart displayed in Table 2. Amount of HPMC (mg) (A), amount of PVP (mg) (B), volume of ethyl acetate-EA[12] (C), volume of acetone[12] (D), Agitation time (h) (E), amount of SLS (mg) (F), and amount of Poloxamer (mg) (G) factors were selected.

Experimental Design: A Taguchi OA was used in this investigation to find parameters that had a significant influence on the size and VS of the GLM-NS. Taguchi OAs were often classified as having fractional factorial designs. The main advantage of using a factorial design was the decreased number of experiments required to gather maximum information.[1] Because of its nature of the solvent evaporation process used for nanoparticle production in this investigation, a seven variable, four-level values matrix (Table 2) was designed, with the PS (the diameter in nanometers), CDR%, and VS as the desired output parameters. Table 2 showed the Taguchi array, which resulted in an optimum combination after eight tests. All of the variable values were established and employed as input functions in our statistical model based on our thorough pre-optimization tests by using BBD as RSM, which yielded preliminary point estimates for the variables as shown in **Table 3**. To get the accuracy required for the final statistical conclusion, all trials were replicated three times. Finally, the statistically optimized run was performed three times

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Table 1. Application of CMAs and CPPs to evaluate the targets.

Quality target product profile (QTPP)		Parameters		
TPP	Target	TPQP	CMAs materials	CPPs
Formulation type	Nanosusepnsion	Particle size	PVP [mg]	Agitataion time [h]
		Viscosity		
		X-ray diffraction		
		PDI		
		Particle morphology:TEM		
Solubility	Enhancement of solubility	In vitro drug release Method AssesSment: USP type II apparatus	Poloxamer [mg]	
		Method of assessement: kinetics		

Table 2. Taguchi orthogonal array design table.

Run	Factor 1 A:HPMC	Factor 2 B:PVP	Factor 3 C:EA	Factor 4 D:Acetone	Factor 5 E:AT	Factor 6 F:SLS	Factor 7 G:Poloxamer	Response 1 Particle Size	Response 2 CDR %	Response 3 Viscosity
	[mg]	[mg]	[mL]	[mL]	[h]	[mg]	[mg]	[nm]	[%]	[Cps]
1	5	50	20	20	3	4	15	267	95	0.85
2	5	25	10	10	1	4	15	278	82	0.94
3	10	25	20	10	3	4	20	271	87	0.89
4	10	50	10	10	3	8	15	230	93	0.92
5	5	25	10	20	3	8	20	280	86	1.43
6	5	50	20	10	1	8	20	295	99	1.75
7	10	25	20	20	1	8	15	270	84	1.54
8	10	50	10	20	1	4	20	300	90	1.68

Table 3. Box–Behnken design and characterization of GLM-NS.

Run	Formulation code	Factor 1 A:PVP	Factor 2 B:Agitation time	Factor 3 C:Poloxamer	Response 1 PS	Response 2 CDR	Response 3 Viscosity
		[mg]	[h]	[mg]	[nm]	[%]	[cps]
1	F1	50	2	20	246.8	93	0.87
2	F2	50	1	17.5	295	87.3	0.89
3	F3	50	2	15	281.1	89	0.91
4	F4	37.5	3	20	258	98.4	1.002
5	F5	25	1	17.5	267	90.4	0.978
6	F6	37.5	2	17.5	246	91.6	1.52
7	F7	37.5	3	15	265	92	1.105
8	F8	25	2	15	226	91.3	0.78
9	F9	37.5	2	17.5	245	92.7	1.54
10	F10	25	3	17.5	268	96.9	1.079
11	F11	37.5	2	17.5	243	92.4	1.56
12	F12	37.5	2	17.5	247	92.4	1.55
13	F13	25	2	20	261	98	1.45
14	F14	37.5	2	17.5	245	92.3	1.54
15	F15	37.5	1	15	264	86.2	0.79
16	F16	50	3	17.5	278	93.4	0.68
17	F17	37.5	1	20	277	92	1.56

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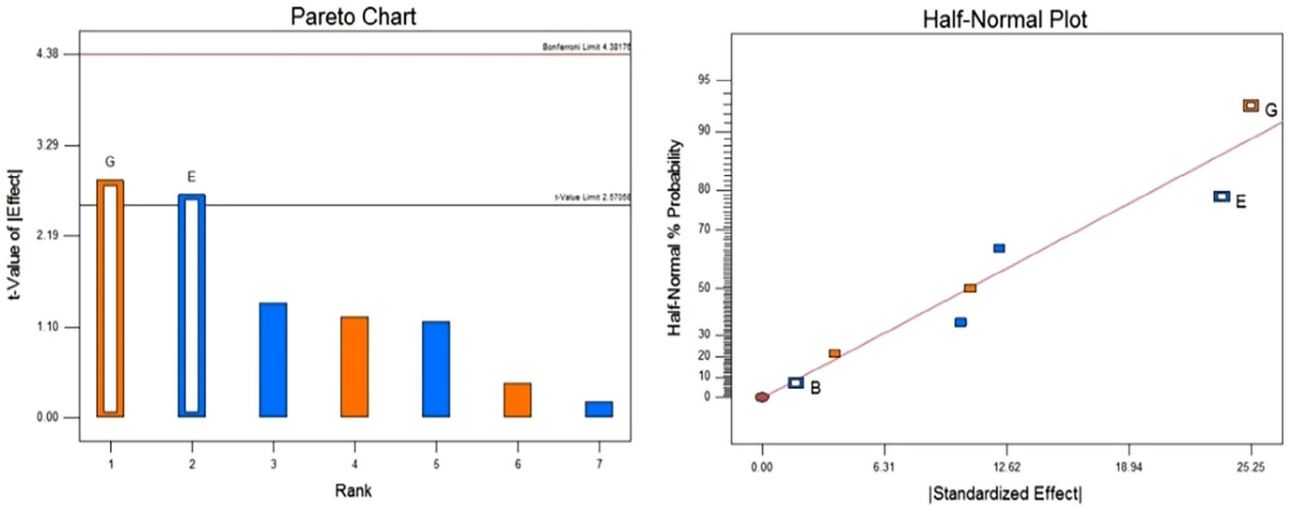

Figure 1. Pareto chart of CDR%.

with and without drug to confirm the estimated optimal variable value setting.

2.2.3. Characterization of Glimepiride Nanosuspension

Particle Size Analysis: A dynamic laser light scattering technique (Horiba scientific SZ-100, Japan) was used for determining the PS and zeta potential of nanosuspension. Polydispersity index (PDI) was used to study the breadth of particle size dispersion of prepared NS. The ideal PDI should be in the range of 0–1. PS, size distribution, morphological, topographical, compositional, and crystalline information were studied by using high resolution powerful microscope.

Transmission Electron Microscopy (TEM): Prepared NS were imaged by using TEM (200 kV TEM JEOL 2000FX). The NS were dropped on copper–gold carbon grids and dried at room temperature, followed by taking photographing at suitable magnification.

Fourier Transform Infrared: FTIR analysis was done by using Agilent Cary 630 bench top FTIR scanned in the range of 400–4000 cm^{-1}.

Differential Scanning Calorimetry: DSC analysis was done on 2–4 mg samples weighed in a Sartorius BP 210 S electronic microbalance, Goettingen, Germany, using a DSC-60 Shimadzu (Japan). The samples were heated in open aluminum pans at a rate of 10 °C min^{-1}. Indium was taken as reference and the hold temperature was maintained at 300 °C.

X-Ray Diffraction: X-ray diffraction patterns of GLM and GLM-NS were obtained using a D8-Advance Bruker AXS diffractometer with Cu Kα radiation, at a wavelength of 1.5406 Å at 25 °C from a generator operating at 40 kV and current of 40 mA.

Rheopexy: Flow response of the suspension with respect to the applied force was studied by Brookfield Rheometer (LVD-VNext) using spindle number 61 at the temperature of 24 °C. Rheopexy was obtained to study the rheological characteristics of the prepared suspension.

In Vitro Dissolution: Drug release characteristics of GLM-NS were examined using a USP type II dissolution equipment (Electro lab dissolution Tester USP EDT 08L) at 50 revolutions per minute and phosphate buffer pH 6.8 as the dissolution medium at 37.5 °C. A 5 mL sample was obtained at intervals ranging from 1 to 24 h. The equal volume of new dissolution media was added. The quantity of GLM-NS released was determined spectrophotometrically at max 228 nm

3. Result and Discussion

3.1. Factor Screening Study

Pareto charts evident that, the factors amount of Poloxamer, amount of PVP, and agitation time have profound influence on response factors, PS, CDR%, and VS (**Figures 1–3**). The "*t*" value of the factors was between *t* value limit and Bonferroni limit, which indicates the significant influence of the factors.[13] In the pareto chart, the color orange denotes a positive influence, while the color blue denotes a negative influence of the corresponding screening factors on the response factors. Hollow blocks indicate screening variable selected for the study. The order in which variables affect the response factors are indicated by the rank axis. Half normal probability plot was used to study the importance and influence of CAAs along with pareto chart.[14] More the deviation from the zero value of *X* axis indicates better the significance of the variable. From the screening study, it was evident that amount of Poloxamer (surfactant), amount of PVP (polymer), and agitation time had significant ($p < 0.05$) influence on CAAs, hence they were chosen as CMP for the optimization study.

3.2. ANOVA

The Model *F*-value of 7.75, 8.73, and 36.79 respectively for responses PS, CDR%, and VS implies the model is significant. There is only a 2.94%, 2.34%, and 0.70% noise probability for the selected models respectively. Model terms were significant when "Prob > *F*" is less than 0.0500. Agitation time and amount of Poloxamer amount of PVP are found to be significant model terms as per the ANOVA.

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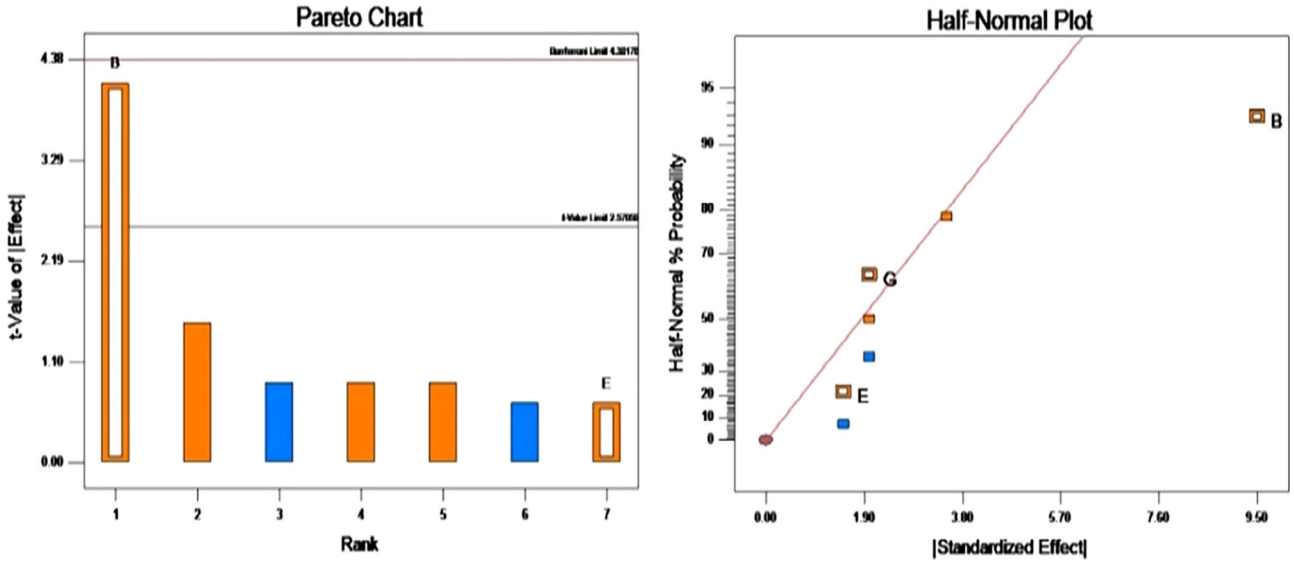

Figure 2. Pareto chart of particle size.

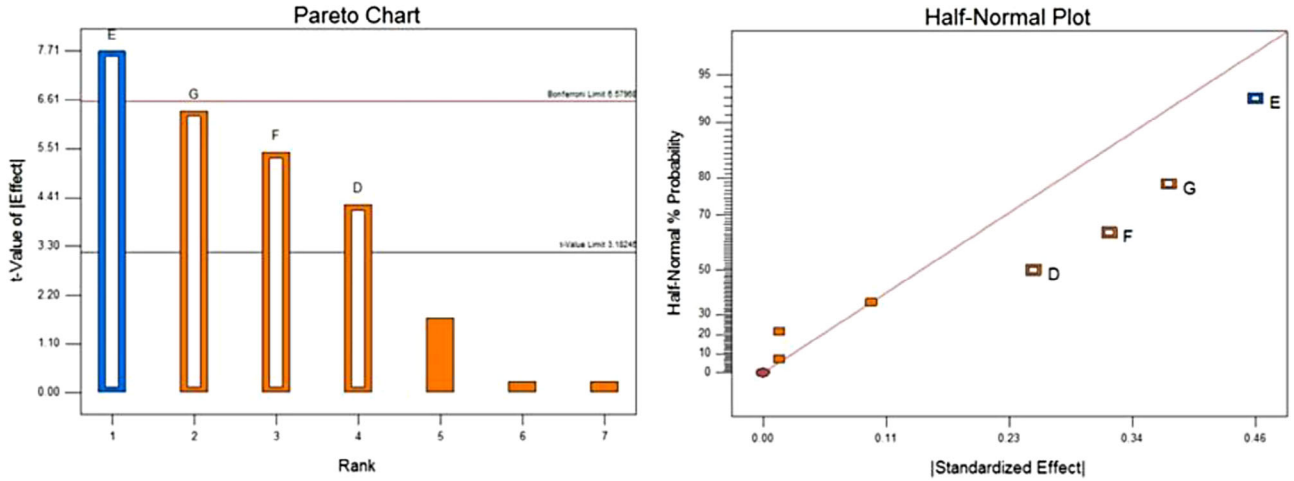

Figure 3. Pareto chart of viscosity.

3.3. Optimization of Glimepiride by Box–Behnken Design

3.3.1. Effect of Independent Variable on CDR% (Cumulative Drug Release)

In source A-PVP, B-Agitation time, and C- Poloxamer shows significant *p*-value (<0.0001) and in interaction effect AC shows *p*-value as significant compared to AB and BC as shown in ANOVA (**Table 4**).

$$\text{CDR} = +92.28 - 1.74\,\text{A} + 3.10\,\text{B} + 2.86\,\text{C} - 0.10\,\text{AB} - 0.68\,\text{AC} + 0.15\,\text{BC} + 0.20\,\text{A}^2 - 0.48\,\text{B}^2 + 0.35\,\text{C}^2 \quad (1)$$

AB, AC, and BC were tested for their effects on CDR% using the factor tool. The results of the study claimed that two variable factor AB and BC show the curve in a additive fashion and parallel to one another. As a result, AB and BC have no interaction effect. However, AC (PVP and Poloxamer) affect CDR% (response variable). PVP and Poloxamer (AC) have no additive effect on CDR%. As the CDR% declines from 98% to 93% at high concentrations of Poloxamer (20 mg) with higher slope as that of low concentration of Poloxamer (15 mg) CDR% goes down from 91% to 90% with less slope (**Figure 4**A). It was caused by the increasing diffusional path length in high concentrations of Poloxamer. Because the central block is hydrophobic and the two end blocks are hydrophilic, the poloxamer acts as a polymer surfactant.[15] According to the graphs, AC (two orders of interaction) has antagonistic effects on CDR%.

3.3.2. Effect of Independent Variable on Particle Size (PS)

The *p*-value (<0.0001) indicates (Table 4) that PVP (A), agitation time (B), and their combination respectively, have a synergistic and antagonistic effect on particle size as a response

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Table 4. ANOVA outcomes and statistical values of the QbD model.

Parameters	Source	Sum of squares	df	Mean square	*F* value	*p*-value	
CDR%	Model	170.09	9	18.9	128.37	<0.0001	Significant
	A-PVP	24.15	1	24.15	164.06	<0.0001	
	B-Agitation time	76.88	1	76.88	522.23	<0.0001	
	C-Poloxamer	65.55	1	65.55	445.28	<0.0001	
	AB	0.04	1	0.04	0.27	0.6183	
	AC	1.82	1	1.82	12.38	0.0097	
	BC	0.09	1	0.09	0.61	0.4599	
	A^2	0.16	1	0.16	1.12	0.3259	
	B^2	0.96	1	0.96	6.52	0.0379	
	C^2	0.51	1	0.51	3.45	0.1055	
	Residual	1.03	7	0.15			
	Lack of fit	0.36	3	0.12	0.72	0.5885	Not significant
	Pure error	0.67	4	0.17			
	Cor total	171.12	16				
PS	Model	4859.62	9	539.96	272.66	<0.0001	Significant
	A-PVP	778.15	1	778.15	392.93	<0.0001	
	B-agitation time	144.5	1	144.5	72.97	<0.0001	
	C-Poloxamer	5.61	1	5.61	2.83	0.1362	
	AB	81	1	81	40.9	0.0004	
	AC	1200.62	1	1200.62	606.27	<0.0001	
	BC	100	1	100	50.5	0.0002	
	A^2	401.29	1	401.29	202.64	<0.0001	
	B^2	2044.85	1	2044.85	1032.57	<0.0001	
	C^2	6.45	1	6.45	3.26	0.1141	
	Residual	13.86	7	1.98			
	Lack of fit	5.06	3	1.69	0.77	0.5692	Not significant
	Pure error	8.8	4	2.2			
	Cor total	4873.48	16				
VS	Model	1.77	9	0.2	397.01	<0.0001	Significant
	A-PVP	0.11	1	0.11	221.6	<0.0001	
	B-agitation time	0.015	1	0.015	31.27	0.0008	
	C-poloxamer	0.21	1	0.21	424.59	<0.0001	
	AB	0.024	1	0.024	48.82	0.0002	
	AC	0.13	1	0.13	254.47	<0.0001	
	BC	0.19	1	0.19	384.72	<0.0001	
	A^2	0.59	1	0.59	1186.02	<0.0001	
	B^2	0.29	1	0.29	582.49	<0.0001	
	C^2	0.12	1	0.12	234.28	<0.0001	
	Residual	3.47E-03	7	4.95E-04			
	Lack of fit	2.59E-03	3	8.62E-04	3.92	0.11	Not significant
	Pure error	8.80E-04	4	2.20E-04			
	Cor total	1.77	16				

CDR%, cumulative drug release percentage; df, degree of freedom; PS, particle size; VS, viscosity.

variable. The combination of AB (PVP and Agitation time), AC (PVP and Poloxamer), and BC (Agitation time and Poloxamer) shows a greater antagonistic effect on particle size. It was asserted by respective *p*-value and coded equation. In addition to that the coded factor claims that a synergistic effect was observed in binate amount of constrained independent variable such as A and B.

$$\text{Particle size (PS)} = +245.20 + 9.86\,A - 4.25\,B + 0.84\,C - 4.50\,AB - 17.33\,AC - 5.00\,BC + 9.76\,A^2 + 22.04\,B^2 - 1.24\,C^2 \quad (2)$$

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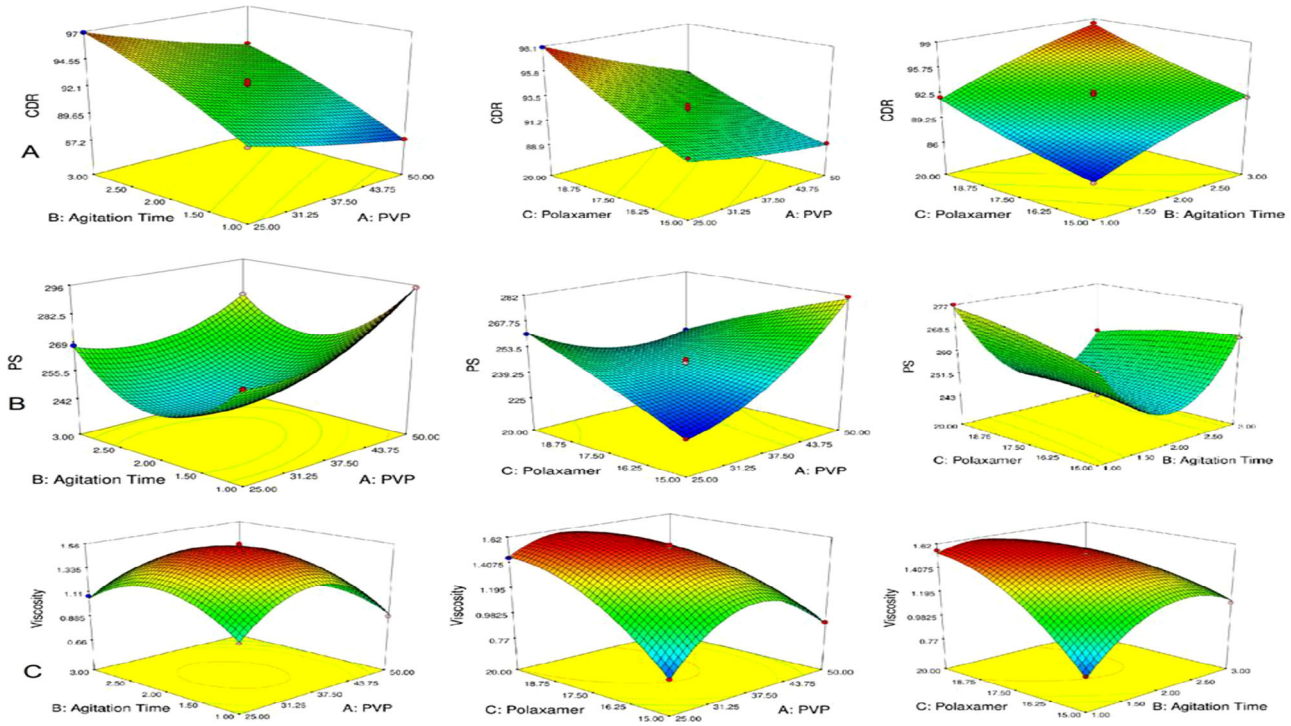

Figure 4. Response surface plots showing the simultaneous influence of independent variables on response parameters A) CDR%, B) PS, and C) VS of GLM-NS within the experimental design.

Graph (3D surface) claims that term AC (PVP and Poloxamer) was associated with negative effects on particle size under constrained conditions as PVP increases,particle size leads to an antagonistic effect with higher level of Poloxamer, results in a reduction of particle size from 261 to 247 nm, (Figure 4B). As contrast the low level of poloxamer shows synergestic effect on PS,as witnesed in increasing the particle size range from 225 to 280 nm. Hence, forth the anatgostic effect was caused by higher concentration of poloxamer, reduce the interfacial tension between the solvent and the particles,in addition to that agitation time resulted in a smaller particle size.[16] Shear forces due to the collision of particles and high velocity help to fracture particles with inherent crystal defects.

In term BC (Agitation time and Poloxamer) identified with antagonstic effects on PS under constrained conditions of increasing agitation time with higher level of poloxamer. Decrease in particle size from 277 to 257.5 nm was evident from the impressed negative effect in coded factor as well as in graphical representations (3D surface).The above illustrated effect was caused by gradual increasing in agitation time and that causes detructive breakage and abrasion,[17] that may diminish the powder particle size with increasing effective surface area, Besides that, higher levels of poloxamer act as polymer surfactants and help the powder to suspend. Both of these mechanisms made sure that the drug particle will crumble and communicate longer with agitation, which was directly related to size reduction.[18]

Based on the analysis of the three second order interactions (AB, AC, and BC), two of them (AC, BC) shown greater influence on response factor (particle size), which suggested they were considered as risk factors in formulation.

3.3.3. Effect of Independent Variable on Viscosity (VS)

The *p*-value (<0.0001) demonstrated that PVP (A), Agitation time (B), and poloxamer (C), individually and in combination, have a synergistic and antagonistic influence on VS as a response variable. The interaction was investigated using a quadratic model, as indicated by the design of expert. The model explicitly asserts that the lack of fit was non-significant (0.1100), implying that the input variable has a considerable effect on response factor. The combination of AB (PVP and Agitation time), AC (PVP and Poloxamer), and BC (Agitation time and Poloxamer) had the strongest antagonistic effect on viscosity. A *p*-value and coded equation were used for assertion. Furthermore, the coded factor claims that a negative effect was observed in binate amounts of constrained independent variables such as A, B, and C.

$$\text{Viscosity} = +1.54 - 0.12\,A - 0.044\,B + 0.16\,C - 0.078\,AB - 0.18\,AC - 0.22\,BC - 0.37\,A^2 - 0.26\,B^2 - 0.17\,C^2 \quad (3)$$

In term BC (Agitation time and Poloxamer) identified negative impact on VS under constrained conditions. As factor B increases with high level of factor C. The viscosity drop down. The graphs represented the decline of VS from 1.53 to 1.01 cps (Figure 4C). According to viscosity theory, this can be due to increased agitation time, which effects small structural break down in the fluid, leading to a decrease in viscosity. Shear thinning mechansim helps to explain this phenomenon.[19]

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Table 5. Cross-validation of actual and predicted values of the optimized GLM-NS under optimal conditions.

S. No	Optimized formulation code	Response	Predicted value	Experimental value	% prediction error
1.	**O-GLM-NS**	PS	258.16	254.13	1.56
		CDR %	98.52	99.24	0.73
		Viscosity	1.087	1.073	1.28

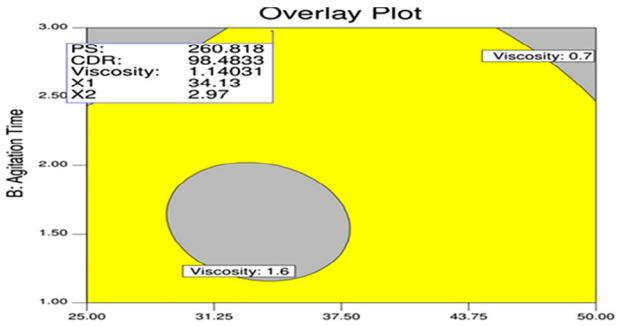

Figure 5. Overlay plot of optimized GLM-NS.

The model for AB (PVP and Agitation Time) under constrained conditions of as factor A increases VS decreases at both high level and low level of factor B,results shown two parabolic parallel curves in elision fashion (open downwards). The graphs confirm there are no input variables that influence the response factor and conclude there is no impact on viscosity for AB order of interaction.

A study using AC (PVP and Poloxamer) with constrained conditions as factor A increases VS decreases at both level of factor C. It was confirmed AC factor does not display an interaction effect on VS.

3.3.4. Optimization

From the desired surface response function, the optimized method can be derived directly from the higher desired value. The desirability feature was observed to be higher (close to 1) for the optimized formula suggesting the suitability of the formulations. The GLM-NS was formulated with 34.1 mg of PVP (A), agitation time as 2.97 h (B), and Poloxamer 20 mg (C) as mentioned in overlay plot, which are considered as optimized input variables for the response factors (**Figure 5**).

3.3.5. Prepration of Checkpoint from Overlay Plot

Check point were selected from the overlay plot of response. The amount of PVP, Agitation time, and Poloxamer were given in the overlay plot flag (Figure 5).

Overlay plot shown the appropriate hatching operation zone (yellow color) for producing the specified CDR%, PS, and VS. A minimum 86.2 hatching percentage and maximum 98.4% for CDR, for PS minimum 226 nm and maximum 295 nm, and for VS minimum 0.68 and maximum 1.56 hatching cps (**Table 5**) is set to ensure economical operation. The bright yellow (default) on the plot shown where the entire range of all intervals meets the specified criteria.

3.4. Characterization

3.4.1. Particle Size and Zeta Potential

The particle size and zeta potential of nanosuspension samples were measured at 25 °C. The PS of GLM in NS for the optimized formulation was found to be 254.1 ± 2 nm. The zeta potential of nanoparticles was observed as −25.2 ± 1 mV. Because of electrostatic repulsion, negative zeta potential values imply that nanoparticles are stable.[20] The electrophoretic mobility value was found to be −0.000196 cm^2 Vs^{-1}. The less electrophoretic mobility is accounted to better viscosity of the prepared NS. Polydispersity index (PDI) was found to be 0.157 which indicate homogenous distribution of polymer chain molecular weights in the NS, uniformity in cross-linking and network formation with more orderly arrangement.[21] The results of particle size and zeta potential are depicted in **Figures 6** and **7**.

3.4.2. TEM Analysis

According to (Farkas and Kramar, 2021), TEM results are well correlated with the particle size determined by dynamic light scattering. (**Figure 8**) shows TEM images of the GLM-NS in a spherical and uniform shape, with an even particle sizes distribution of <250 nm. There was no evidence of nanoparticle aggregation. A TEM analysis showed that the GLM nanoparticles contained in the prepared NS were uniform and spherical.

3.4.3. FT-IR

FTIR spectra of Glimepiride shown characteristic peak at 3288 and 3369 cm^{-1} corresponding to N—H stretching. 1707 and 1674 cm^{-1} correspond to carbonyl stretching, 1345 cm^{-1} CH stretching, 1153 cm^{-1} showing S = O stretching. FTIR spectra of drug admixed with excipient and NS of the GLM shown peaks at similar wavenumbers exhibited by the standard drug, but the % transmittance value was found to be less for admixture and NS compared to the pure drug (**Figure 9**). This decrease in the % transmittance can be accounted for chemical interaction or bond formation of the drug with excipients.[22]

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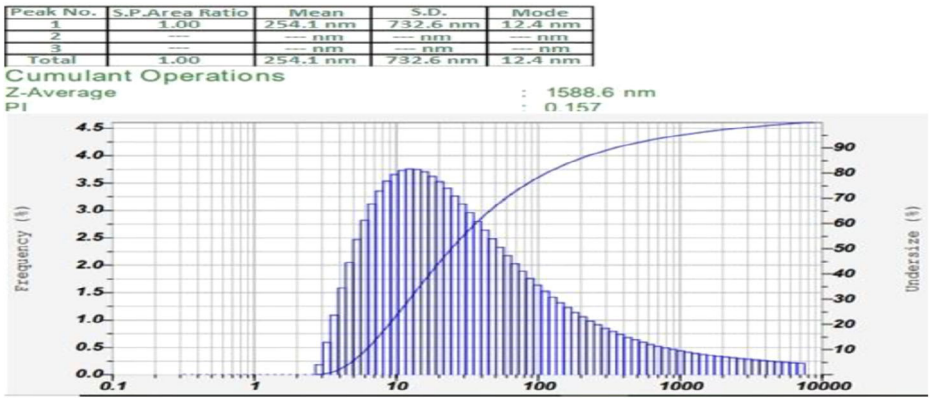

Figure 6. Particle size of optimized GLM-NS.

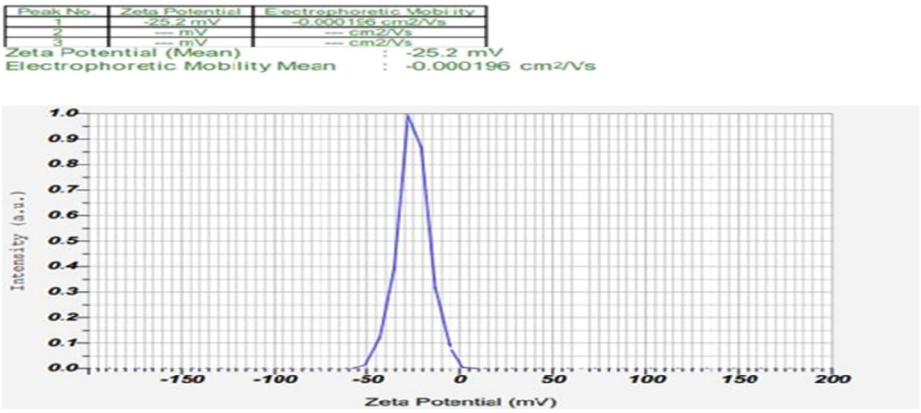

Figure 7. Zeta Potential of optimized GLM-NS.

3.4.4. DSC Analysis

Thermal behavior of pure drug and GLM-NS are depicted (**Figure 10**). The DSC curve of GLM profiles a sharp endothermic peak at 234.18 °C with heating rate of 96.01 J kg^{-1} corresponding to its melting point, indicating its crystalline nature. However, the characteristic endothermic peak, corresponding to drug melting was slightly shifted to 264.34 °C at heating rate of 87.23 J kg^{-1}. The shift in endothermic peak may be accounted due to interaction of GLM with hydrophilic polymers in the formulation.[23] This interaction might lead to enhancement in aqueous solubility of GLM.[24]

3.4.5. X-Ray Diffractometry

Figure 11 shows the X-ray diffraction patterns of GLM and its NS. Crystallinity can be determined by comparing peak heights

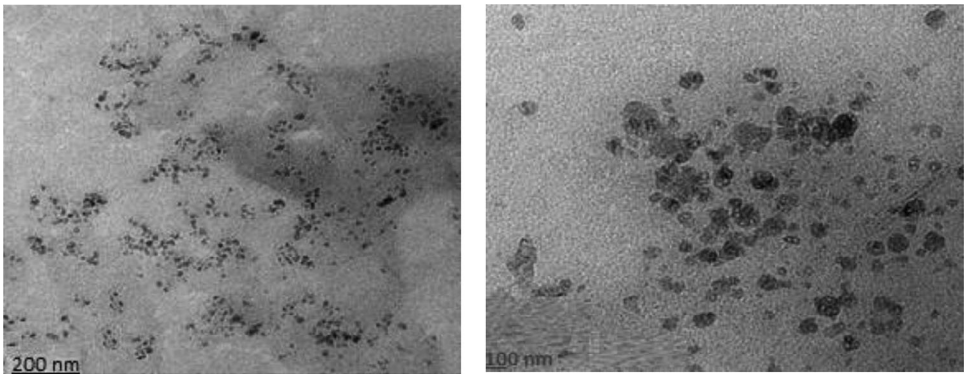

Figure 8. TEM of optimized GLM-NS.

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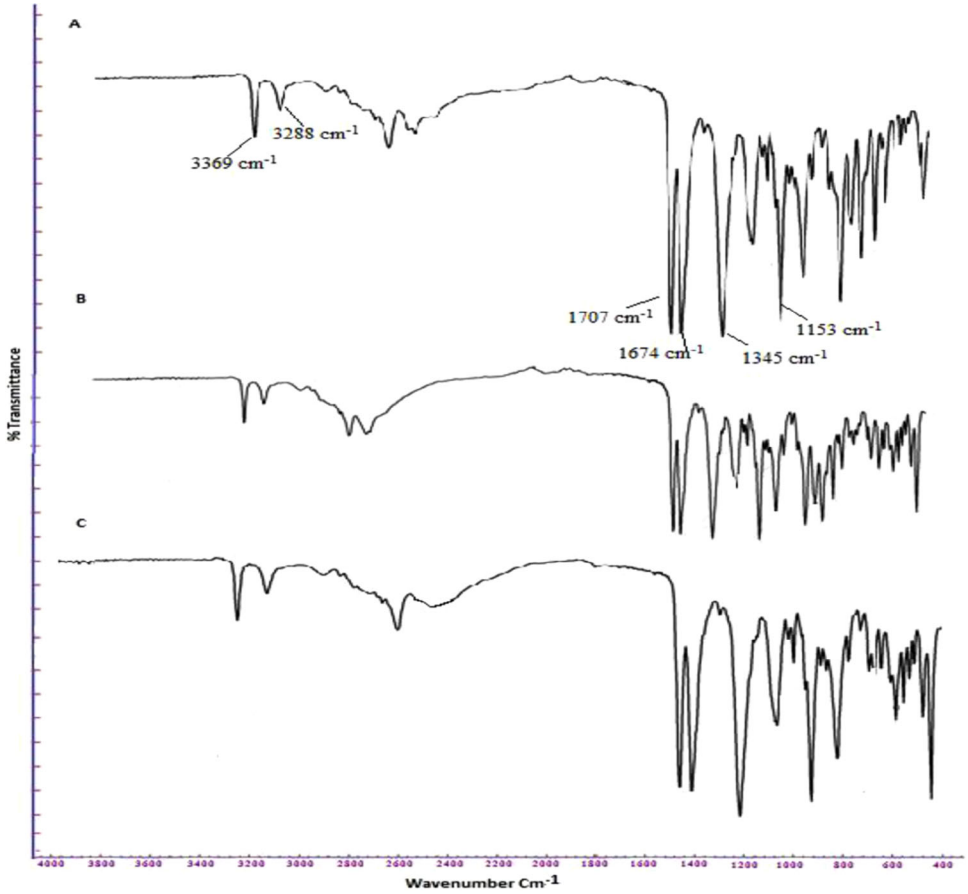

Figure 9. FTIR spectrum of A) free Glimepiride (GLM), B) excipients, and C) optimized GLM-NS.

of characteristic peak in the diffraction pattern of GLM–NS sample with that of a standard. Relative Degree of Crystallinity (RDC) is used to calculate the crystallinity. The 2θ value of characteristic peak in pure drug was found to be 24.52°. Similarly, 2θ value of prepared nanosuspension was found to be at 24.12° (Figure 11). But the intensity of the peak in NS decreased noticeably compared to that of standard drug. The decrease in peak intensity can be accounted to reduction in crystallinity due to inclusion of excipients with amorphous nature.

The RDC value is calculated by measuring the ratio of the intensity of standard 2θ value (Cs) with that of NS 2θ value (Ct).[25] The RDC value of the NS was found to be 0.558. This reduction in crystallinity is attributable to low crystallinity indicating chemical bond formation with excipients which is more water soluble. RDC value is measured by the following formula.

$$\text{RDC} = \frac{\text{Ct}}{\text{Cs}} \tag{4}$$

3.4.6. Rheopexy

The Rheopexy was plotted against speed in rpm Vs Viscosity (**Figure 12**). VS of the suspension was found to increase gradually with respect to the increase in speed of the system. The consistency curve shown that obtained flow was dilatant. The resultant increment in VS owes to, an open form of particles packing, and the bulk of the system expands or dilates, leading to increase in void volume and insufficient number of vehicles to fill the void. Henceforth, the particles are neither wet nor lubricated and produce flow resistance.

3.5. In vitro Drug Release Studies and Release Kinetics

Depending on the independent factors, the percent CDR ranged from 86.2% to 98.4% for up to 24 h (**Figure 13**). A greater amount of agitation time and poloxamer, as well as moderate levels of PVP, were associated with the highest percent CDR (98.4%). However, with a lower amount of agitation duration and polaxmer, the lowest percent CDR (86.2%) was recorded. It was critical to understand the release mechanism and kinetics when attempting to develop a NS. GLM-NS had biphasic drug release profiles, with the faster drug release phase occurring first (burst effect). The unbound drug and drug molecules that adsorbed on the GLM-NS surface could be responsible for the burst effect (**Figure 14**). The rate of release of the entrapped GLM is determined by the rate of diffusion of GLM from the polymer and surfactant matrix.The partitioning of GLM between the lipid phase and the aqueous dissolution media is also important for GLM diffusion from polymer and surfactant matrix.

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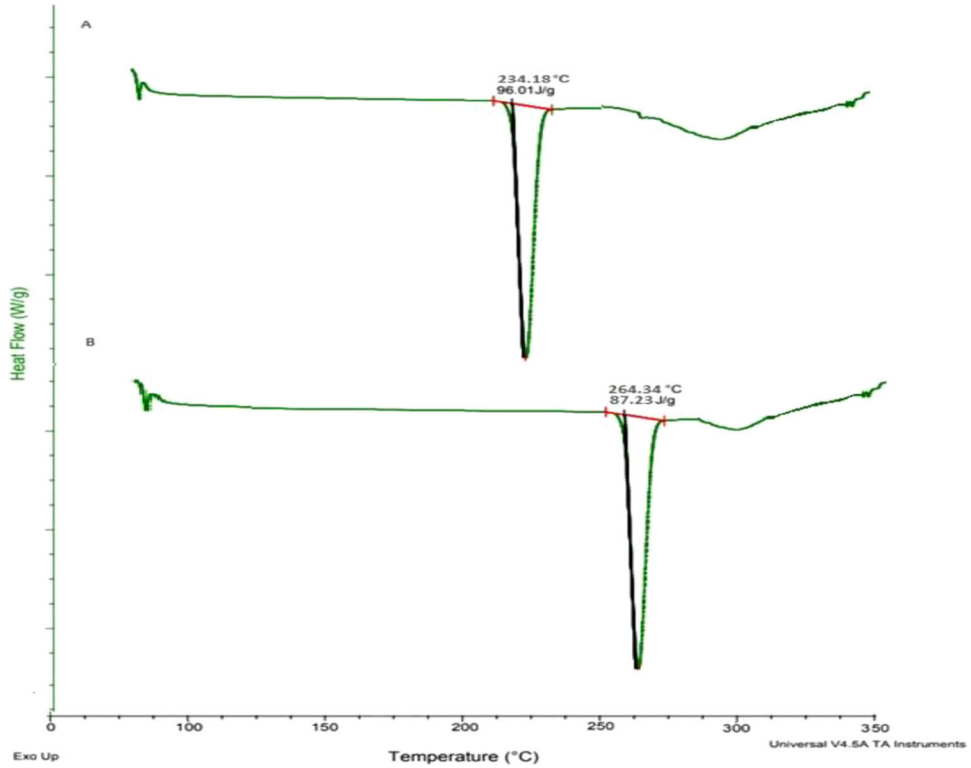

Figure 10. DSC thermogram of A) GLM, B) optimized GLM-NS.

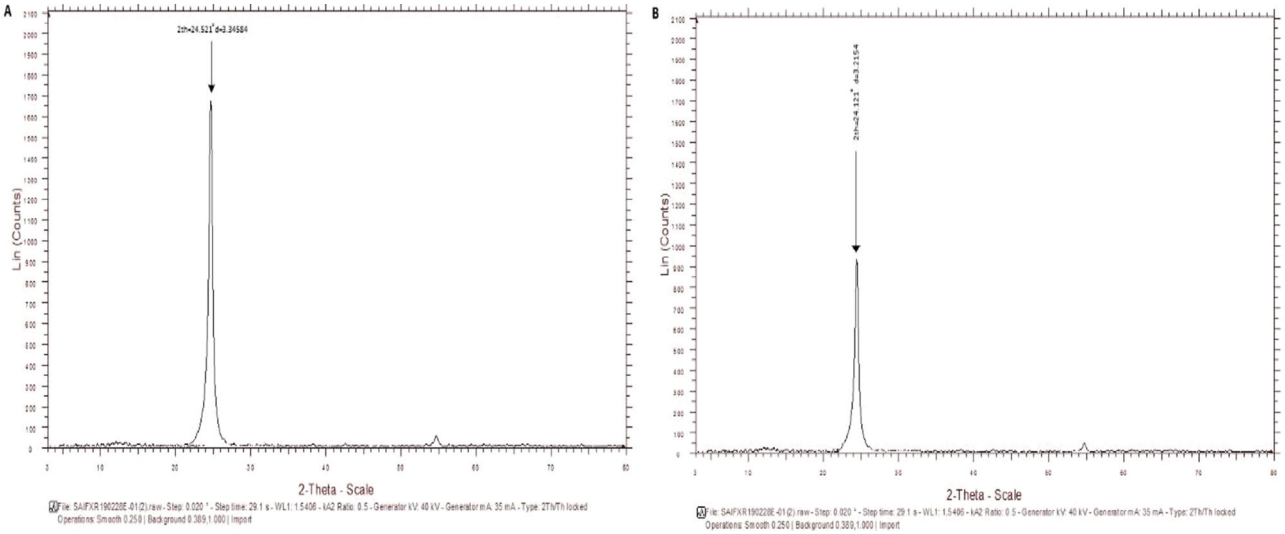

Figure 11. XRD of A) GLM, B) optimized GLM-NS.

The drug release kinetic is controlled by one or more processes that are determined by the matrix composition, geometry, manufacture technique, and dissolving medium of drug release. To study the drug release characteristics, and to know the release mechanism,various mathematical model used in the study and quality of fit of a model measured by R^2 value. The in vitro drug release data from GLM-NS were fitted into zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. **Table 6** shows the drug release kinetics data. To confirm the diffusion process, the data were fitted to Korsemeyerpeppa's equation with (n) values ranging from 0.88 to 1.0, revealing that during released, from a zone of highly swollen diffusion and separated from a region of dry, glassy diffusion by a sharp boundary. This suggests that the drug is released in a non-fickian manner by following both diffusion and relaxation mechanisms.

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Table 6. In vitro release kinetics of GLM-NS formulations up to 24 h.

FMLN code[a)]	Zero order		First order		Higuchi	Korsmeyer–Peppas	
	K_0	R	K_1	R	R	n	R
F1	2.643	0.968	−0.02	0.973	0.8787	0.888	0.828
F2	3.034	0.889	−0.031	0.9729	0.977	0.916	0.78
F3	3.560	0.995	−0.037	0.9057	0.930	0.997	0.901
F4	3.687	0.966	−0.053	0.818	0.984	0.919	0.799
F5	3.409	0.964	−0.038	0.975	0.991	0.915	0.797
F6	3.425	0.991	−0.045	0.650	0.962	0.904	0.818
F7	3.404	0.971	−0.036	0.926	0.987	0.937	0.826
F8	3.516	0.989	−0.036	0.883	0.925	0.918	0.824
F9	3.842	0.996	−0.030	0.876	0.918	1.005	0.939
F10	3.741	0.984	−0.004	0.767	0.906	0.987	0.874
F11	3.623	0.992	−0.036	0.845	0.921	1.035	0.911
F12	3.483	0.992	−0.035	0.856	0.944	0.984	0.878
F13	4.196	0.989	−0.051	0.787	0.892	1.024	0.937
F14	3.559	0.971	−0.042	0.966	0.989	0.912	0.795
F15	3.534	0.999	−0.031	0.926	0.937	1.094	0.943
F16	3.718	0.991	−0.038	0.848	0.909	1.036	0.908
F17	3.711	0.996	0.039	0.913	0.955	1.022	0.893

[a)] Additional information is attached in supplementary file.

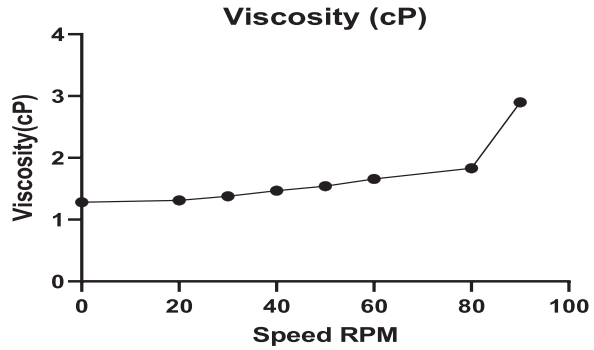

Figure 12. Consistency curve of optimized GLM-NS.

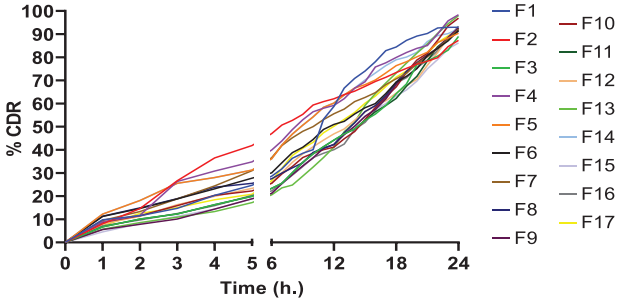

Figure 13. In vitro release profiles of GLM-NS up to 24 h F1–F17.

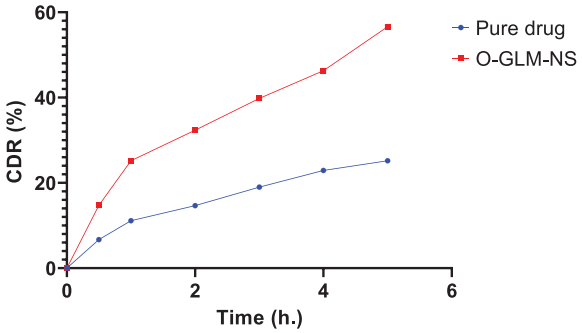

Figure 14. In vitro dissolution studies of Optimized Glimepiride nanosuspension and pure drug.

4. Conclusion

GLM-NS with increased solubility was successfully developed and evaluated with the aid of QbD technique. The technique was used to better understand the influence of CMAs and CPPs on CQAs and to improve formulation quality and safety. The impact of process and formulation factors on CQAs of GLM-NS, as well as CDR%, PS, and VS, was optimized using through Taguchi and BBD within a QbD framework. Because of the reduced particle size, increased surface area, and hydrophilicty of the GLM-NS, the dissolution velocity and release rate were significantly increased. Optimized Nano formulation had the PS, CDR% and VS as 254.13 nm, 99.24%, 1.073 cPs, respectively. The in vitro drug release kinetics confirmed that GLM was released from the NS by a non-Fickian process. GLM-NS could be utilized to deliver GLM in order to provide controlled drug release in systemic circulation, which could improve solubility and reduce toxicity for effective diabetic therapy. Furthermore, with the implementation of the QbD idea in this study, relatively few experimental runs are utilized for the optimization of NS, indicating a reduction in manufacturing cost. The lowest residual error values obtained indicate a reduction in manufacturing variability. Based on the current study, it is possible to infer that QbD is a useful tool

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in NDDS for reducing production variability, improving quality and safety, and lowering manufacturing costs.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

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

Keywords

glimepiride, non-Fickian kinetics, quality-by-design, response surface methodology, viscosity

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