FORMULATION DEVELOPMENT OF GLIBENCLAMIDE (GBC) LOADED NANOSTRUCTURED LIPID CARRIERS (NLCS) USING BOX-BEHNKEN DESIGN

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Abstract

The formulation development of Glibenclamide (GBC) loaded nanostructured lipid carriers (NLCs) using Box-Behnken design is the focus of this study. GBC is a widely used antidiabetic drug, and the utilization of NLCs as carriers can enhance its bioavailability, stability, and targeted delivery. The Box-Behnken design, a statistical experimental design, is employed to optimize the formulation variables for achieving desirable NLC characteristics. In this study, NLCs were prepared using a solvent injection method. The independent variables considered for optimization were the concentration of solid lipid Glycerylmonostearate, concentration of liquid lipid (Caprol PGE-860, and the concentration of surfactant Pluronic F127. The dependent variables evaluated were entrapment efficiency and particle size. The Box-Behnken design allowed the investigation of 17 experimental runs, including both factorial and axial points. The obtained data were analyzed using response surface methodology to establish mathematical models correlating the independent and dependent variables. The models were validated, and the optimized formulation was determined using desirability function. The particle size of GBC-loaded NLCs for formulation F1 to F17 was found to be between 86.74 to 126.65nm respectively The Entrapment efficiency of GBC-loaded NLCs for formulation F1 to F17 was found to be between 60.74 to 73.32 percentages respectively. The Zeta Potential value of formulations OF1, OF2, and OF3 were found to be -35.65, -41.38 and -40.65 mV respectively.In comparison to all formulations, formulation OF3 showed sustain drug release from formulation up to 24 hrs. The formulation showed 13.25, 18.95, 22.23, 36.65, 48.85, 59.98, 69.89, 86.65 and 98.85 percentage after 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hrs. respectively. This study highlights the successful utilization of the Box-Behnken design in the formulation development of GBC-loaded NLCs. The optimized formulation exhibited desirable characteristics, indicating its potential as a delivery system for GBC in the treatment of diabetes. Further in vitro and in vivo studies are warranted to evaluate its pharmacokinetic and therapeutic efficacy.

Keywords: Glibenclamide, Nanostructured lipid carriers, Box-Behnken design, Optimization, Antidiabetic, Formulation development.

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Introduction

Nanostructured lipid carriers (NLCs) are a promising class of lipid-based nanocarriers that have gained significant attention in the field of pharmaceutical and biomedical research. NLCs offer numerous advantages as drug delivery systems, including enhanced drug solubility, improved stability, controlled drug release, and potential targeting capabilities. These attributes make NLCs attractive for the delivery of various drugs, including hydrophobic and poorly water-soluble compounds (1).

The development of NLCs involves the incorporation of lipids, such as solid lipids and liquid lipids, to form a structured matrix with nanoscale dimensions. The unique composition and structure of NLCs contribute to their superior performance compared to conventional lipid carriers, such as liposomes and solid lipid nanoparticles (SLNs). NLCs exhibit improved drug loading capacity, reduced drug expulsion, and enhanced physical stability, making them suitable for a wide range of therapeutic applications (2).

The incorporation of solid lipids in NLCs provides a solid matrix that helps prevent drug expulsion and offers improved drug entrapment efficiency. However, the inclusion of liquid lipids in the formulation further enhances the flexibility and deformability of the lipid matrix, thereby improving drug encapsulation and release characteristics. The combination of solid and liquid lipids in NLCs results in a more optimized lipid carrier system with improved performance (3).

The versatility of NLCs allows for the incorporation of different types of drugs, including small molecules, peptides, proteins, and nucleic acids. The surface of NLCs can be modified with ligands, targeting moieties, or other functional molecules to achieve site-specific drug delivery and enhance therapeutic efficacy. Additionally, the small particle size of NLCs facilitates their passive targeting to certain tissues and enhances their cellular uptake (4).

The formulation of NLCs can be optimized using various techniques, including statistical experimental designs such as the Box-Behnken design. This approach enables systematic investigations of multiple formulation variables and their effects on NLC characteristics, helping to identify optimal formulation conditions (5).

Overall, NLCs represent a promising and versatile platform for drug delivery with potential applications in various therapeutic areas. The ability to tailor their composition, size, and surface properties makes NLCs an attractive option for improving the solubility, stability, and targeted delivery of a wide range of drugs.

Glibenclamide (GBC) is a widely prescribed antidiabetic drug belonging to the sulfonylurea class. It is used in the management of type 2 diabetes mellitus to lower blood glucose levels by stimulating insulin secretion from pancreatic β-cells (6). However, GBC is associated with poor aqueous solubility, low bioavailability, and limited tissue penetration, which can result in suboptimal therapeutic outcomes (7).

To overcome these challenges and improve the delivery of GBC, various drug delivery systems have been investigated. One promising approach is the utilization of nanostructured lipid carriers (NLCs), which are lipid-based nanocarriers designed to encapsulate hydrophobic drugs like GBC (8). NLCs offer several advantages, including enhanced drug solubility, improved stability, prolonged drug release, and the ability to target specific sites (9).

In the formulation development of GBC-loaded NLCs, optimization of formulation variables is crucial to achieve desirable characteristics such as particle size, entrapment efficiency, and drug release. The Box-Behnken design, a statistical experimental design, is widely used to systematically investigate the effects of multiple variables and optimize the formulation process (10). It allows for the determination of the optimal levels of each variable and the establishment of mathematical models correlating the variables with the desired responses.

The objective of this study is to utilize the Box-Behnken design to optimize the formulation variables for the development of GBC-loaded NLCs. By employing this design, we aim to achieve NLC formulations with desirable characteristics, including small particle size, high entrapment efficiency, and sustained drug release. The optimized formulation holds the potential to improve the therapeutic efficacy of GBC in the treatment of diabetes.

Material and Methods

A design of experiment (DOE) (2^3 full factorial design) has been used in order to look into the key

effects, as well as interactions of three critical process parameters (CPPs), namely, solid lipid (Glycerylmonostearate) amount (A), liquid lipid (Caprol PGE-860) amount (B) and concentration of surfactant (C), on the prepared NLC critical quality attributes (CQAs), namely, % EE (R1), Particle Size (R2).

Formulation development of Glibenclamide (GBC) loaded nanostructured lipid careers

Glibenclamide loaded nanostructured lipid careerswere prepared by the solvent injection method. An accurately weighed amount of drug along with the lipid (Glycerylmonostearate (GMS) and Caprol PGE-860) was dissolved in ethanol (1 mL) at 40◦C as per table 1. The whole lipid phase was rapidly injected using a 1 mL glass syringe and into the aqueous phase (30 mL) containing measured amount of Pluronic F127 (surfactant) pre-stirred at a specific speed on the magnetic stirrer (REMI, India). The stirring was continued for 2 h to evaporate the organic solvent. The resulting dispersion was filtered (0.45) to remove any excess lipids. Samples were withdrawn for particle size analysis followed by separation of NLCs by centrifugation at 50,000 rpm for 1 h. The recovered NLCs were washed twice and re-suspended in 2 mL of deionized water. They were subsequently frozen at −80 ◦C and freeze–dried in Dry/Shell Freeze System at -10◦C for 48 h using 5% mannitol as cryoprotectant. The obtained freeze–dried particles were stored in refrigerator for further analysis (11).

Freeze-drying of NLC dispersion

GBC-NLCs dispersions were lyophilized to obtain formulation in dry form. The obtained GBC-NLC was frozen using freezer at -20ºC for overnight; GBC-NLCs were transferred to the lyophilizer at the temperature at -70ºC for 48 h. After lyophilizationGBC-NLC lyophilized form was collected from lyophilizer and was subjected to physicochemical characteristics, in-vitro studies.

Table 1: Formation design according to 2^3 factorial designs

Std	Run	Factor 1: Glycerylmonostearate (mg)	Factor 2: Caprol PGE-860 (mg)	Factor 3: Pluronic F127 (%)
17	1	225	100	1
8	2	300	100	1.5
7	3	150	100	1.5
14	4	225	100	1
9	5	225	50	0.5
1	6	150	50	1
5	7	150	100	0.5
4	8	300	150	1
3	9	150	150	1
15	10	225	100	1
11	11	225	50	1.5
13	12	225	100	1
6	13	300	100	0.5
10	14	225	150	0.5
16	15	225	100	1
2	16	300	50	1
12	17	225	150	1.5

Final Equation in Terms of Coded Factors

Entrapment Efficiency =+68.71+1.95A+2.41B+2.47C-3.16AB+0.7750AC+2.53BC+1.71 A^2-1.83 B^2-1.55 C^2.

Final Equation in Terms of Actual Factors

Entrapment Efficiency = +50.76375-0.047227Glycerylmonostearate-+0.282815 Caprol PGE-860+2.61650 Pluronic F127-0.000841 Glycerylmonostearate * Caprol PGE-860+0.020667 Glycerylmonostearate * Pluronic F127+0.101100 Caprol PGE-860 * Pluronic F127+0.000304 Glyceryl monostearate².

Final Equation in Terms of Coded Factors

Particle Size = +118.84+6.48A -3.05B-5.52C-1.37 AB-2.30 AC-16.73 BC-10.50 A^2-5.62 B^2-1.44 C^2.

Final Equation in Terms of Actual Factors

Particle Size = -95.14375+1. Glycerylmonostearate+1.13990Caprol PGE-860+81.19500Pluronic F127-0.000365Pluronic F127+0.000667Glycerylmonostearate * Caprol PGE-860-0.061267Glycerylmonostearate * Pluronic F127-0.669100Caprol PGE-860 * Pluronic F127-0.001867Glyceryl monostearate²-0.002248Caprol PGE-860²-5.76500Pluronic F127².

Evaluation of prepared GBC-NLC

Entrapment efficiency

Entrapment efficiency (EE) of GBC-NLC was determined by measuring the concentration of unentrapped drug in aqueous medium by centrifugation method. The nanoparticles were centrifuged in a high speed cooling centrifuge (C-24, Remi) using Nanosep centrifuge tubes with ultrafilter having molecular weight cutoff 100 KD (Pall life sciences, India) at 5,000 rpm for 15 min at 4◦C, and the supernatant was separated. The amount of drug in the supernatant was determined at 234nm using UV-Vis spectrophotometer (Labindia 3000+) after appropriate dilution (12). The percentage entrapment efficiency (% EE) was calculated by using the following formula:

$$\%EE = \frac{\text{Total drug content} - \text{Free drug Total drug content}}{\text{Total druntg conte}} x\ 100$$

Determination of particle size

The average particle size of GBC-NLCwas measured using dynamic light scattering, Malvern zetasizer (Malvern zetasizer, Worcestershire, UK) from SAIF RGPV, Bhopal. The sample was kept in polystyrene cuvette and observations were found at 90 fixed angle. The dispersion sample was diluted to 1:9 v/v with de-ionized water and distilled water to ensure that the light scattering intensity was within the instrument sensitivity range. Polydispersity index is a range of measurement of particles sizes within measured sample (13). The polydispersity index calculated as the average weight divided by the number average molecular weight. It is used to indicate vesicles diameter distribution range.

Experimental results with predicted responses:

One way to evaluate the quality of predicted responses is to compare them to actual responses from a subset of the experiments. This is often done by randomly selecting a portion of the experimental runs to serve as a validation set, and using the remaining runs to build a predictive model. To evaluate the impact of using predicted responses on the experimental results, the analysis can be conducted both with and without the predicted responses. This allows the effects of the predicted responses to be isolated and quantified.

Based on the Design of Experiments (DOE) formulation, four optimized formulations have been selected for the preparation of nanostructured lipid carriers. These formulations were chosen because the experimental values for their composition were found to be very similar to the predicted values, and they were also within the acceptable limit. This indicates that the predictive model used in the DOE formulation was accurate and reliable. Therefore, the use of these optimized formulations is likely to result in nanostructured lipid carriers with desirable properties. It is important to note that any report on the use of these formulations should acknowledge the use of DOE and clearly state the basis for their selection. Additionally, it should provide details about the experimental values and their comparison to the predicted values to support the decision-making process.

Table 5.19: Experimental results with predicted responses

Formulation	Run Order	Composition Glycerylmonostearate (mg)/Caprol PGE-860 (mg)/Pluronic F127 (%)	Response	Predicted value	Experimental value
OF1	3	150/100/1.5	% EE	68.61	69.05
			Particle Size	97.20	96.65
OF2	9	150/150/1.0	% EE	71.86	72.21
			Particle Size	94.55	94.85
OF3	17	225/150/1.5	% EE	73.14	72.74
			Particle Size	86.49	86.74

Zeta Potential

The zeta potential of the NLC was analyzed by an electrophoretic light-scattering (ELS) spectro=-photometer (Malvern zetasizer, Worcestershire, UK). The samples were directly added in a quartz cuvette, and all measurements were carried out at 25°C (14).

Determination of drug content

The NLC sample 10mg should be properly prepared by dispersing it in a 10ml methanol, to extract the drug from the lipid matrix. Take 0.1 ml of the extracted drug and dilute up to 10ml with methanol. Take the absorbance of this solution and take the absorbance with UV-Vis spectrophotometer (Labindia 3000+) and calculate the drug content using celebration curve method (14).

In vitro drug release study

To perform the dissolution study of NLC, an appropriate volume (Equivalent to 10mg) of the NLC suspension was added to a dialysis bag, and the bag was placed in the release medium

(100ml). The release medium was stirred at 100 rpm on a magnetic stirrer. During the initial 2 hours, the NLC were exposed to artificial gastric juice with a pH of 1.2. After 2 hours, the release medium was replaced by pH 6.8 phosphate buffer. At specific time intervals, 3 mL of the dispersion was withdrawn from the release medium, and an equal volume of fresh release medium was added promptly to maintain a constant volume. The samples were analyzed using the UV method under the same analytic conditions as described previously (15).

Release Kinetics of Optimized formulation OF3

Release kinetics models are commonly used to describe the behavior of drug release from a formulation over time. The most frequently used models include zero order, first order, Higuchi, and Peppas.

Zero-order kinetics:

Formula: $Q = k0 * t$

Where Q is the amount of drug released at time t, and k0 is the zero-order release rate constant.

Interpretation: Zero-order kinetics indicates that the drug release rate is constant over time, regardless of the drug concentration. This model suggests that the drug release is primarily controlled by a diffusion barrier or the degradation of the polymer matrix.

First-order kinetics:

Formula: $\ln(Q) = -kt + \ln(Q0)$

Where Q is the amount of drug released at time t, Q0 is the initial amount of drug in the formulation, k is the first-order rate constant, and ln is the natural logarithm.

Interpretation: First-order kinetics assumes that the drug release rate is proportional to the drug concentration and that the release rate decreases over time. This model suggests that the drug release is primarily controlled by the degradation of the polymer matrix or drug molecule.

Higuchi kinetics:

Formula: $Q = kh * t^{(1/2)}$

Where Q is the amount of drug released at time t, and kh is the Higuchi dissolution constant.

Interpretation: Higuchi kinetics assumes that the drug release rate is proportional to the square root of time. This model suggests that the drug release is primarily controlled by the diffusion of the drug through the polymer matrix.

Korsmeyer-Peppaskinetics:

Formula: $Q = k * t^n$

Where Q is the amount of drug released at time t, k is the Peppas release rate constant, and n is the Peppas exponent.

Interpretation: Peppas kinetics is a power law model that describes the release behavior of drug formulations that exhibit non-Fickian or anomalous diffusion. This model suggests that the drug release is controlled by both the polymer matrix and the drug molecule.

Results and Discussion

The particle size of NLCs is a critical parameter that influences their stability, drug release kinetics, and biodistribution. Smaller particle sizes are often desired as they offer several advantages, including increased surface area, improved cellular uptake, and potential for enhanced tissue penetration. Additionally, smaller particles tend to exhibit improved physical stability, as they are less prone to aggregation or sedimentation. The particle size of GBC-loaded NLCs for formulation F1 to F17 was found to be between 86.74 to 126.65nm respectively table 2. In the case of GBC-loaded NLCs, achieving a small and uniform particle size is important to ensure their effective delivery and distribution at the target site. By optimizing the formulation variables, such as the concentrations of solid and liquid lipids, as well as the homogenization speed, it is possible to control the particle size of NLCs. The use of the Box-Behnken design facilitates the identification of optimal conditions for achieving the desired particle size.

Entrapment efficiency refers to the amount of drug that is successfully encapsulated within the lipid matrix of NLCs. It is a crucial parameter that determines the drug loading capacity and efficacy of the NLCs as drug delivery systems. Higher entrapment efficiency indicates that a larger proportion of the drug is encapsulated and protected from degradation or premature release. The Entrapment efficiency of GBC-loaded NLCs for formulation F1 to F17 was found to be between 60.74 to 73.32 percentages respectively table 2.

The entrapment efficiency of GBC-loaded NLCs can be influenced by multiple factors, including the lipid composition, drug-lipid interactions, preparation method, and processing conditions. By optimizing these variables, it is possible to maximize the entrapment efficiency of GBC

within the NLCs, leading to improved drug stability and controlled release.

The factors affecting the drug encapsulation within the lipid matrix. It would address how the selected lipid composition, drug-lipid interactions, and processing conditions influenced the entrapment efficiency of GBC. The discussion would also consider any possible strategies to improve the entrapment efficiency, such as modifying the formulation variables or incorporating other excipients. Particle size and entrapment efficiency in GBC-loaded NLCs would provide insights into the formulation development process, optimization strategies, and the potential implications of the obtained results for the therapeutic efficacy of the NLCs as a drug delivery system. The particle size and entrapment efficiency of GBC-loaded NLCs would typically involve comparing the obtained results with the desired or targeted values. It would address the influence of the formulation variables on these parameters and how they can be optimized to achieve optimal particle size and entrapment efficiency.

Zeta potential is a measure of the electrical charge present on the surface of nanoparticles, including NLCs. It plays a crucial role in the stability and behavior of colloidal systems, as it determines the degree of repulsion or attraction between particles. A high absolute value of zeta potential indicates a strong repulsive force, leading to increased stability and reduced particle aggregation or flocculation. On the other hand, a low absolute value of zeta potential may result in particle aggregation and instability.

In the context of GBC-loaded NLCs, the zeta potential of the optimized formulations (OF1, OF2, and OF3) would be an important parameter to assess the stability and potential for particle aggregation. A higher absolute value of zeta potential would indicate stronger repulsive forces between the NLCs, promoting their stability and preventing particle aggregation. Conversely, a lower absolute value could suggest a higher risk of particle aggregation and potential instability.

The zeta potential can also provide insights into the surface charge characteristics of the NLCs. Depending on the nature of the lipid components used in the formulation, the NLCs may exhibit a positive or negative zeta potential. This surface charge can influence the interactions of NLCs with biological components, such as cell membranes or proteins, potentially affecting their cellular uptake and targeting capabilities. The Zeta Potential value of formulations OF1, OF2, and OF3 were found to be -35.65, -41.38 and -40.65 mV respectively Table 3.

In vitro drug release studies are essential to evaluate the release profile and kinetics of a drug from the NLC formulation. These studies provide insights into the release mechanism, duration, and extent of drug release, which are crucial for assessing the formulation's efficacy and potential therapeutic applications.

In vitro drug release study results for OF1, OF2, and OF3 would typically involve comparing the release profiles and characteristics among the different formulations. It would address factors such as the initial burst release, sustained release, and total release of GBC from the NLCs over the study duration.

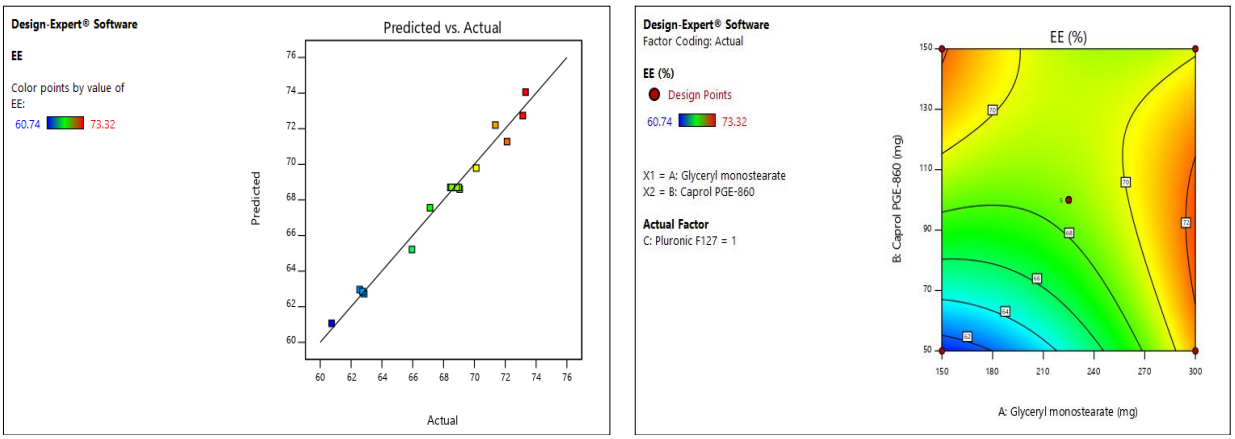
The optimized formulations OF1, OF2, and OF3 may exhibit different drug release profiles due to variations in the formulation variables, such as the lipid composition, surfactant choice, or drug loading capacity. In comparison to all formulation, formulation OF3 showed sustain drug release from formulation up to 24 hrs. The formulation showed 13.25, 18.95, 22.23, 36.65, 48.85, 59.98, 69.89, 86.65 and 98.85 percentage after 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hrs. respectively table 4.

The drug release data suggests a sustained release pattern over the studied time period. Different Release kinetics models Zero-order kinetics, First-order kinetics, Higuchi model and Korsmeyer-Peppas Model. The maximum r^2 value was found 0.978 in Korsmeyer-Peppas model. The formulation follow Korsmeyer-Peppas release kinetics table 5 & 6.

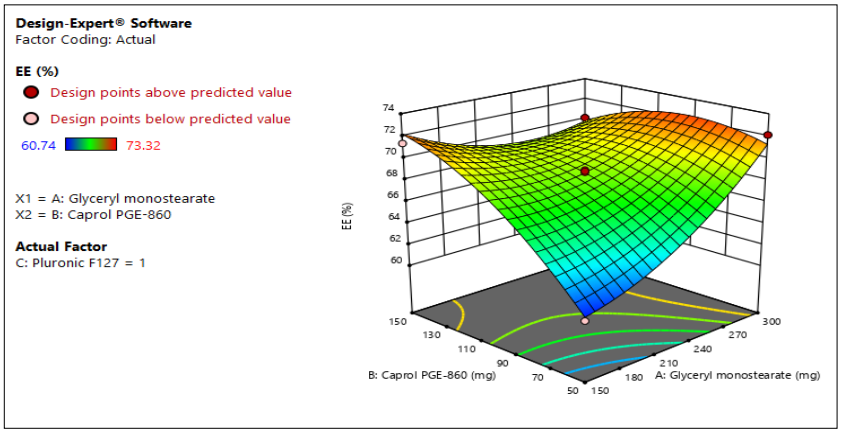
Table 2: Results of particle Size and entrapment efficiency

F. Code	Response 1: % EE	Response 2: Particle Size (nm)
F1	68.52	118.85
F2	73.32	105.25
F3	69.05	96.65
F4	68.74	117.74
F5	62.56	103.36
F6	60.74	97.85

F7	65.95	103.95
F8	70.12	104.85
F9	71.36	94.85
F10	68.85	118.85
F11	62.74	126.65
F12	68.98	119.98
F13	67.12	121.74
F14	62.85	130.36
F15	68.44	118.78
F16	72.12	113.32
F17	73.14	86.74

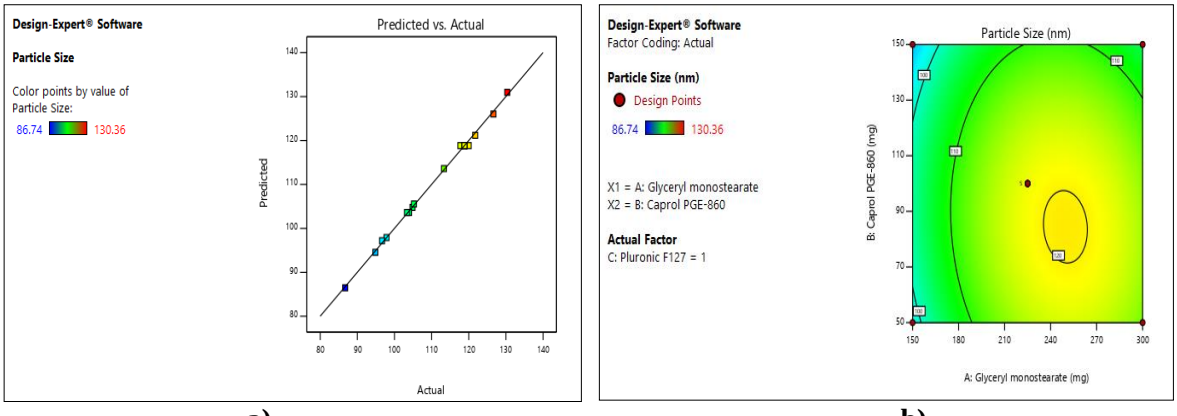


(a) **(b)**



(c)

Figure 1: a) Response surface plots for Entrapment Efficiency, b) Contour graph of Entrapment Efficiency (GlycerylmonostearatevsCaprol PGE-860), c)3D surface plot of Entrapment Efficiency (GlycerylmonostearatevsPluronic F127)



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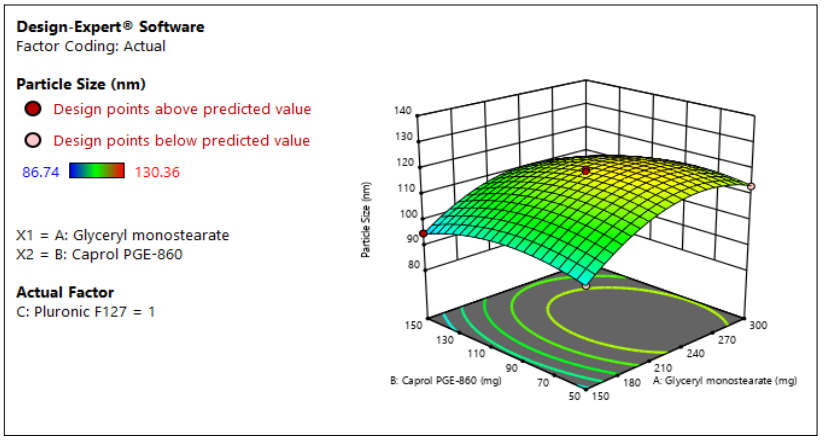


c)

Figure 2: Response surface plots for particle size (Predicted vs. Actual), b)Figure 17: Contour graph of particle size (GlycerylmonostearatevsCaprol PGE-860), c)3D surface plot of particle size (GlycerylmonostearatevsPluronic F127)

Table 3: Results ofZeta Potential of optimized formulation OF1, OF2 and OF3

S.No.	Formulation Code	Zeta Potential (mV)
1.	OF1	-35.65
2.	OF2	-41.38
3.	OF3	-40.65

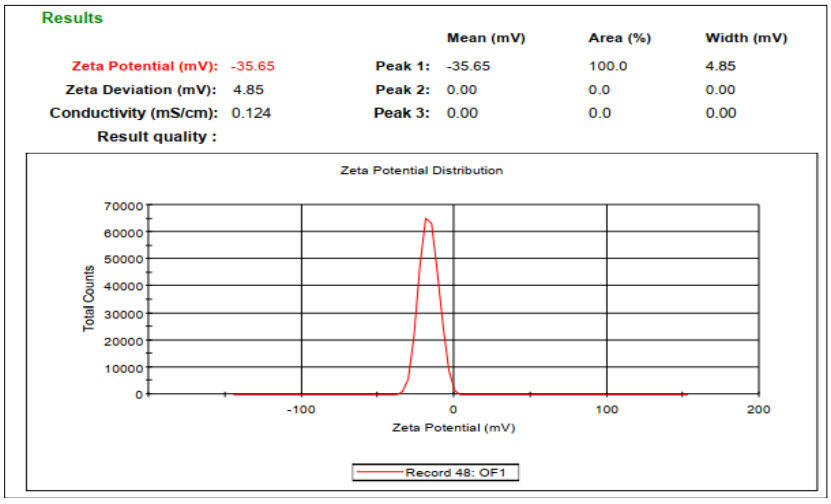


Figure 3: Graph of Zeta Potential of formulation OF1

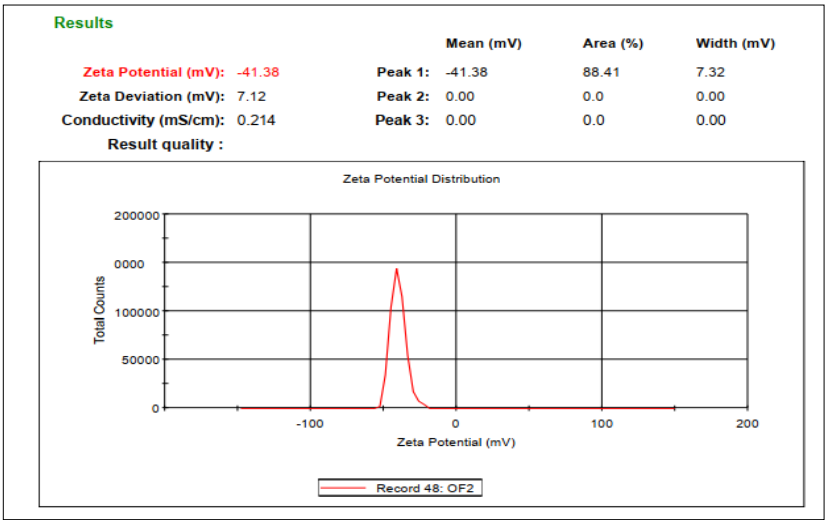


Figure 4: Graph of Zeta Potential of formulation OF2

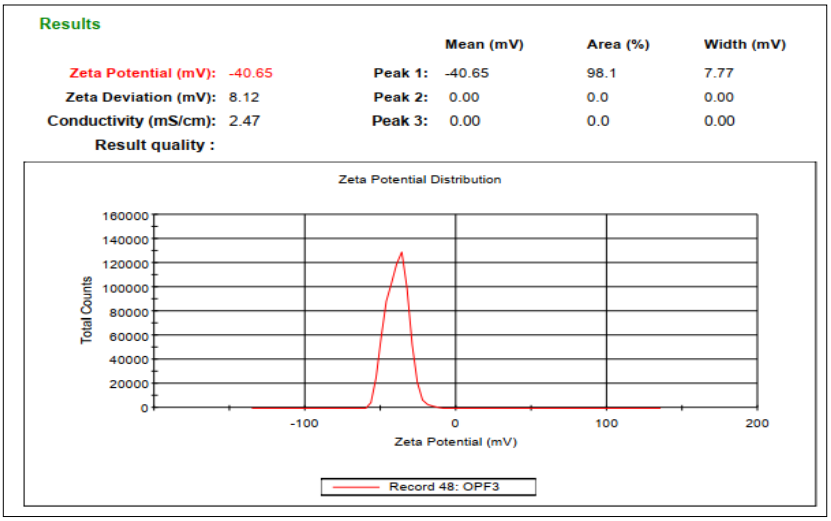


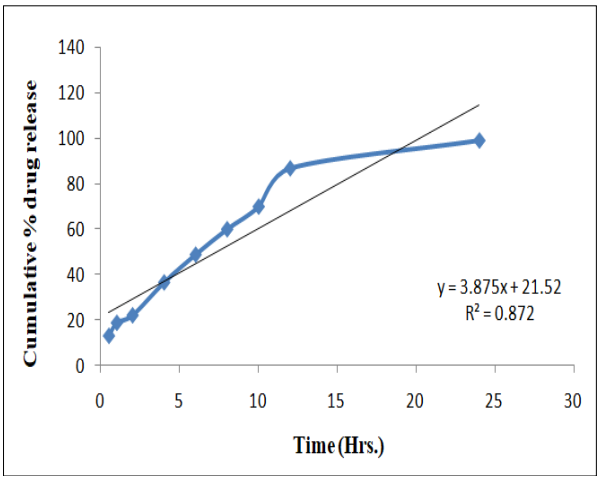
Figure 5: Graph of zeta potential of formulation OF3

Table 4: In vitro drug release study of formulation OF1, OF2 and OF3

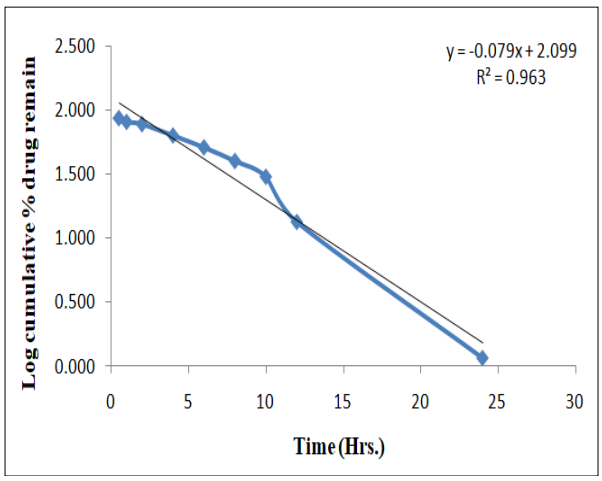
Time (Hrs.)	Formulation Code		
	OF1	OF2	OF3
0.5	22.32	20.23	13.25
1	36.65	32.25	18.95
2	46.65	45.65	22.23
4	55.85	53.32	36.65
6	69.98	65.85	48.85
8	83.36	82.12	59.98
10	92.25	90.25	69.89
12	98.85	96.69	86.65
24	99.12	98.12	98.85

Table 5: Results of In vitro drug release kinetics data of formulation OF3

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	13.25	1.122	86.75	1.938
1	1.000	0.000	18.95	1.278	81.05	1.909
2	1.414	0.301	22.23	1.347	77.77	1.891
4	2.000	0.602	36.65	1.564	63.35	1.802
6	2.449	0.778	48.85	1.689	51.15	1.709
8	2.828	0.903	59.98	1.778	40.02	1.602
10	3.162	1.000	69.89	1.844	30.11	1.479
12	3.464	1.079	86.65	1.938	13.35	1.125
24	4.899	1.380	98.85	1.995	1.15	0.061



a)



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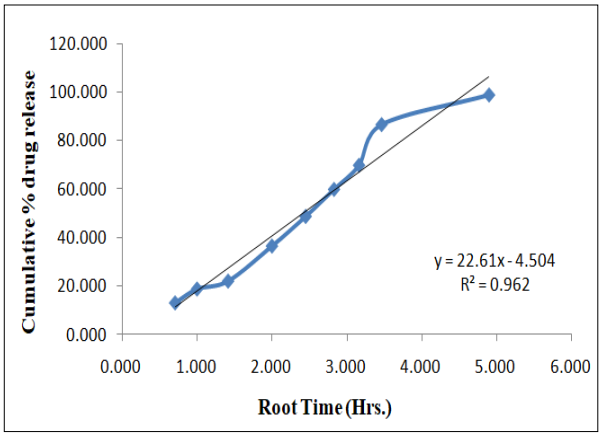

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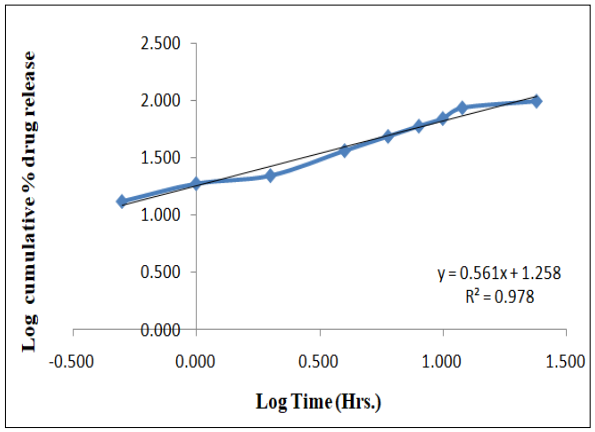

d)

Figure 6: Release Kinetics Graph of of formulation OF3, a) Zero order, b) First order, c) Higuchi, d) KorsmeyersPeppas

Table 6: Regression analysis data of optimized formulation OF3

Batch	Zero Order	First Order	Higuchi's Model	KorsmeyersPeppas Equation
	R^2	R^2	R^2	R^2
OF3	0.872	0.963	0.962	0.978

Conclusion

The Box-Behnken design is a response surface methodology that enables the systematic optimization of multiple formulation variables to achieve desired product characteristics. After evaluating different formulation variables and their levels, OF3 was determined to be the optimized formulation based on predefined criteria, such as particle size, entrapment efficiency, zeta potential, or in vitro drug release. The specific parameters used for optimization may vary depending on the objectives of the study. The conclusion of the formulation development study highlights the successful application of the Box-Behnken design in optimizing the formulation of GBC-loaded NLCs. OF3 represents the formulation with the most desirable characteristics among the evaluated formulations. The formulation OF3, identified as the optimized formulation, holds promise for further development and potential applications in drug delivery systems involving GBC. Future studies may focus on evaluating its performance in terms of stability under different storage conditions, in vitro and in vivo efficacy, targeted drug delivery, and compatibility with various administration routes. The formulation development of GBC-loaded NLCs using the Box-Behnken design resulted in the identification of the optimized formulation, OF3. This conclusion sets the stage for further research and development of this formulation as a potential drug delivery system for GBC, with the aim of improving therapeutic outcomes and patient compliance.

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