Formulation and Optimization of Glyburide Loaded Graphene Oxide Nanocomposites for Sustained Release Drug Delivery: 3^2 Factorial Approaches

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ABSTRACT

Glyburide has a high plasma protein binding (99.8%) and less oral bioavailability, both of which indicate that its first-pass effect is anticipated to be minimal. The work aims to investigate the design and optimization of glyburide loaded graphene oxide nanocomposites for sustained release delivery. The Hummer's method was employed to synthesize graphene oxide. Then the glyburide was loaded into the synthesized graphene oxide. A 3^2 response surface methodology design was employed to optimize the formulation. In this approach, the quantities of glyburide (X_1) and graphene oxide (X_2%) served as the independent factors, while entrapment efficiency (Y_1%) and drug release (Y_2%) were evaluated as the dependent responses. The prepared beads were characterized by employing entrapment efficiency (EE), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), drug release (DR). The percent encapsulation efficiency was found to be as 70.21-88.12 % while % DR was in the range of 85.19-92.39 % for a duration of 12 hrs. The graphene oxide nanocomposites containing glyburide were effectively formulated and appear to offer a promising sustained-release profile lasting up to 12 hours. Formulated novel system exhibits delayed release of drug release action up to 12 hrs, with subsequent elimination of frequent dosing issues of glyburide for enhanced patient compliance.

Keywords: Glyburide; Graphene oxide; Design Expert; Sustained release; Bioavailability.

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Introduction

The nanotechnology field has emerged as the highly potential candidate to deliver the drug during the last few decades. Metal oxide composites have established their value in the delivery of medications, in medical diagnosis, in imaging, etc. They serve as drug carriers in the majority of research studies and have strong biocompatibility or biodegradability[1]. Graphite is the source of the carbon nanomaterial known as graphene oxide (GO). Graphene is considered the thinnest two-dimensional material because its carbon atoms are arranged in a honeycomb pattern, forming hexagonal, benzene-like rings with a carbon atom at each corner[2]. An oxidized form of graphene, Graphene Oxide (GO), is inexpensive and simple to make from graphene a with huge surface area. Graphene oxide is a commonly used nanocarrier in pharmacology and is well tolerated by the human body. Because graphene oxide has two exposed surfaces, it can hold a larger amount of drug molecules. In pharmaceutical settings—particularly drug-delivery systems—it provides several advantages: high loading capacity, good biocompatibility, suitability for preclinical and clinical evaluation, the ability to release drugs in a controlled and site-specific manner, effective tumor targeting, and overall low cost. Without harming the body or triggering cell death, functional graphene only degrades and is expelled from it[3,4].

Due to a changed way of life, altered dietary habits, and inherited factors, diabetes mellitus is a disease that affects almost every individual regularly. There are many different medications on the market. However, because of the short half-life, medication is needed twice or three times per day. Long-term usage of these anti-diabetic medications can have negative side effects, including kidney failure, lower limb amputation, heart disease, stroke, and other conditions[5]. For the efficient treatment of diabetics, the standard dose forms of some medications

require frequent dosing to achieve plasma peak levels. Drugs that have been loaded into biocompatible polymers have recently been used to eliminate side effects and the problem of repeated dosing[6].

The most widely used sulphonylurea of the antihyperglycemic class, glyburide (GBL), has an oral half-life of 1.5 to 1.8 hours and is used to treat type II diabetes. By activating pancreatic beta cells and blocking ATP-sensitive K+ (KATP) channels, GBL aids in the release of insulin. Patients with active ischemic heart disease and chronic kidney disease should refrain from receiving long-term GBL medication. GBL has a high plasma protein binding (99.8%) and less oral bioavailability, both of which indicate that its first-pass effect is anticipated to be minimal[7].

In the current study, we used improved Hummer's method for synthesizing GO, and we employed the resulting GO for drug transporter as nano-composites for glyburide's prolonged release. The statistical design was utilised to analyse how different glyburide and graphene oxide concentrations affected the effectiveness of drug encapsulation and release. Using Design Expert software, an experimental analysis was performed using the 3^2 (three level-two factors) response surface methodology[8]. The morphology, encapsulation effectiveness, Fourier transform-infrared (FTIR), particle size analysis, Differential Scanning Calorimetry **(**DSC), X-ray diffractometry, and in vitro drug release of the produced glyburide-loaded GO-nanocomposites were evaluated. The created glyburide-loaded GO-nanocomposites (GBL-GO NC) are discovered to be the most promising method for glyburide medication delivery with a sustained release.

Materials and Methods

Materials

The Glyburide (GBL) which is active drug was purchased from Swapnroop drug distributor, Aurangabad, India. Graphite flakes were as generous sample from Rankem Pvt. Ltd., India. Hydrogen peroxide, potassium permanganate were purchased from Himedia Pvt. Ltd., Mumbai, India. All other chemicals were of analytical grade.

Methods

Graphene oxide nanoparticles synthesis:

A Hummer's process was enhanced to create graphene oxide. The three neck-round bottom flasks were filled with 3 g of graphite flakes, 9:1 ratio of 360 ml of sulfuric acid and 40 ml of orthophosphoric acid along with 15 minutes of stirring. Over the course of 60 minutes, the suspension received 18 g of KMnO4 progressively while being constantly stirred. The reaction mixture's temperature rose to 35–40°C owing to exothermic nature of reaction. The reaction mixture was then decanted into a beaker with 400 gm ice with 3ml (30%) hydrogen peroxide after being stirred for an additional 12 hours at 45–50°C and cooling at room temperature. The suspension was first filtered through a polyester cloth, and the resulting liquid was then centrifuged at 5000 rpm for 20 minutes at room temperature. The collected solid was washed three times: once with 200 mL of water, twice with 200 mL of 30% HCl, and once with 200 mL of ethanol. To promote solidification, 200 mL of ether was added. The final product was dried under vacuum and heated at 60 °C for 24 hours[9-11].

Development of Drug-Loaded GO Nano composites(GBL-GO NC)

An adequate amount of GO, as given by design expert software, was treated subjected to 45 min of ultrasound in the bath sonicator in 20 ml of water. The required quantity of Glyburide (GBL) **(as given in Table 1)** was dissolved in ethanol, added dropwise in aforementioned mixture, and stirred continuously at 35-37^{0}C. for 6 to 8 hours. After that, the mixture was centrifuged for 10 minutes. After being lyophilized for 24 hours, the resulting suspension was employed to characterize a freeze-dried product[12, 13].

Table 1: Formulation composition of the nanocomposite of GO-GBL

Experimental run	Independent variable		Dependent variables	
	GlyburideX1 (mg)	Graphene oxide (mg) X2	% EE Y1	% DR Y2
F1	0	0	84.36	85.51
F2	-1	1	85.84	89.04
F3	-1	-1	82.65	90.48

F4	0	-1	75.77	90.33
F5	0	0	84.84	86.12
F6	0	0	84.59	85.73
F7	0	0	84.15	85.19
F8	1	0	73.08	88.36
F9	1	-1	70.21	88.14
F10	0	1	88.12	86.94
F11	1	1	84.14	92.39
F12	0	0	82.64	86.98
F13	-1	0	86.43	88.98

Levels with their codes

Independent Variable	Low Level (-1)	Medium Level (0)	High Level (+1)
X1 : Glyburide	100.00	150.00	200.00
X2: Graphene oxide	100.00	150.00	200.00

Design of Experiment for optimization

Design-Expert® software (Stat-Ease Inc., Minneapolis, MN, USA) was employed to carry out the statistical experimental design. A 3² response surface methodology (three-level, two-factor design) was applied to optimize the formulation and to evaluate the influence of independent variables on the measured responses[14]. During the formulation of glyburide (GBL)-loaded nanocomposites, multiple preliminary trials were performed to enhance drug entrapment efficiency while minimizing drug release. These initial experiments involved systematic variation in drug concentration and graphene oxide content, which resulted in corresponding changes in entrapment efficiency and drug release characteristics. Based on these preliminary findings, two independent variables were selected: amount of glyburide (X_1) and percentage of graphene oxide (X_2), each studied at three coded levels—low (−1), medium (0), and high (+1). Entrapment efficiency (Y_1) and percentage drug release at 12 h (Y_2) were chosen as the dependent responses. The experimental matrix defining the selected independent and dependent variables is presented in Table 1. For optimization purposes, the relationship between the independent variables (X_1, X_2) and the responses (Y_1, Y_2) was described using the following polynomial equation:

$$Y = \beta_0 + \beta_1 x_1 + \beta_1 x_1 + \beta_3 x_1 x_2 + \beta_4 x_1^2 + \beta_5 x_2^2 \quad (1)$$

Where,

Y shows the response, β_0 is the intercept and $\beta_1 - \beta_5$ is the regression coefficient. x_1, x_2 are individual effects. $x_1 x_2$ is the interaction effect and x_1^2, x_2^2 are the quadratic effects. The significance of the model was evaluated at $P < 0.05$ level using One-way ANOVA[15].

Characterization

Entrapment Efficiency (EE)

The GBL-loaded GO nano composite, which was precisely weighed at 10 mg, was dissolved in DCM (5 ml) before being mixed with PBS (pH 6.8) to extract GBL. In order to encourage the organic solvent evaporation, 30 min continuous stirring was employed. After proper dilution with PBS, the % EE of GBL from filtrate was evaluated by UV analysis. For this, the suitable dilutions of the sample put in a cuvette with proper positione in UV chamber. The scanning of the sample was done using a UV-spectrophotometer at 225 nm. Using appropriate standard to record readings[16,17]. The encapsulation efficiency of the samples was calculated . The percent encapsulation efficiency was calculated using:

$$EE\ (\%) = \frac{Weight\ of\ drug\ determined\ (mg)}{Weight\ of\ drug\ added\ (mg)} \times 100$$

Drug Loading (% DL)

A GBL-loaded GO nanocomposite that was precisely weighed at 10 mg was individually distributed in enough DCM to dissolve. For the drug extraction, an appropriate amount of PBS of pH 6.7 was mixed to this solution. Up until the DCM started to evaporate, stirring was continued. Following suitable dilution, the dispersion was passed through Whatman filter paper, and the resulting filtrate containing the drug was analyzed using a UV–visible spectrophotometer at a λmax of 225 nm[18]. From the absorbance, DL was calculated by using the following equation.

$$DR\ (\%) = \frac{Amount\ of\ drug\ determined}{Amount\ of\ drug\ in\ nanocomposites} \times 100$$

Fourier Transform Infrared Spectroscopy (FTIR)

A Fourier transform infrared (FTIR) spectrophotometer (Shimadzu, FTIR-8400) was employed to evaluate the compatibility between glyburide (GBL) and graphene oxide (GO). FTIR spectra of GO, GBL, and the GBL-loaded GO nanocomposite were obtained using the potassium bromide (KBr) pellet technique. Each sample was thoroughly mixed with KBr and compressed into a transparent pellet using a KBr press. The spectra of individual components and the formulated nanocomposite were recorded over the wavenumber range of 4000–400 cm^{-1}[19] .

Differential Scanning Calorimetry (DSC)

The thermal behavior of graphene oxide (GO), glyburide (GBL), and the GBL-loaded GO nanocomposite was evaluated using differential scanning calorimetry (DSC) (DSC-60, Shimadzu and DSC-821, Mettler Toledo). Accurately weighed samples (5–10 mg) were placed in sealed aluminum pans and subjected to thermal scanning over a temperature range of 30–200 °C at a heating rate of 10 °C/min[20].

Particle Size and Polydispersity Index (PDI) Measurements

The mean particle size and polydispersity index (PDI) of the optimized glyburide-loaded graphene oxide (GBL–GO) nanocomposite were determined using a particle size analyzer (Malvern Zetasizer ZS-200, Worcestershire, UK). The nanocomposite was dispersed in distilled water and analyzed at ambient temperature using the instrument.

X-ray diffraction (XRD) analysis

Powder X-ray diffraction (PXRD) analysis of pure glyburide (GBL), graphene oxide (GO), and the GBL-loaded GO nanocomposite was performed using an X-ray diffractometer (Bruker D8 Advanced, Germany) equipped with Cu Kα radiation operated at 40 kV and 20 mA. Diffraction patterns were recorded over a 2θ range of 4–80° to examine the crystalline characteristics of the polymers and formulations. Data acquisition was carried out in step-scan mode at a scanning rate of 0.03 s^{-1}.

In-Vitro drug release studies (DR)

The GBL-loaded GO nanocomposite and pure GBL performed in vitro drug release testing in dissolution apparatus (Type-II, Paddle technique, Electrolab, TDT 06; 37°C/75 rpm/12 hours). The release kinetics were conducted in a fresh 900 ml PBS upto 12 hours (pH 1.2 for the first 2 hours because the formulation was intended for oral dosage form, then pH 6.8 for the next 10 hours). The phosphate buffer dissolution medium (pH 1.2 and pH 6.8) was created in accordance with the Indian Pharmacopoeia 2007. To 900 mL of dissolving medium, 10 mg of a GBL-loaded GO nanocomposite were added after being precisely weighed. To maintain sink condition, aliquot equal 5 ml was removed and immediately replaced using fresh fluid at specified time intervals. Each sample was filtered via a membrane filter before being subjected to a UV-vis spectroscopy analysis using a standard cell at a maximum wavelength of 225 nm to determine its drug content. The analysis was done out in triplicate to ensure the correctness of the findings, and the mean was used to create the graph[21].

In- vitro drug release kinetics and mechanism

The in vitro drug release data of the glyburide-loaded graphene oxide (GBL–GO) nanocomposite formulations were analyzed by fitting them to various mathematical release kinetic models, including zero-order, first-order, Hixson–Crowell, Higuchi, and Korsmeyer–Peppas models[22] .

In the **zero-order model**, the drug release profile is described by the equation $Q = kt + Q_0$, where Q represents the cumulative amount of drug released at time t, Q_0 denotes the initial drug amount, and k is the zero-order release rate constant.

The **first-order model** follows the equation $Q = Q_0 e^{kt}$, where Q is the amount of drug released at time t, Q_0 is the initial drug concentration, and k corresponds to the first-order rate constant.

For the **Higuchi model**, drug release is expressed as $Q = k\, t^{1/2}$, indicating diffusion-controlled release, where Q is the amount of drug released at time t and k is the Higuchi dissolution constant.

The **Korsmeyer–Peppas model** is represented by the equation $Q = k\, t^n$, where Q denotes the drug released at time t, k is the kinetic constant, and n is the release exponent indicative of the underlying drug release mechanism.

The goodness of fit and predictive capability of each model were evaluated using the coefficient of determination (R^2). The Korsmeyer–Peppas model was further applied to interpret the drug release mechanism from the formulated beads, allowing differentiation among Fickian diffusion, non-Fickian (anomalous) transport, and relaxation-controlled (case-II) release. Release behavior was classified as Fickian when the diffusional exponent n was ≤ 0.43. Values of n between 0.43 and 0.85 indicated non-Fickian (anomalous) transport, whereas an n value ≥ 0.85 suggested case-II transport (relaxation-controlled release)[23].

Stability studies of GBL-loaded GO nanocomposite

In a stability chamber, the GBL-loaded GO nanocomposite was subjected to accelerated storage conditions for three months at 20^0C/RH75%. (Mack Pharmatech, India). All organoleptic as well as drug release characteristics were observed initially and after an interval of per month[24, 25].

Results and discussions

Encapsulation Efficiency (EE) and Drug Loading (DL)

The 3D contour plot (Figure 8b) generated by Design-Expert illustrates the combined impact of glyburide and graphene oxide concentrations on entrapment efficiency (EE). Across all 13 experimental runs, EE values ranged from 70.21% to 88.12%. Entrapment efficiency increased with decreasing glyburide amount (X_1) and increasing graphene oxide concentration (X_2). Drug loading (DL) values for the same 13 runs varied between 53.48% and 73.15%.

Fourier transform infrared spectroscopy (FTIR) Analysis:

FTIR analysis was conducted to evaluate the potential interactions between glyburide and the excipients. The FTIR spectra of pure GBL, GO, and the GBL-loaded GO nanocomposite are presented in **Figure 1.** The spectrum of GBL exhibited characteristic absorption bands at 1661.74 cm^{-1} (C=O stretching), 3391.2 cm^{-1} (O–H and N–H stretching), 2817.5 cm^{-1} and 2926.4 cm^{-1} (C–H stretching), and 712.2 cm^{-1} (C–Cl stretching). In the spectrum of GO, prominent peaks were observed at 3625.8 cm^{-1} (O–H stretching), 1828.5 cm^{-1} (C=O stretching), 1724.2 cm^{-1} (C=C/C–C), 1202.2 cm^{-1} (C–O stretching), 951.3 cm^{-1} (epoxy groups), and 779.2 cm^{-1} (C–O–C vibrations). However, in the FTIR spectra of GBL-loaded GO nanocomposite, most of the peaks resembled to spectra of pure GO, which indicates the presence of GBL in GO nanocomposites.

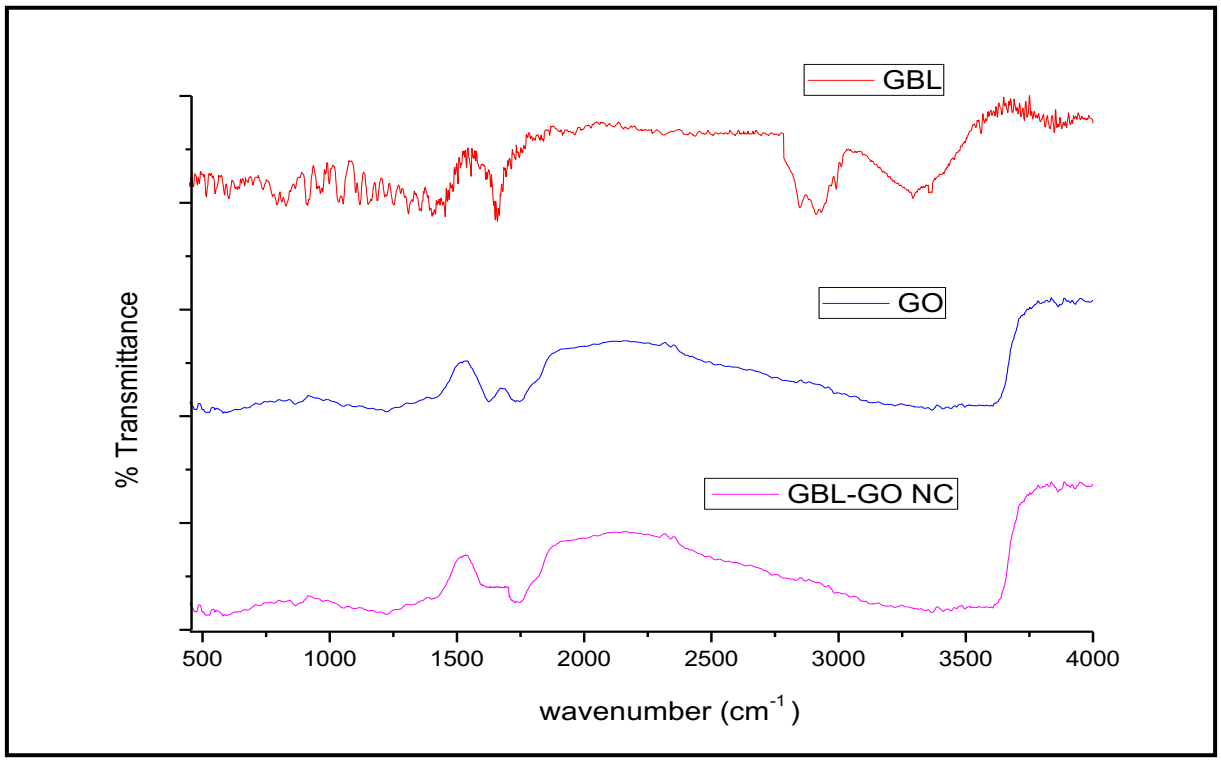


Figure 1: FTIR spectra of GBL, GO, and GBL-loaded GO nanocomposite (GBL-GO NC)

Differential Scanning Colorimetry

Differential scanning calorimetry was carried out to assess the interaction between the drug and the carrier. The DSC thermograms of pure GO, GBL, and the GBL-loaded GO nanocomposite are depicted in **Figure 2.** The thermogram of GBL displayed a distinct sharp endothermic peak at 167.2 °C, corresponding to the melting point of pure glyburide.In DSC thermograms of GO, a broad endothermic peak is observed in the range between 40 and 60^0C. The subsequent pronounced exothermic event is found in the range between 150- 180^0 C. Due to the interaction of GBL and GO, as well as the change in crystalline state to amorphous state, the characteristic endothermic peak of GBL disappeared in the thermogram of GBL-loaded GO nanocomposite

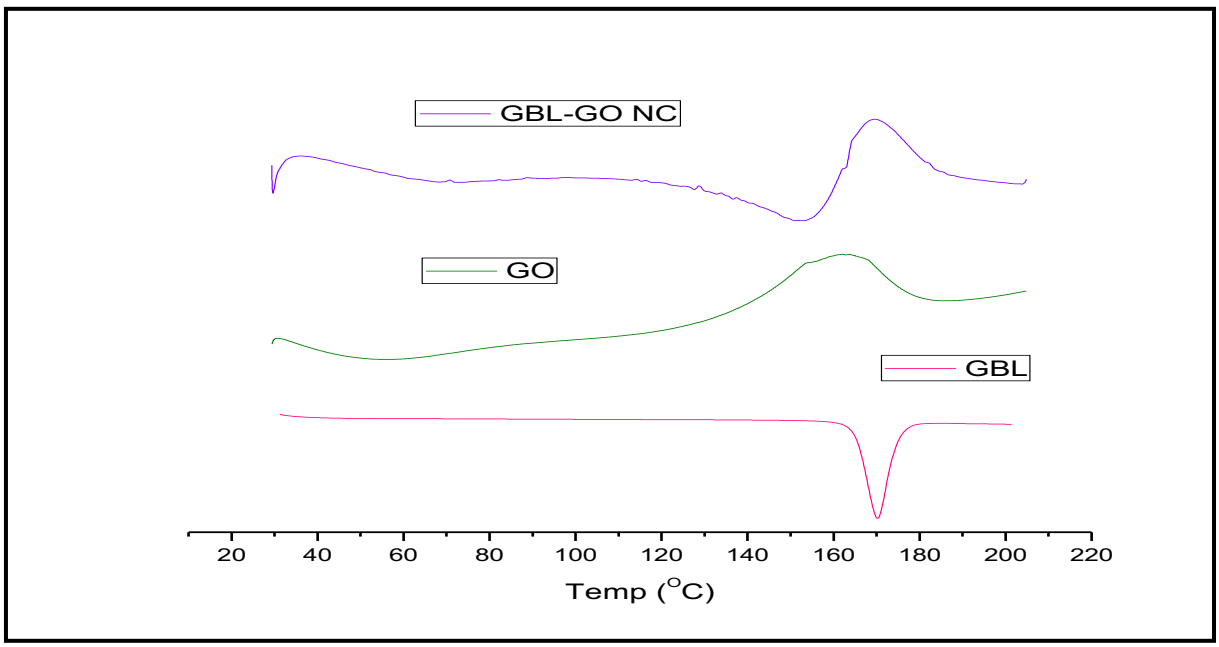
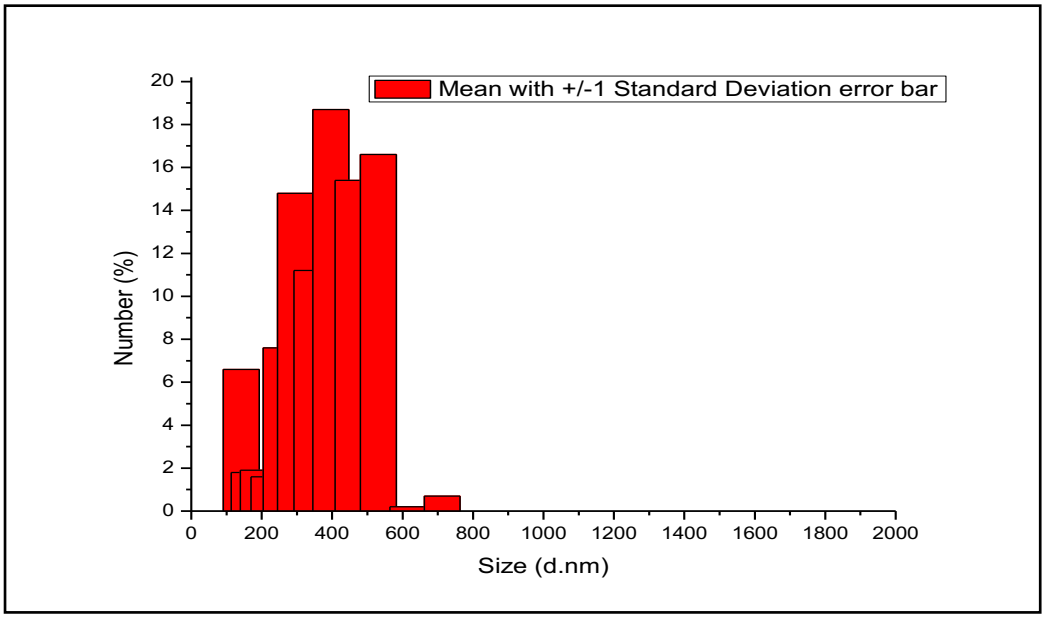


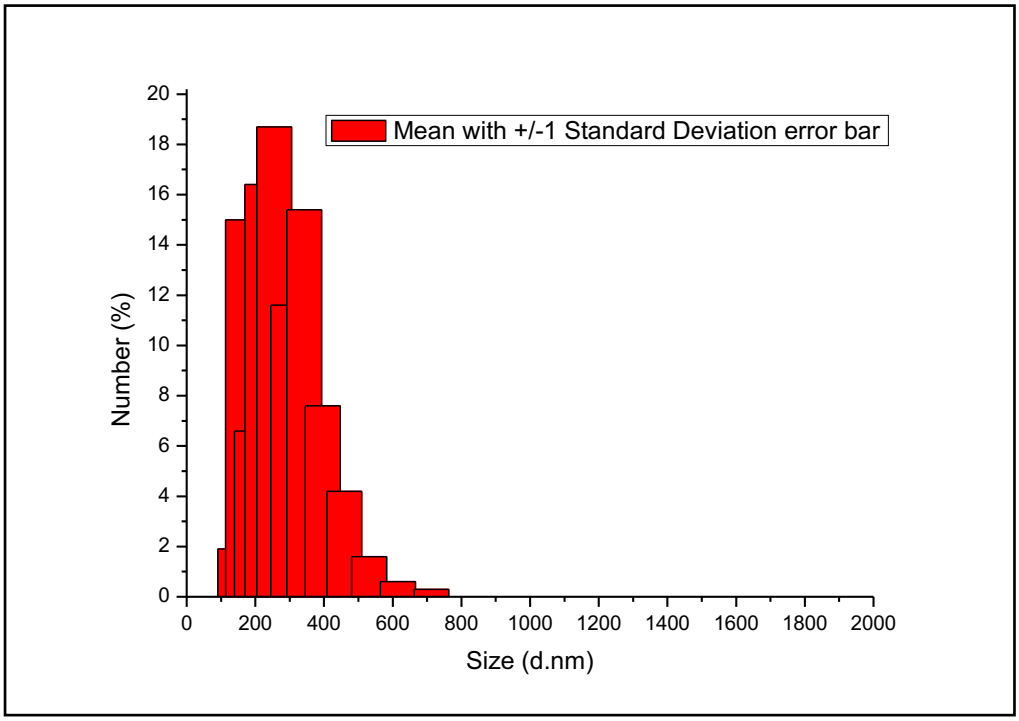
Figure 2*:* DSC thermograms of pure GO, GBL, and GBL-loaded GO nanocomposite (GBL-GO NC)

Particle Size Distribution

The mean size (d. nm) of pure GBL along with the optimized batch of GBL-loaded GO nanocomposite was about 382.2 nm (**Figure 3a**) and 496.4 nm (**Figure 3b),** respectively.



(a)



(b)

Figure 3: Average size (d. nm) of(a) pure GBL (b) GBL loaded GO nanocomposite

XRD

The XRD spectra of pure GBL, GO, and GBL-loaded GO nanocomposite(GBL-GO NC) are shown in **Figure 4.** XRD patterns of pure GBL show sharp 2Ø-scattered angles at 9.65 °, 10.88°, 11.27°,12.77°, 15.68°, 16.43°, 19.93°and 23.03°. These peaks indicated crystalline form. In the XRD pattern of the GBL-loaded GO nanocomposite, the characteristic crystalline peaks of glyburide were significantly reduced or disappeared. The reduction in peak intensity suggests a decrease in the drug's crystallinity, indicating that glyburide is present in a more amorphous state following encapsulation within the nanocomposite.

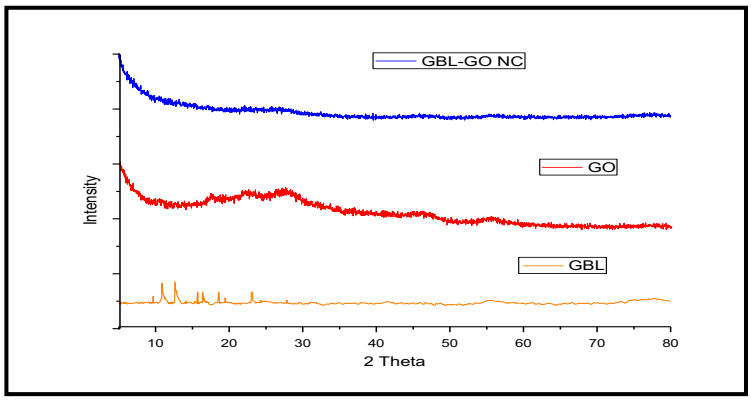


Figure 4: XRD spectra of pure GBL, GO, and GBL-loaded GO nanocomposite

***In-Vitro* drug release studies**

The profile correlates the drug release of different formulation. The release of different batches was found to be in the range of 85.19-91.98 % upto 12 hrs (**figure 5**). All batches of prepared GBL-loaded GO nanocomposites show sustained and slower release action for 12 hrs.

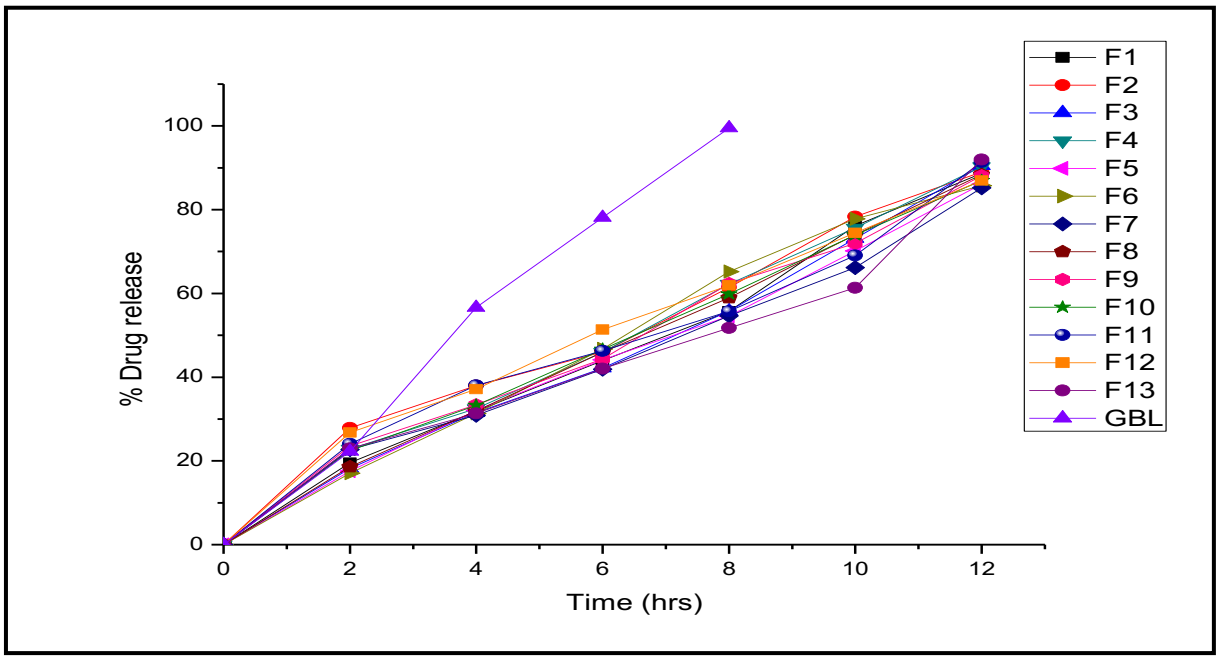


Figure 5:Dissolution profiles of pure GBL and all 13 batches of GBL-loaded GO nanocomposite

The release profiles were analyzed using various kinetic models to determine the drug release mechanism. The in vitro drug release data best fitted the first-order kinetic model, as indicated by the highest coefficient of determination (R^2) compared to the other models. The release mechanism for each batch is summarized in **Table 2**.

Table 2: Release Kinetics for formulation batches F1 to F13

Time	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
ZERO	0.993	0.978	0.994	0.991	0.995	0.992	0.985	0.998	0.989	0.992	0.974	0.979	0.956
FIRST	0.902	0.909	0.863	0.9	0.906	0.976	874	0.9174	0.9037	0.929	0.794	0.935	0.718
HIGUCHI	0.961	0.951	0.957	0.967	0.972	0.988	0.942	0.9803	0.9628	0.975	0.931	0.981	0.873
KORSEMEYER	0.988	0.96	0.9899	0.979	0.996	0.996	0.965	0.9977	0.9762	0.988	0.971	0.987	0.936

Stability of GBL-loaded GO nanocomposite(GBL-GO NC)

An optimised formulation's physical characteristics, percent drug content, and *in vitro* dissolution study were not significantly altered under accelerated stability conditions.

Optimization of nanocomposites:

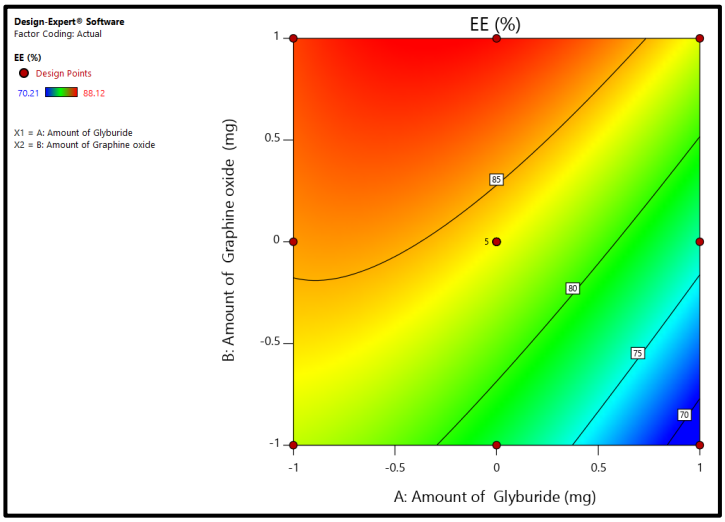
A 3^2 response surface methodology was utilized to evaluate the influence of the independent variables (X_1, X_2) on the responses (Y_1, Y_2). To assess the effects of each factor, both 2D contour plots **(Figures 6a and 7a)** and 3D response surface plots (**Figures 6b and 7b**) were generated. The 3D surface plots are particularly helpful in visualizing the main and interaction effects of the variables. The independent variables selected were the amount of glyburide (X_1) and the percentage of graphene oxide (X_2), while the dependent responses were entrapment efficiency (EE, %) and drug release (DR, %).

The contour and surface plots for EE indicated that EE increased when glyburide content (X_1) decreased and graphene oxide concentration (X_2) increased. Conversely, drug release (DR) decreased with increasing glyburide (X_1) and decreasing graphene oxide (X_2). Across all 13 experimental runs, EE values ranged from 70.21 to 88.12%, and DR values ranged from 85.19 to 92.39%, as summarized in **Table 1**.

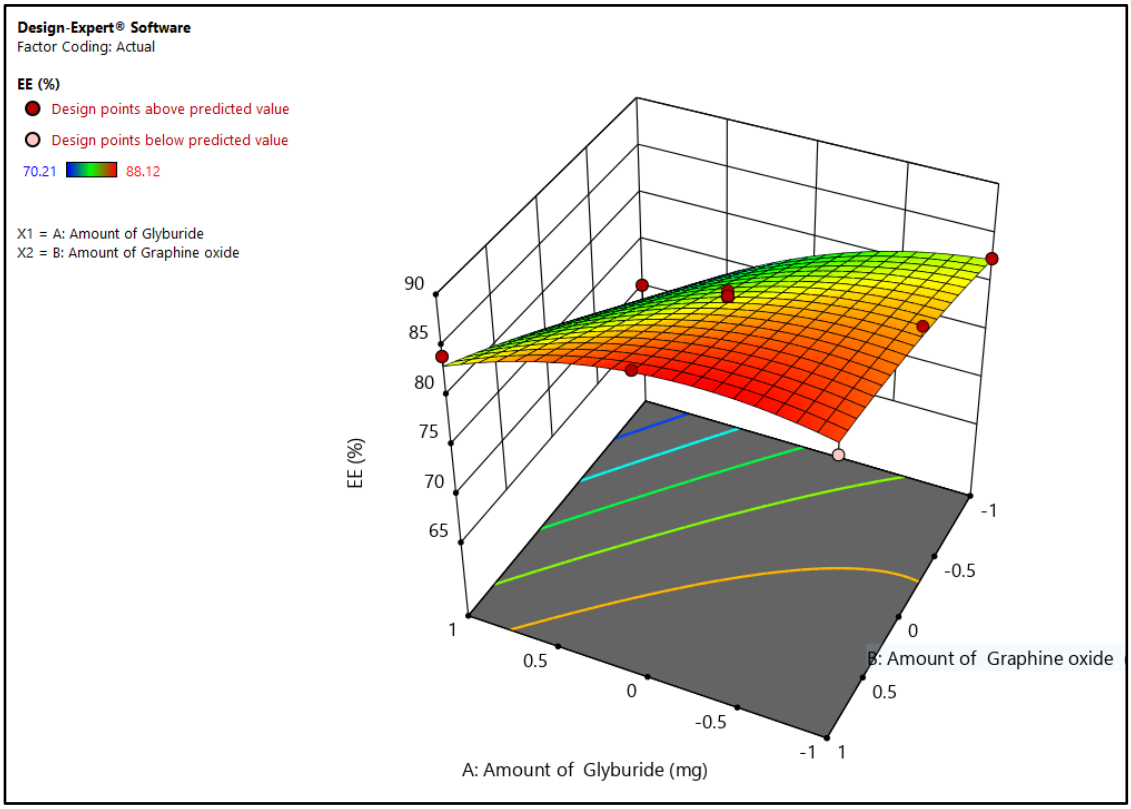
The relationship between the independent variables and responses was modeled using polynomial equations, supported by contour plots. For the quadratic model, the coefficients of determination (R^2) for Y_1 and Y_2 were 0.8681 and 0.8049, respectively, indicating a good model fit (**Table 3**). The polynomial equations obtained for EE (Y_1) and DR (Y_2) are presented below.

$$Y1 = +83.6848 + -4.58167x_1 + 4.91167x_2 + -2.8519x_1^2 + -0.661897x_2^2 + 2.685\,x_1x_2. \quad (3)$$

$$Y2 = 86.0972 + 0.065\,x_1 - 0.0966667x_2 + 2.09466x_1^2 + 2.05966x_2^2 + 1.4225\,x_1x_2. \quad (4)$$

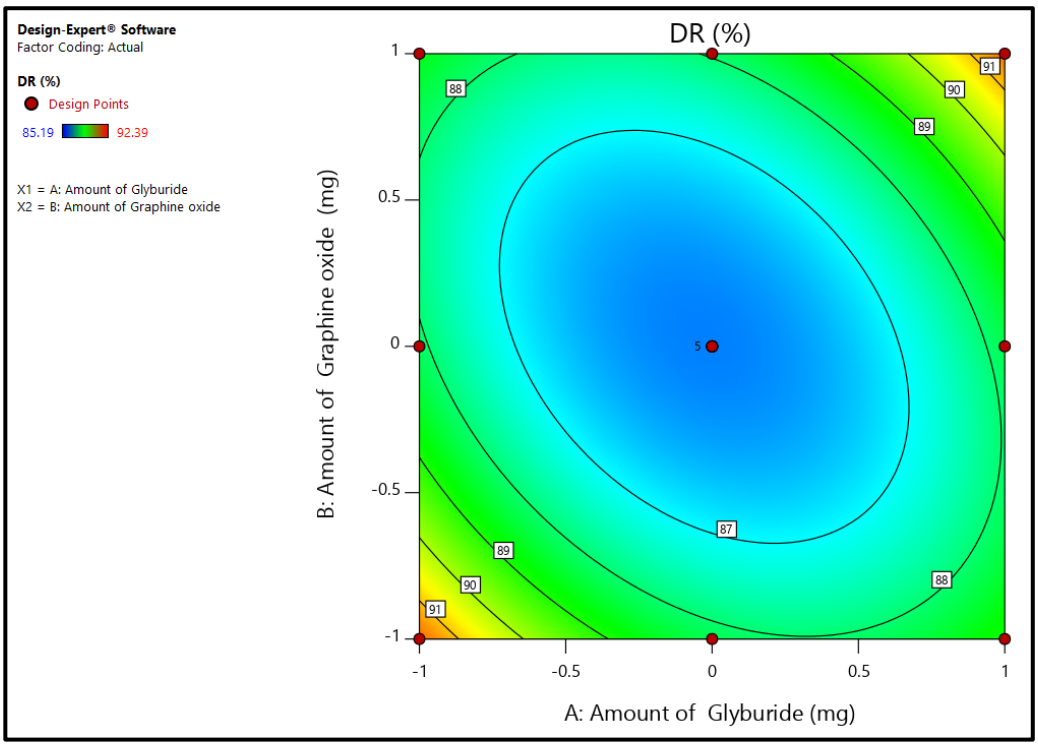


(a)

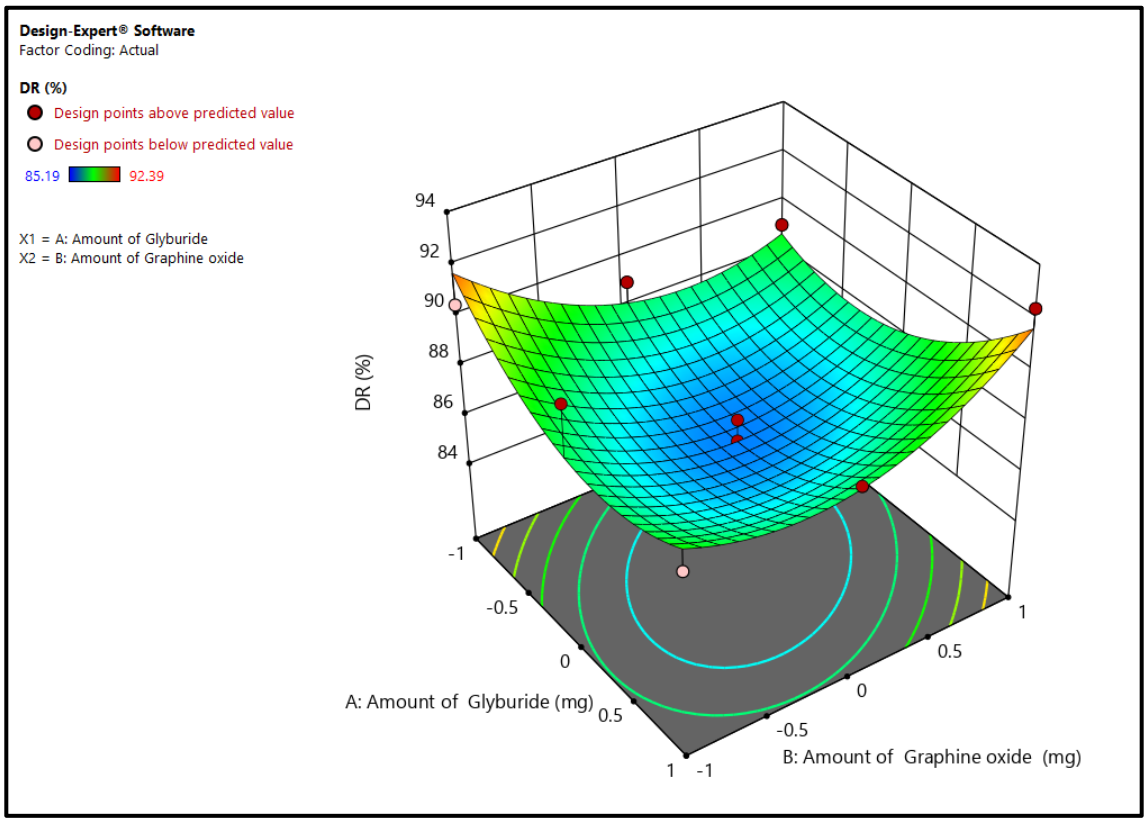


(b)

Figure 6*:* 2D contour plot (a) and 3D response surface plot (B) for entrapment efficiency



(a)



(b)

Figure 7: 2D contour plot (a) and 3D response surface plot (B) for drug release

In the equations above, positive coefficients indicate synergistic effects, whereas negative coefficients indicate antagonistic effects. The ANOVA results for responses Y_1 and Y_2 are presented in **Table 4.** For entrapment efficiency (Y_1), the quadratic model demonstrated that EE was significantly influenced by the terms X_1, X_2, X_1^2, X_2^2, and the interaction term X_1X_2. Similarly, the drug release response (Y_2) followed a quadratic model and was affected by X_1, X_2, X_1^2, X_2^2, and X_1X_2.

The influence of these independent variables on both EE and DR was statistically significant ($P < 0.05$). The models were also significant, with F-values of 16.80 and 5.78 for Y_1 and Y_2, respectively ($P < 0.05$). **Table 5** summarizes diagnostic statistics, including the actual, predicted, and residual values for each response. Prediction error was calculated by comparing the experimental results with the values predicted by the model. Since the difference between observed and predicted values was minimal, the model demonstrated a strong fit.

Table 3:Summary of regression analysis.

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	Remark
Y1 Response						
Linear	2.98	0.7529	0.7035	0.4744	188.96	
2FI	2.58	0.8331	0.7775	0.4909	183.04	
Quadratic	**1.99**	**0.9231**	**0.8681**	**0.3522**	**232.89**	**Suggested**
Cubic	1.39	0.9729	0.9351	-1.1903	787.47	
Y2 Response						
Linear	2.41	0.0014	-0.1983	-1.0266	117.52	
2FI	2.35	0.1410	-0.1454	-2.4881	202.26	
Quadratic	**1.27**	**0.8049**	**0.6656**	**-0.6234**	**94.14**	**Suggested**
Cubic	0.8032	0.9444	0.8665	-1.7046	156.83	

Table 4: ANOVA of models

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remark
Response Y1						
Model	331.87	5	66.37	16.80	0.0009	significant
x_1	125.95	1	125.95	31.87	0.0008	
x_2	144.75	1	144.75	36.63	0.0005	
x_1^2	28.84	1	28.84	7.30	0.0306	
x_2^2	22.46	1	22.46	5.68	0.0486	
x_1x_2	1.21	1	1.21	0.3062	0.5972	
Response Y2						
Model	46.68	5	9.34	5.78	0.0199	significant
x_1	0.0253	1	0.0253	0.0157	0.9038	
x_2	0.0561	1	0.0561	0.0347	0.8575	
x_1^2	8.09	1	8.09	5.01	0.0602	
x_2^2	12.12	1	12.12	7.50	0.0290	
x_1x_2	11.72	1	11.72	7.25	0.0310	

Table 5: Diagnostics case statistics

Run Order	Actual Value	Predicted Value	Residual	Run Order	Actual Value	Predicted Value	Residual
Response Y1				**Response Y2**			
1	73.08	76.25	-3.17	1	88.36	88.26	0.1031
2	84.59	83.68	0.9052	2	85.73	86.10	-0.3672
3	82.65	82.53	0.1240	3	90.48	91.71	-1.23
4	84.14	83.19	0.9540	4	92.39	91.64	0.7476
5	70.21	67.99	2.22	5	88.14	88.99	-0.8507
6	75.77	78.11	-2.34	6	90.33	88.25	2.08
7	85.84	86.98	-1.14	7	89.04	88.67	0.3726
8	86.43	85.41	1.02	8	88.98	88.13	0.8531
9	84.15	83.68	0.4652	9	85.19	86.10	-0.9072
10	84.84	83.68	1.16	10	86.12	86.10	0.0228
11	84.36	83.68	0.6752	11	85.51	86.10	-0.5872
12	88.12	87.93	0.1854	12	86.94	88.06	-1.12
13	82.64	83.68	-1.04	13	86.98	86.10	0.8828

Conclusion

The GBL-loaded GO nanocomposite was successfully formulated in this study. The developed glyburide system demonstrated sustained drug release for up to 12 hours. A 3^2 (three-level, two-factor) response surface methodology was employed to evaluate the influence of independent variables on the responses. Entrapment efficiency values across the experimental runs ranged from 70.21% to 88.12%, while drug release was sustained up to 12 hours, ranging from 85.19% to 92.39%. The average particle size of pure glyburide and the optimized GBL-loaded GO nanocomposite was approximately 496.4 nm and 382.2 nm, respectively. XRD analysis indicated that the drug existed in a predominantly amorphous state after encapsulation. No significant interactions between the drug and carrier were observed in the DSC and FTIR analyses. Overall, the novel formulation exhibited prolonged release over 12 hours, potentially reducing the need for frequent dosing and improving glyburide therapy for diabetes management.

Disclosure statement

No potential conflict of interest was reported by the authors.

AUTHOR CONTRIBUTION

Swapnil Bhanudas Gadekar: Performed experimental work, manuscript writing, and editing
Dr. Ashok Vithal Bhosale: Project supervision, Conceptualization

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