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Formulation, Characterization and Optimization of Glibenclamide Loaded Nanosponges

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ABSTRACT
The present study aimed to formulate optimized nanosponges of Glibenclamide. The nanosponges were prepared by the emulsion solvent evaporation method using high-speed homogenization. Nine formulations were prepared using Ethyl cellulose (EC) and Polyvinyl alcohol (PVA). The formulations were designed and optimized with response surface methodology using design expert software. The particle size was obtained in the range of 600-800 nm with good zeta potential. The percentage yield was found to be between 68 to 82%. The formulation GECN1, GECN5, and GECN9 showed Cumulative % drug release from 82±1.22, 80±1.34, 84±1.32. All the formulations followed zero-order release kinetics. Stability study of optimized formulation OPGECN1 and OPGECN2 resulted in good stability.

Keywords: Nanosponges, Glibenclamide, Optimization, High-speed homogenizer,

I. INTRODUCTION

The use of barely soluble medicines in treatment continues to be a challenge for research. Limited solubility drugs will have low absorption and bioavailability [1]. Almost 40% of newly discovered medicines are poorly soluble in water, necessitating the development of appropriate dosage forms for therapy [2]. Hydrophobicity is required for target receptor affinity [3] and intracellular targeting [4]. Poorly water-soluble drugs are classified as Class II in the Biopharmaceutical Classification System (BCS). Low water solubility and high permeability define drugs in this family [5]. The drug's dissolving rate becomes a limiting step for absorption [6] It's a sulfonylurea derivative used to treat type 2 diabetes. It boosts the sensitivity of peripheral tissue to insulin by stimulating insulin release from pancreatic cells. [7] As Glibenclamide is essentially insoluble in water, it has a 45 percent oral bioavailability. There are certain methods for enhancing its solubility. One of the methods is formulating as nanosponges. Glibenclamide nanosponges prepared by emulsion solvent diffusion method with high-speed homogenizer [8]. Other nanoparticle production methods rely on the development of nano-drug delivery systems. The purpose of the study was to formulate nanosponges containing Glibenclamide with controlled release for the management of diabetes.

II. METHODOLOGY

Table no1: List of ingredients used

Ingredients	Company
Glibenclamide	Yarrow chemical Ltd., Mumbai.
Ethylcellulose (EC)	Yarrow chemical Ltd., Mumbai.
Polyvinyl alcohol (PVA)	Yarrow chemical Ltd., Mumbai.
Dichloromethane	S D. Fine-chem. Ltd.; Mumbai

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Determination of Absorbance maxima of Glibenclamide in 0.1 N HCl of pH 1.2 and phosphate buffer 7.2 pH [9]

Absorption maxima for Glibenclamide in 0.1 HCl of pH 1.2, and phosphate buffer 7.2 pH were determined by scanning the 20 mcg/ml concentration of drug solution within a range of 400 to 200 nanometres using a UV-Visible spectrophotometer

Standard Calibration curve of Glibenclamide in 0.1 N HCl of pH 1.2

Preparation of standard stock solution-A

Accurately weighed 50 mg of Glebenclamide was transferred into 100 ml volumetric flask, then dissolved and made up to 100 ml with of 0.1N HCl of pH 1.2, to get a concentration of 500 mcg /ml.

Preparation of standard stock solution-B

Accurately pipetted 4 ml from stock solution-A and transferred into 100 ml volumetric flask made up to 100 ml with of 0.1N HCl of pH 1.2 to results in a concentration of 20 mcg /ml.

Dilutions

2,4,6,8 and 10 ml of standard stock solution B was diluted with 10 ml to obtain concentrations of 4,8,12,16 and 20 mcg/ml and absorbance is measured at 269 nm UV-Visible Spectrophotometer,

The absorbance values thus obtained was plotted against respective concentration to obtain the standard calibration curve. The procedure was repeated three times and the average values of absorbance were calculated. The data obtained were statistically evaluated to obtain the standard deviation of the said values and regression coefficient.

All the steps repeated for the standard calibration curve of Glibenclamide in phosphate buffer 7.2 pH

Solubility detrminations10

Solubility of Glibenclamide was determined in solvents: 0.1N HCl of pH 1.2, and phosphate buffer 7.2 pH. An excess amount of sample was added in 10 ml of solvent with sonication for one hour, at a temperature of 25 ± 0.5°C for 48 h, and sonicated using a sonicator (ElectrolabTM) for two-hour. Samples were filtered and assayed spectrophotometrically for drug content at absorbance maximum of Glibenclamide

Determination of melting point [10]

The sample, packed in a capillary tube is attached to the thermometer and held through a thread. The Thiele tube is heated using a Bunsen burner and the rate of temperature increase was carefully controlled. and the melting point is noted.

Preparation of nanosponges emulsion solvent diffusion method with high speed homogeniser[11]

A high-speed homogenizer was used to create nanosponges with an anti-diabetic medication as the core ingredient. Glibenclamide (50 mg) was accurately weighed and dissolved in acetone (5 mL) and polymer EC in dichloromethane (10 mL) separately. The mixture of drug and polymer was mixed and added to the aqueous phase (100 mL distilled water) containing PVA using a high-speed homogenizer of rpm of 15,000 to 20,000 for one hour with an ice bath. The added organic solvent evaporated by stirring at 2000 rpm for three hours at room temperature, following which the nanoparticles were recovered by centrifugation for 15 minutes at 5000 rpm. Supernatant separated. The residue mass was washed with distilled water (with saturated drug) to remove excess PVA and centrifuged at 5000 rpm, Purified Nanoparticles were dried in a hot air oven at 40°C.

Designing of experiments[12]

Response Surface Methodology (RSM): Design-Expert® Software (Version-12.0.0.1, Stat-Ease Inc., Minneapolis,) which allows Evaluation by nine experiments to limit the number of experiments. Response Surface Methodology (RSM) was used for the analysis of results. The amount of Methyl Cellulose (X1, mg) and PVA as a surfactant (X2, %w/v) concerning the drug was selected as independent variables. Selected statistical models were used to evaluate the effect of independent variables on the dependent variables like % Cumulative percentage of drug release (% CDR) (Y1), percentage of % Drug loading(%DL) (Y2). A group of statistical and mathematical methods helps in carrying out a systematic analysis of the formulations. The method allows optimizing the numerical parameters that can influence the response surface, using response surface methodology; existing relation between numerical parameters can be quantified at different levels with obtained response surfaces.

Fourier transforms infrared spectroscopy (FT-IR)[13,14]

Drug polymer interactions were studied by using an FTIR spectrophotometer (Shimadzu, FT-IR-8400). FTIR analysis was carried out by the KBr pellet method. The sample was mixed with KBr and compressed into a disc in a manual press. The spectrum was scanned from 4000 to 400 cm1

Differential Scanning Calorimetry / Thermo Gravimetric Analysis (DSC/TGA) [15]

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The thermal behavior of the sample was determined by Simultaneous Differential Scanning Calorimetry/ Thermo Gravimetric Analysis-DSC/TGA (TA Instruments SDT-Q600 Simultaneous TGA / DSC). Accurately weighed samples (5-10 mg) were sealed in an aluminum pan and scanned at a temperature range of 30°C to 600 °C at the rate of 10°C/min under a dry nitrogen atmosphere purge of 50 mL/min.

Particle morphology by Field Emission Scanning electron microscopic (FE-SEM)[16]

The morphology of the samples was carried out using scanning electron microscopy (FE-SEM, Type-II, Model-S-4800, Hitachi, Japan). The surface morphology was analyzed at a working distance of 8.7-8.8 mm and 1.0 kV accelerating voltage

Particle size distribution and Zeta Potential[17]

The particle size distribution and zeta potential were determined in water as a dispersion medium by laser diffraction size analyzer, Malvern Zetasizer (Model: ZS 200).

Drug loading[18]

Weighed amount (50 mg) of Glibenclamide-loaded nanosponges were dispersed in 10 ml methanol and sonicated for one hour, centrifuged at 5,000 rpm for half an hour the supernatant was withdrawn and suitably diluted with 0.1N HCl and were subjected to UV spectrophotometric analysis against blank 0.1 N HCl. With the help of the standards curve. The percentage % Drug loading was calculated by the following equation

Percentage Drug loading

$$= \frac{\text{actual drug contentinnanosponges}}{\text{Theoretical drug content}} \times 100$$

Percentage yield[19]

The percentage yield of the nanosponges was determined by calculating accurately the initial weight of the raw materials and the last weight of the nanosponge obtained

Percentage yield

$$= \frac{\text{Practical mass of nanosponges}}{\text{Theoretical mass (drug-polymer)}} \times 100$$

***In- vitro* drug release studies[20,21]**

The *in-vitro* release of Glibenclamide Loaded nanosponges (50 mg equivalent) was placed in a dialysis bag secured with a clamp at each end and immersed in dissolution media. Dissolution is performed using USP type II dissolution test apparatus (Electro lab, India) in 900 ml of 0.1 N HCl of 1.2 pH for the first two hours and then in phosphate buffer (7.2 pH) for 3, 4, 5, 6, 8, 12, 24, 36 and 72 h at 37 ± 0.5 °C and stirring rate of 50 rpm. Samples (5 ml) were collected periodically and replaced with an equal volume of fresh dissolution medium on each occasion. After filtration through Whatman Grade No. 41 Quantitative Filter Paper (pore size 25 µrn.), the concentration of Glibenclamide was determined spectrophotometrically using UV-Visible spectrophotometer (Jasco V530, Japan). In vitro release profile was analyzed by various kinetic models (zero-order, first-order, and Higuchi) using PSP Disso software.

Model generation using Design Expert Software [22,]

Design-Expert® Software (Version-12.0.0.1, Stat-Ease Inc., Minneapolis,). Response Surface Methodology (RSM) was used for designing of experiments and optimization

The significance of the model was determined by the comparisons of statistical parameters, and the best model (suggested) was decided based on the reasonable agreement between predicted and actual response values and the Model p-value was should be less than 0.05.

A group of statistical and mathematical methods helps in carrying out a systematic analysis of the formulations. The method allows optimizing the numerical parameters that can influence the response surface, using response surface methodology; existing relation between numerical parameters can be quantified at different levels with obtained response surfaces. The implied model can be explained by the following equation exhibiting coefficient effects, interactions, and polynomial terms.

$$Y = b0 + b1X1 + b2X2 + b12X1X2 + b11X12 + b22X22+\ldots$$

Where Y is a measured response associated with each factor level combination, b0 is an intercept, b1 to b22 are regression coefficients computed from the observed experimental values of Y1, Y2, and X1, X2 are the coded levels of independent variables.

Optimization of nanosponges by Design Expert Software[23]

Numerical optimization uses the models to search the factor space for the best trade-offs to achieve multiple goals. Constrains of optimization for the factors X1 and X2 were set 'in range' and for the response, % drug loading, and %CDR, the goal was set to be “maximize" between a selected minimum

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and maximum levels constraints. A desirability value near '1' is selected for each goal.

Evaluation of drug release kinetics[23]

To investigate the mechanism of Glibenclamide release from nanosponge tablets the release data was analysed for zero order, first order, Higuchi model, and Korsmeyer-Peppas model. The data was presented in the following graphical representation and regression analysis was performed. Mt versus t (zero-order), Log cumulative % of the drug remained versus t (first-order) was calculated by linear regression analysis.

Stability studies on optimized formulation [23]

The accelerated stability studies were carried out on optimized formulation using a sealed vial containing optimized formulation placed in stability chambers maintained at 25 °C ± 2 °C/60% RH ± 5% RH and 30 °C ± 2 °C/65% RH ± 5% RH The formulations subjected to stability tests were analyzed for zero, one, three and six months for its % drug loading, %CDR and Particle size (nm).

III. RESULTS

Table no. 2: Solubility study of Glibenclamide

Drug	Medium	pH	Tem.^{0}C	Concentration mg/ml
Glibenclamide	0.1N HCl	1.2	25	0.966
	Phosphate Buffer 7.2 pH	7.2	25	0.023

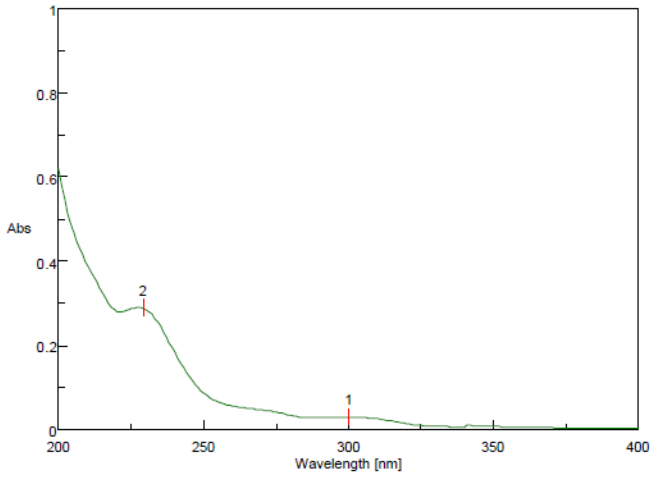

Figure 1: UV spectra of Glibenclamide in 0.1N HCl of pH 1.2 with absorbance maxima at 229 nm

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Table No.3: Absorbance Values of Glibenclamide in 0.1 HCl of 1.2 pH and Phosphate Buffer 7.2 pH

	0.1 HCl of 1.2 pH			Phosphate Buffer 7.2 pH		
Sl.no	Concentration in mcg/ml	Absorbance*	Standard Deviation (±)	Concentration in mcg/ml	Absorbance*	Standard Deviation (±)
1	0	0.000	0	0	0	0
2	4	0.170	±0.012	4	0.266	±0.034
3	8	0.320	±0.003	8	0.409	±0.012
4	12	0.480	±0.011	12	0.619	±0.002
5	16	0.608	±0.015	16	0.790	±0.047
	20	0.740	±0.019	20	0.968	±0.004

*** Average of three determinations**

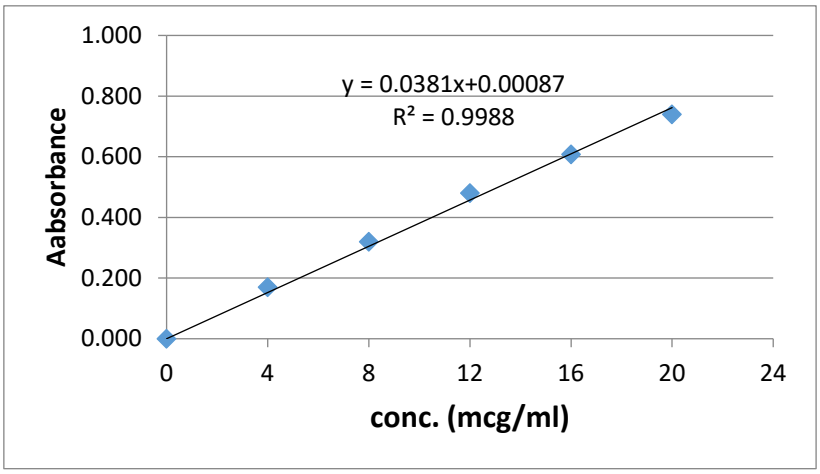

Fig. No.2: Calibration Curve of Glibenclamide in 0.1 HCl of pH 1.2

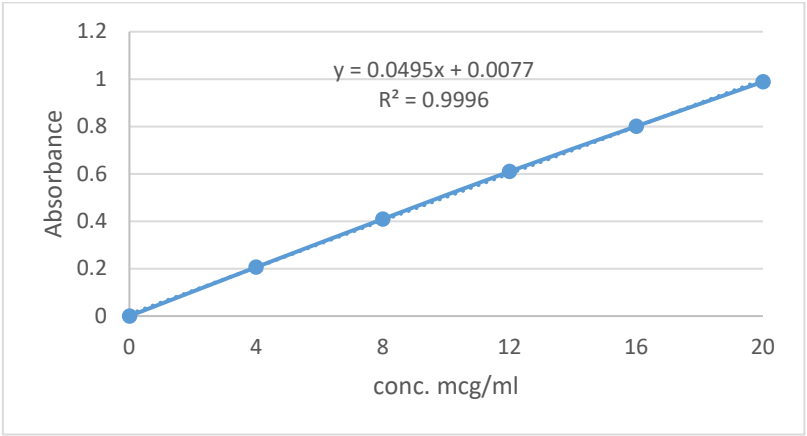

Fig. No.3: Calibration Curve of Glibenclamide in Phosphate Buffer 7.2 pH

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Table 4: Wavelength of maximum absorption (λmax) and Calibration curve R^2 values of Glibenclamide in different dissolution media

Sl.no	Solvent	λmax (nm)	Slope	Intercept	R^2
1	0.1 HCl of pH 1.2	229	0.0381	0.00087	0.998
2	Phosphate buffer 7.2 pH	226	0.0495	0.0077	0.999

Table no.5: Melting point of Glibenclamide

Melting point	Reported	observed
Glibenclamide	172 176ºc	176ºc

Table no 6: levels of independent variables.

Independent variables	The level used (actual, coded)			
	Low (mg)	High (mg)	Low coded	High coded
Factor (EC) -X_1	100	400	-1.00	1.00
Factor (PVA) -X_2	0.15	0.45	-1.00	1.00

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Table no 7: Independent and dependent variables of response surface methodology

Code	Std	Run	Type	Independent variable	
				Factor 1 A: EC mg	Factor 2 B: PVA mg
GECN1	1	1	Fact.	100.00	0.20
GECN2	5	2	Axial	37.87	0.30
GECN3	7	3	Axial	250.00	0.16
GECN4	8	4	Axial	250.00	0.44
GECN5	3	5	Fact.	100.00	0.40
GECN6	6	6	Axial	462.13	0.30
GECN7	2	7	Fact.	400.00	0.20
GECN8	9	8	Cent.	250.00	0.30
GECN9	4	9	Fact	400.00	0.40

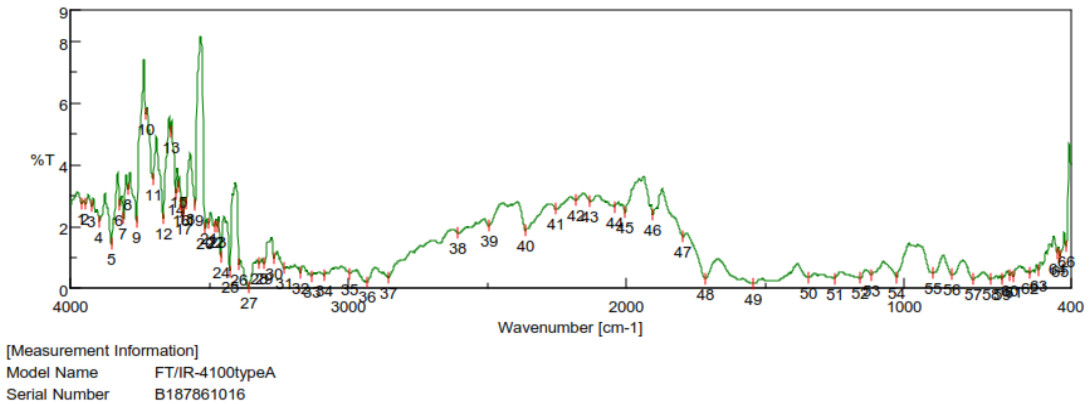

Figure 4: FT-IR spectra of Glibenclamide

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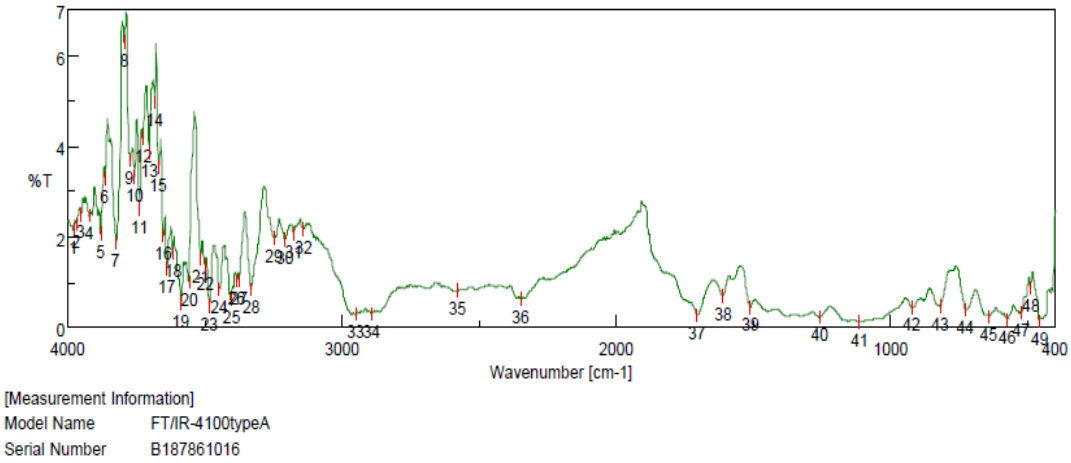

Figure 5: FT-IR spectra of GECN2 nanosponge of Glibenclamide

Table 8: Peaks observed in FT-IR spectra of Glibenclamide

Description	Pure Glibenclamide cm-$^{-1}$	Nanosponges of Glibenclamide cm^{-1}
C=O stretching	1690, 1630, 1714	1705.73
N-H stretching	3500, 3300, 3552	3500.17
S=O asymmetric stretching	1372,1168, 1554	1259.29
C=C stretching	1680, 1620, 1613	1613.16

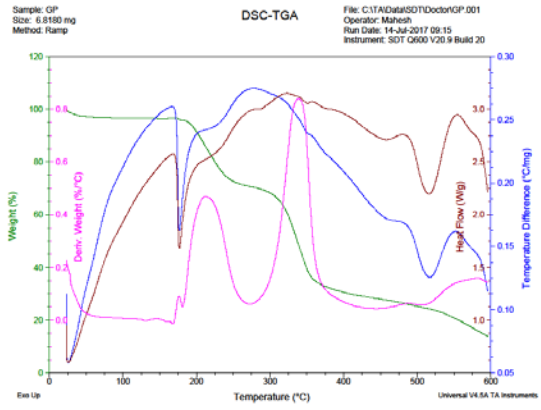

Figure no 6: DSC-TGA analysis of Glibenclamide

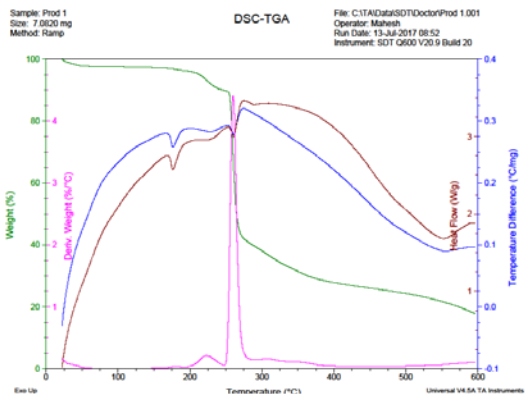

Figure no 7: DSC-TGA analysis of GECN1 nanosponge of Glibenclamide

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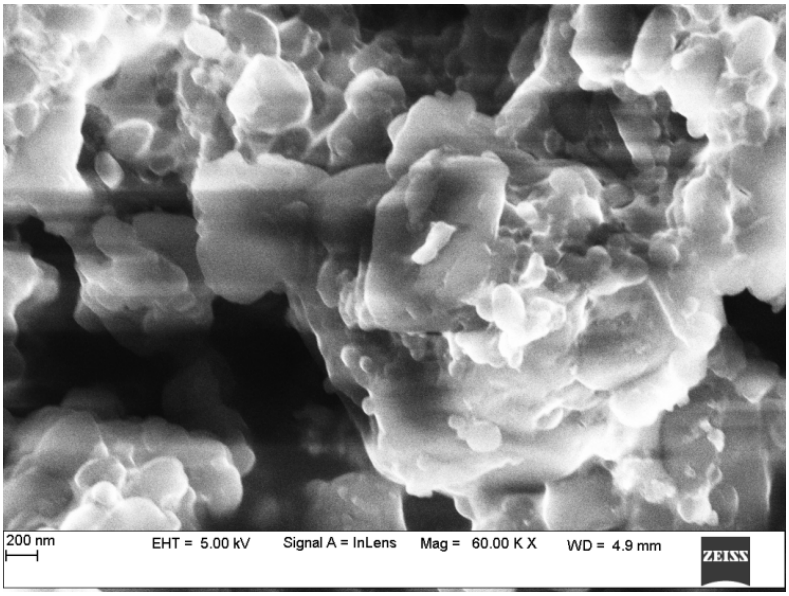

Figure no 8: FE-SEM of prepared GECN1 nanosponge of Glibenclamide

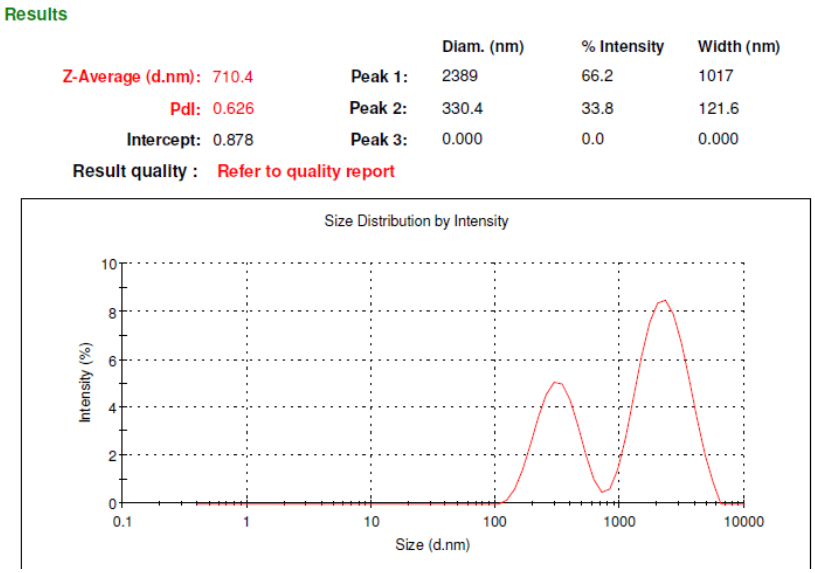

Figure no.9: Particle size analysis of sample GECN1

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Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -4.44	Peak 1:	-3.42	95.7	9.80
Zeta Deviation (mV): 11.2	Peak 2:	-33.7	4.3	3.52
Conductivity (mS/cm): 0.0229	Peak 3:	0.00	0.0	0.00

Result quality : Good

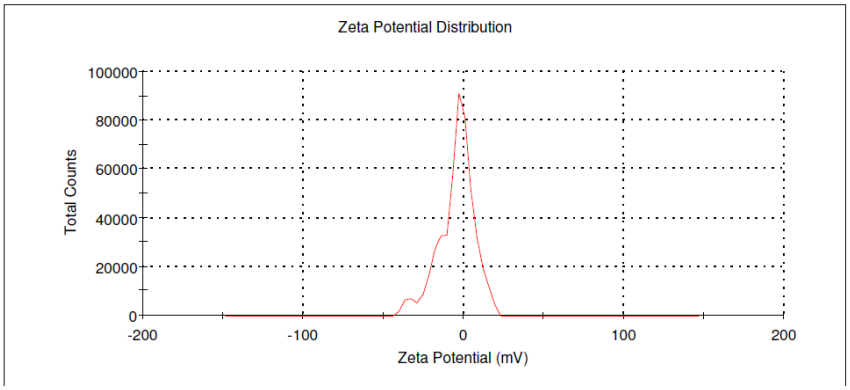

Figure no.10: Zeta potential analysis of sample GECN1

Results

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 816.3	Peak 1:	848.1	84.3	235.7
PdI: 0.648	Peak 2:	206.3	15.7	43.45
Intercept: 0.870	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report

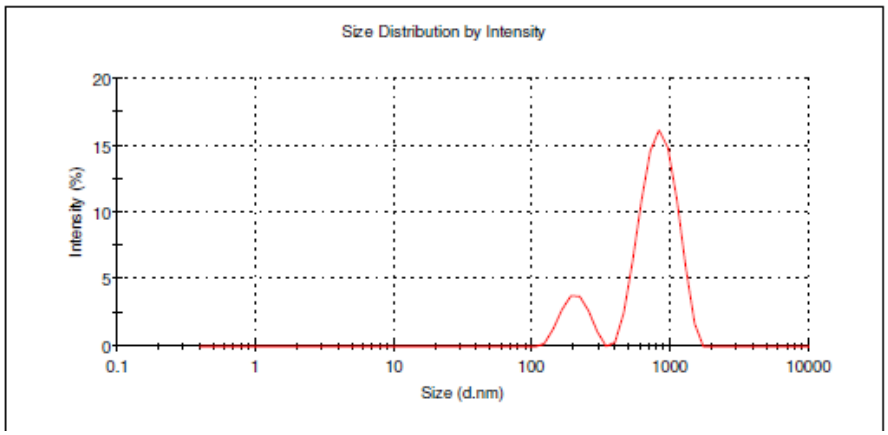

Figure no.11: Particle size analysis of sample GECN2

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Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -8.39	Peak 1:	-0.885	55.6	5.54
Zeta Deviation (mV): 11.2	Peak 2:	-17.9	44.4	7.69
Conductivity (mS/cm): 0.0204	Peak 3:	0.00	0.0	0.00

Result quality : Good

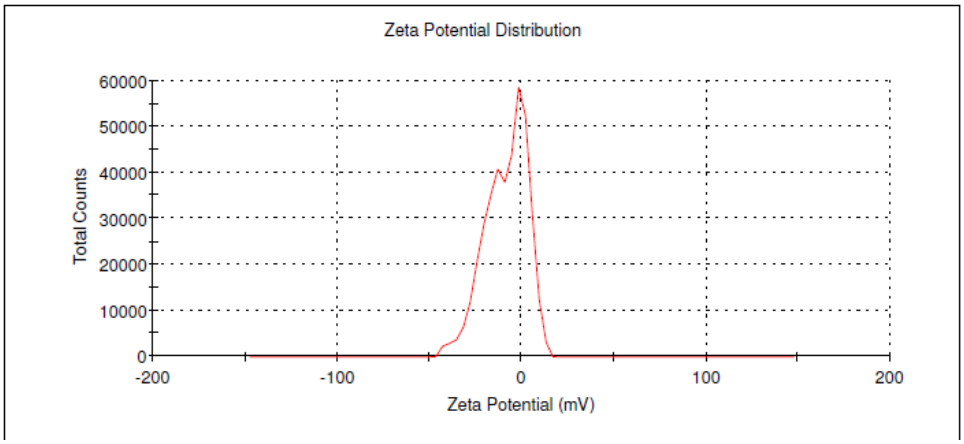

Figure no.12: Zeta potential analysis of sample GECN2

Results

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 708.7	Peak 1:	507.2	89.1	149.0
PdI: 0.603	Peak 2:	165.8	10.9	33.64
Intercept: 0.940	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report

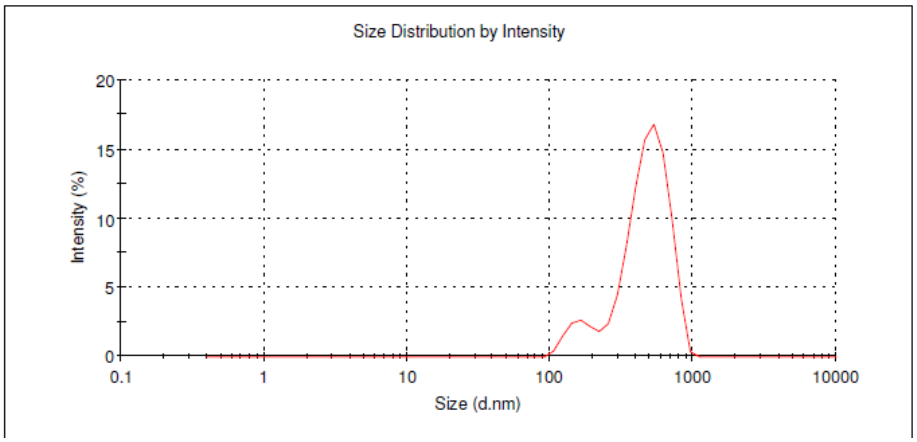

Figure no.13: Particle size analysis of sample GECN3

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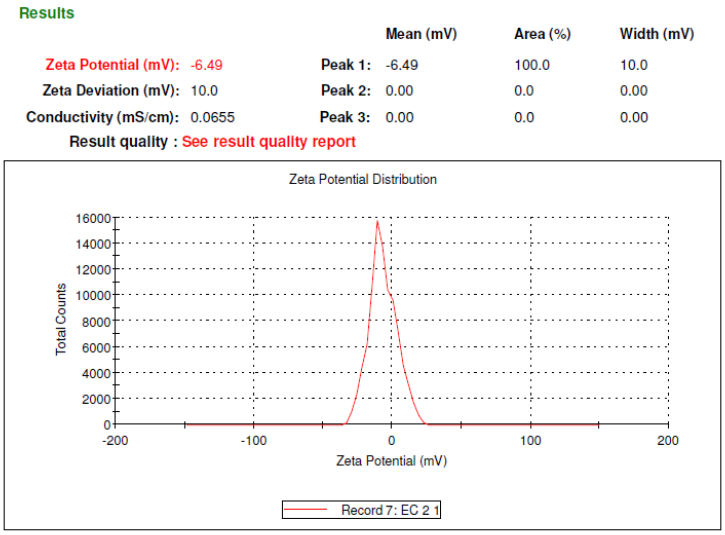

Figure no.14: Zeta potential analysis of sample GECN3

Table no:8 The particle size analysis, zeta potential, polydisperse index, percentage drug loading, and percentage yield of Glibenclamide nanosponges

Code	Particle Average diameter (nm)	Poly dispersive index (PDI)	Zeta potential (mV)	% Drug loading	% yield
GECN1	710.04	0.626	-4.4.0	72.32	68.84
GECN2	816.30	0.648	-8.39	75.34	76.54
GECN3	708.70	0.603	-6.49	80.72	78.43
GECN4	710.60	0.451	-9.26	79.39	71.19
GECN5	765.07	0.526	-7.45	69.21	82.12
GECN6	790.52	0.446	-8.01	72.18	81.32
GECN7	720.41	0.620	-11.02	81.13	82.42
GECN8	601.25	0.408	-10.52	74.26	80.48
GECN9	608.10	0.210	-11.48	70.64	82.80

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Table no –9: Cumulative % drug release of Glibenclamide Nanosponges prepared by emulsion solvent diffusion with high-speed homogenization method in 0.1N HCl of pH 1.2

Time in hr.	% Cumulative drug release*								
	GECN1	GECN2	GECN3	GECN4	GECN5	GECN6	GECN7	GECN8	GECN9
0.0	0	0	0	0	0	0	0	0	0
0.5	20±0.23	22±1.23	23±1.34	19±1.87	16±1.23	27±1.34	21±1.23	18±1.67	26±1.32
1.0	23±0.98	25±0.93	32±1.11	27±1.45	21±1.87	30±1.65	24±1.56	19±1.56	29±1.98
1.5	28±0.45	31±1.67	36±0.91	32±1.32	23±1.39	31±1.34	28±1.34	23±1.78	32±1.45
2.0	32±0.56	32±1.32	42±1.35	33±1.67	28±1.54	33±1.32	32±1.54	25±1.23	33±1.78

*** SD(n=3)**

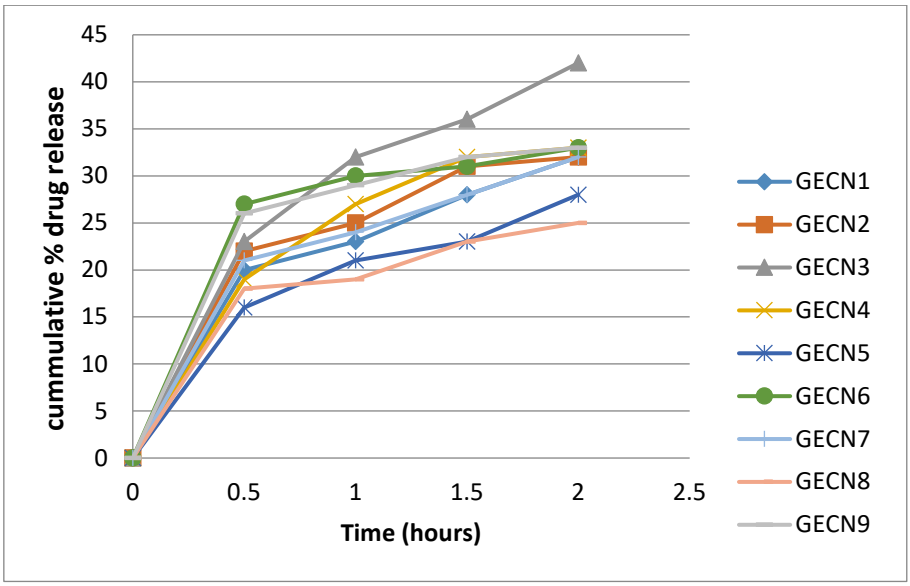

Figure no –15. *In-vitro* Dissolution Profile of Glibenclamide Nanosponges prepared by emulsion solvent diffusion with high-speed homogenization method in 0.1N HCl of pH 1.2

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Table no –10: Cumulative % drug release of Glibenclamide Nanosponges prepared by emulsion solvent diffusion with high-speed homogenization method in Phosphate Buffer 7.2 pH

Time in hours	% Cumulative drug release*								
	GECN1	GECN2	GECN3	GECN4	GECN5	GECN6	GECN7	GECN8	GECN9
0	0	0	0	0	0	0	0	0	0
1	35±1.23	36±0.92	38±0.93	32±1.32	40±2.45	42±1.62	37±1.87	38±1.34	41±1.76
2	45±1.45	44±0.34	40±1.22	35±1.65	46±1.31	43±1.87	39±1.32	39±1.35	46±1.09
3	60±1.56	59±1.23	45±1.65	60±1.34	62±1.54	69±1.98	56±1.54	45±1.33	67±1.40
4	63±1.98	60±1.10	53±2.11	63±1.56	63±1.33	70±1.67	64±1.67	49±1.60	68±1.03
5	68±1.54	63±1.45	59±1.43	67±1.21	68±1.98	71±1.91	70±1.65	66±1.90	70±1,32
10	70±1.76	70±1.65	60±1.89	70±1.23	73±1.34	75±1.65	73±1.11	72±1.54	72±1.20
15	75±1.87	72±1.33	62±1.54	73±1.78	76±1.23	76±1.45	78±1.67	73±1.32	74±1.32
20	79±1.98	79±2.34	62±1.23	68±1.54	76±1.23	77±1.34	79±1.56	73±1.77	77±1,90
24	82±1.22	72±1.32	75±1.87	70±1.98	80±1.34	78±1.34	791.34	73±1.41	84±1.32

*SD(n=3)

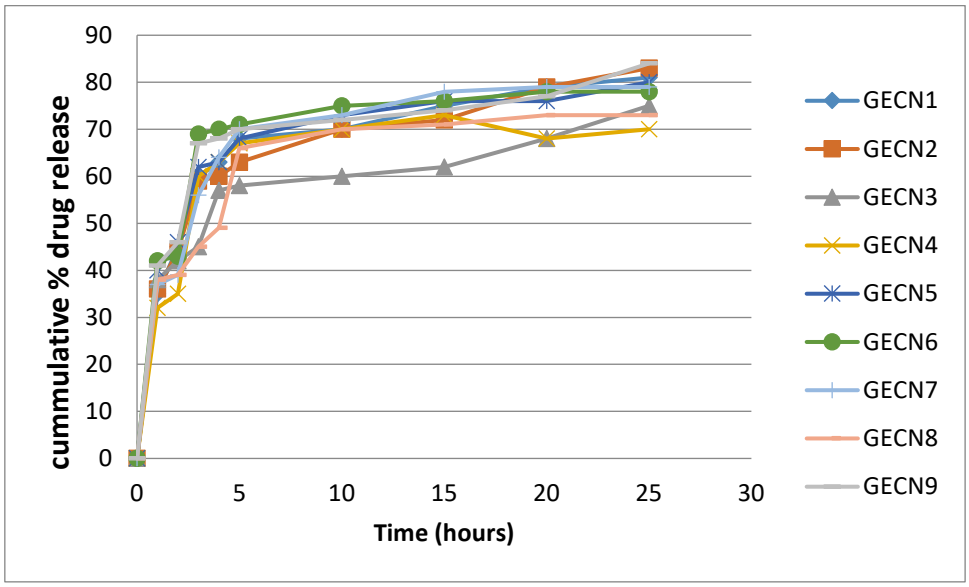

Figure no –16: *In-vitro* Dissolution Profile of Glibenclamide Nanosponges prepared by emulsion solvent diffusion with high-speed homogenization method in Phosphate Buffer 7.2 pH

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Table no 11. Comparison of correlation coefficient (r^2) and rate constant of zero-order and first-order kinetic models of **nanosponge prepared by emulsion solvent diffusion with high-speed homogenization**

Formulation code	Zero-order		First-order	
	r^2	k_0	r^2	K_1
GECN1	0.991	11.45	0.812	0.227
GECN2	0.994	11.34	0.799	0.227
GECN3	0.996	11.76	0.849	0.227
GECN4	0.992	11.34	0.833	0.227
GECN5	0.996	1.167	0.876	0.227
GECN6	0.981	11.98	0.887	0.227
GECN7	0.993	11.56	0.815	0.227
GECN8	0.991	12.78	0.823	0.227
GECN9	0.998	11.49	0.843	0.227

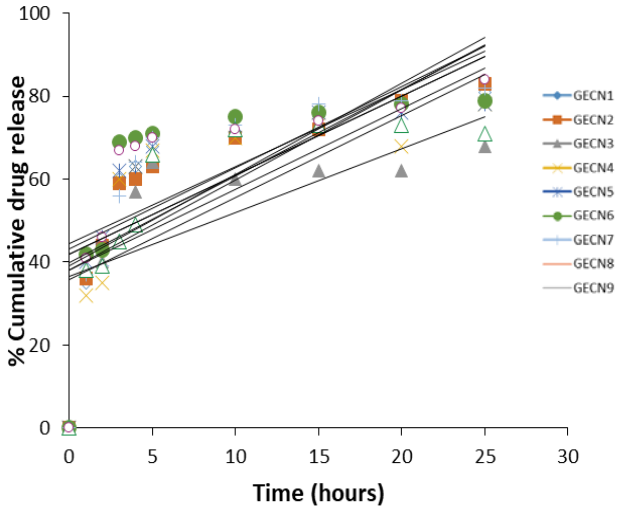

Figure no 17: zero-order kinetics of drug release for GECN1 to GECN9 nanosponges containing glibenclamide

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Table no 12. Central composite design with the independent and dependent variable

Code	Std	Run	Type	Independent variable		Dependent variable	
				Factor 1 A:EC Mg	Factor 2 B: PVA %	Y1 % Cumulative drug release	Y2 % Drug loading
GECN1	1	1	Fact.	100.00	0.20	82	72.32
GECN2	5	2	Axial	37.87	0.30	72	75.34
GECN3	7	3	Axial	250.00	0.16	75	80.72
GECN4	8	4	Axial	250.00	0.44	70	79.39
GECN5	3	5	Fact.	100.00	0.40	80	69.21
GECN6	6	6	Axial	462.13	0.30	78	72.18
GECN7	2	7	Fact.	400.00	0.20	79	81.13
GECN8	9	8	Cent.	250.00	0.30	71	74.26
GECN9	4	9	Fact	400.00	0.40	84	70.64

Table 13: Sequential model sum of squares for % Drug loading

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Mean vs Total	49136.11	1	49136.11			
Linear vs Mean	**177.14**	**2**	**88.57**	**92.41**	**0.0001**	**Suggested**
2FI vs Linear	0.25	1	0.25	0.23	0.6537	
Quadratic vs 2FI	4.01	2	2.01	4.05	0.1405	
Cubic vs Quadratic	0.36	2	0.18	0.16	0.8699	Aliased
Residual	1.13	1	1.13			
Total	49319.00	9	54.79.00			

Table no 14: Model summary statistics for % Drug loading (%)

Source	**Std. Dev.**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**	
Linear	**0.98**	**0.9686**	**9.9581**	**0.9230**	**14.07**	**Suggested**
2FI	1.05	0.9699	0.9519	0.9104	16.38	
Quadratic	0.70	0.9919	0.9783		+	
Cubic	1.06	0.9938	0.9508		+	Aliased

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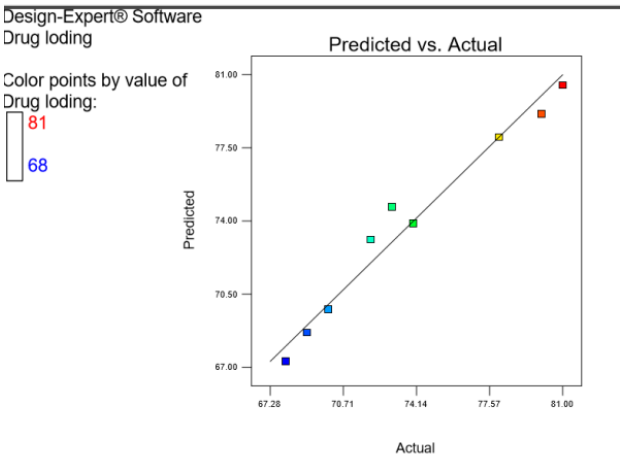

Figure no 18. Predicted vs. Actual correlations of % % Drug loading

Table no 15. The sequential model sum of squares %cumulative drug release

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	53053.44	1	53053.44			
Linear vs Mear	189.42	2	94.71	46.84	0.0002	Suggested
2FI vs Linear	1.00	1	1.00	0.45	0.5324	
Quadratic vs 2	5.56	2	2.78	1.49	0.3545	
Cubic vs Quad	5.58	2	2.79	6.366E+007	< 0.0001	Aliased
Residual	0.000	1	0.000			
Total	53255.00	9	5917.22			

Table no 16: Model summary statistics % cumulative drug release

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	1.42	0.9398	0.9197	0.8816	23.87	Suggested
2FI	1.49	0.9448	0.9116	0.8298	34.31	
Quadratic	1.36	0.9723	0.9262		+	
Cubic	0.000	1.0000	1.0000		+	Aliased

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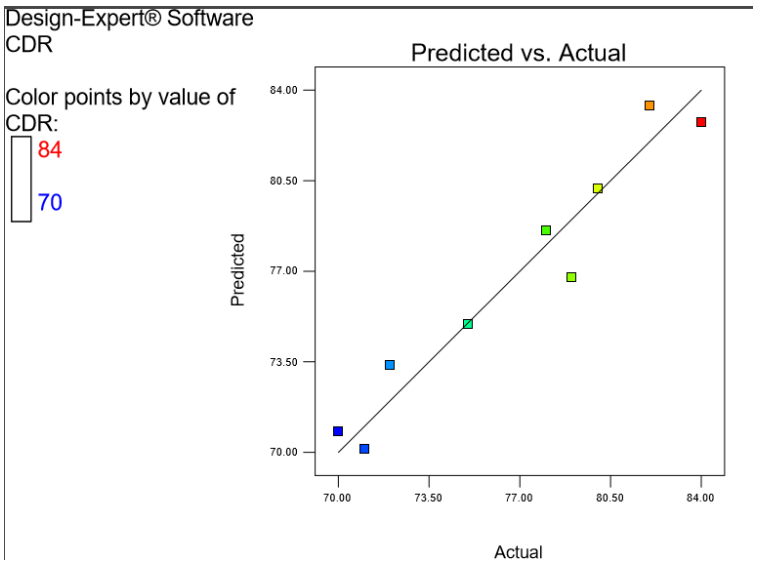

Figure no 19. Predicted vs. Actual for % cumulative drug release

Table no 17: Constraints: A summary spreadsheet to show all of the criteria applied to find the optimal settings and solutions given by software to see which ones best meet the specified criteria.

Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
EC	is in range	100	400	1	1	3
PVA	is in range	0.2	0.4	1	1	3
Drug loding	maximize	68	81	1	1	3
CDR	maximize	70	84	1	1	3

Solutions

Number	EC	PVA	Drug loding	CDR	Desirability	
1	400.00	0.20	79.1138	83.2526	0.900	Selected
2	400.00	0.22	79.0295	82.9808	0.887	

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Table 18. Stability study data of OPGECN1 and OPGECN2 nanosponges

Time days	Temperature /RH*	Formulations code	% CDR	% % Drug loading %
0	**25 °C ± 2 °C/60% RH ± 5% RH**	OPGECN1	82.65%	76.12%
		OPGECN2	82.33%	78.87%
	30 °C ± 2 °C/65% RH ± 5% RH	OPGECN1	82.03%	77.45%
		OPGECN2	82.45%	76.98%
30	**25 °C ± 2 °C/60% RH ± 5% RH or**	OPGECN1	82.00%	76.00%
		OPGECN2	82.21%	78.41%
	30 °C ± 2 °C/65% RH ± 5% RH	OPGECN1	81.99%	76.92%
		OPGECN2	82.13%	76.54%
90	**25 °C ± 2 °C/60% RH ± 5% RH or**	OPGECN1	81.43%	75.98%
		OPGECN2	81.30%	77.67%
	30 °C ± 2 °C/65% RH ± 5% RH	OPGECN1	81.03%	76.03%
		OPGECN2	81.45%	76.06%
180	**25 °C ± 2 °C/60% RH ± 5% RH or**	OPGECN1	81.10%	75.50%
		OPGECN2	81.03%	77.08%
	30 °C ± 2 °C/65% RH ± 5% RH	OPGECN1	82.23%	75.99%
		OPGECN2	82.05%	75.83%

IV. DISCUSSION

Solubility of Glibenclamide in 0.1N HCl of pH 1.2 showed good solubility i.e 0.966 mg/ml whereas it showing poor solubility in water and pH 7.4 phosphate buffer.

The scanning of Glibenclamide for absorbance maxima was with 0.1N HCl of pH 1.2 showing two peaks at 300 nm and 229 nm and the 229 nm was selected for calibration of the standard graph and it showed a R^2 value of 0.998 showing a good correlation

The standard graph range was found to be within beers-lamberts' range of concentration i.e 05 -20 mcg/ml

Solubility of Glibenclamide in 0.1N HCl of pH 1.2 showed good solubility i.e 0.966 mg/ml whereas it showing poor solubility in water and pH 7.2 phosphate buffer.

The standard graph range was found to be within beers-lamberts' range of concentration i.e 4 - 20mcg/ml

The scanning of Glibenclamide for absorbance maxima was with phosphate buffer pH 7.2. showing two peaks at 226 nm was selected for calibration of standard graph and it showed R2 value of 0.996 showing good correlation

The melting point of Glibenclamide

Melting point Glibenclamide was found to be 176 ^{o}C (Table no 2) which complied with I. P. standards, indicating the purity of the drug sample.

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Fourier transforms infrared spectroscopy (FT-IR)

FTIR spectra of pure Glibenclamide, Glibenclamide loaded EC nanosponges-GECN1 formulation shown in Figures 4 and 5. FTIR spectra of the pure drug showed characteristic peaks at 3552 cm-1 and 3500.17 cm-1 corresponding to N-H stretching, 1714 cm-1and 1705 cm-1 due to carbonyl stretching, 1259 cm-1. 1154 cm-1 showing S=O stretching shown in Table 8 confirms the compatibility of the drug with polymers.

Differential Scanning Calorimetry / Thermo Gravimetric Analysis (DSC-TGA) of nanosponges containing Glibenclamide by Emulsion Solvent Diffusion Method

Figure no: 6 and 7. Shows the DSC-TGA thermogram of pure Glibenclamide, and GECN1 nanosponges containing Glibenclamide respectively. DSC Thermogram of pure Glibenclamide and formulation showed a sharp endothermic peak at 176°C which corresponds to the melting point of the drug, which is the same as reported in the literature.

The TGA curve shows an initial 6.2% loss corresponds to moisture content. The lighter substances are removed initially and then heavier materials are removed. The TGA curve shows that major degradation occurs above 210ºC.

Particle morphology by Field Emission Scanning electron microscopic (FE-SEM)

Figure no: 8. Shows the field emission scanning electron micrographs (FE-SEM) of GECN1 nanosponges and were found to be irregular rough with the formation of aggregates in nanosponge

Particle size distribution zeta potential

The particle size analysis of Glibenclamide loaded Nanosponges by Emulsion Solvent Diffusion method showed (table no- 7) and (fig. no. 9 to 14) that the average particle size measured by Dynamic Light Scattering, using Malvern Zetasizer is around 600 nm to 800 nm and the polydisperse index values found in the range 0.30 to 0.80 indicating the prepared nanosponges are polydisperse.

The lodging efficiency of GECN1 to GECN 9 formulation was found to be between 60 to 74 %. The percentage yield of GECN1 to GECN 9 formulation was found to be between 70% to 82% the formulation GECN 7 showed an 80% yield (table no- 8)

In-vitro Dissolution studies of nanosponges in 0.1 HCl of pH 1.2 (up to 2 hours) and phosphate buffer 7.2 pH (up to 24 hours)

The Cumulative % drug release from GECN1 to GECN9 ranges from 32±0.56, 32±1.32, 42±1.35, 33±1.67, 28±1.54, 33±1.32, 32±1.54, 25±1.23, and 33±1.78% respectively in 0.1 HCl of pH 1.2 (up to 2 hours) (table no- 9) (Figure no 15)

When compared with pure Glibenclamide increase in % drug release is observed in a controlled manner in the case of nanosponges containing Glibenclamide.

In the case of phosphate buffer, 7.2 pH (up to 24 hours) Cumulative % drug release from GECN1 to GECN9 ranges from 82±1.22, 72±1.32, 75±1.87, 70±1.98, 80±1.34, 78±1.34, 791.34, 73±1.41, 84±1.32 respectively.

The formulation GECN1, GECN5, and GECN9 showed the highest and controlled manner, and all formulations the release followed a controlled manner. (Table no- 10) (Figure no 16)

Kinetics of drug release

Kinetics of drug release from the nanosponges containing Glibenclamide prepared by emulsion solvent diffusion with high-speed homogenization method is subjected to mathematical treatment. The best fit model with the highest correlation coefficient values (R2) for the formulation codes GECN1 To GECN9 between 0.991 to 0.998 indicating that the release is best fits to zero-order kinetics release model. (Table no- 11) (Figure no 17).

Selection and validation of models:

% Drug loading-ANOVA for quadratic model:

The Model F-value of 92.41 implies the model is significant. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant.

Quadratic Equation

Final Equation in Terms of Coded Factors % Drug loading= +73.89 +4.67 * A -0.55 * B

Final Equation in Terms of Actual Factors:

% % Drug loading= +67.75572 +0.031154 * EC -5.51777 * PVA

The main effects of factors with different levels on response are shows that the main effects of factor A (EC) on a response (% drug loading.) are linear and explained by polynomial equations. (% Drug loading= +67.75572 +0.031154 * EC -5.51777 * PVA) As a factor, A changes from level -1 to +1 the % Drug loading increases linearly.

Based upon the quadratic equation generated, the model is validated. The predicted value and the actual values are linear indicating less error. (Table no- 12,13,14) (Figure no 18).

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Final Equation in Terms of Coded Factors for % CDR = +76.78 +4.69 * A -1.28 * B
Final Equation in Terms of Actual Factors: (Table no- 15,16) (Figure no 19).% CDR = +72.79453 +0.031297 * EC -12.80330 * PVA

Constraints for Numerical Optimization:
The optimization module for a combination of factor levels that simultaneously satisfy the criteria placed on each of the responses and factors. Numerical optimization uses the models to search the factor space for the best trade-offs to achieve multiple goals. For the factors X1, X2, the goal was set 'in range' and for the response, % drug loading, and %CDR, the goal was set to be 'maximize'. Between Minimum and maximum levels which was provided for each parameter included as mentioned in Table 17.
Several solutions were given by the Design Expert® software by deciding upon different constraints to see which ones best meet the specified criteria. Here the final formulation was selected based on the highest % % Drug loading and % CDR and desirability near to 1. The different solutions given by the software using different combinations of independent levels along with desirability are shown in Table 12. The first solution (OPGECN1) with EC (400 mg) and PVA (0.20%) with predicted CDR is 83.25 % and predicted % Drug loading79.11 % w/w was selected which showed the desirability near to 1. The actual % CDR is 82.55 % and % Drug loading 80.12% was observed shows fewer errors. The second solution (OPGECN2) with EC (400 mg) and PVA (0.22%) with predicted CDR is 82.98 % and predicted % Drug loading79.02 % w/w was selected which showed the desirability near to 1. The actual % CDR is 82.01 % and % Drug loading79.12% were observed.

Stability studies
Stability study on optimized nanosponge formulation prepared by emulsion solvent diffusion with high-speed homogenization method is OPGECN1 and OPGECN2 and subjected to stability study table no 18. the formulation analyzed for %CDR, & % Drug loading all the formulations showed good stability.

V. CONCLUSIONS

The scanning of Glibenclamide for absorbance maxima with 0.1N HCl of pH 1.2 was 229. And standard graph R2 value of 0.998
The scanning of Glibenclamide for absorbance maxima was with phosphate buffer pH 7.2. showing peaks of 226 nm. The standard graph showed R^2 value of 0.996
The melting point of Glibenclamide was found to be 176oC, which complied with I. P. standards, indicating the purity of the drug sample
The FT- IR Spectrum of pure Glibenclamide drug was compared with the FT- IR spectrum of physical mixture of Glibenclamide with polymers and product. There was no disappearance of any characteristic peaks. This shows that there is no chemical interaction between the drug and the polymers used in the nanosponges.
Field emission scanning electron micrographs (FE-SEM) of the GECN1 containing Glibenclamide prepared by the solvent evaporation method with high-speed homogenization was found in nano range of sizes 600 -800 nm and rough surface morphology
DSC-TGA Thermogram of pure Glibenclamide showed a sharp endothermic peak at 1760C which corresponds to the melting point of the drug, which is the same as reported in the literature. The TGA curve shows that major degradation occurs above 210ºC.
The prepared nanosponges loaded with Glibenclamide the percentage of loading was found to be between 68 to 85 %
The particle size analysis showed the in a case by emulsion solvent diffusion method was in the range of 608 nm to 816 nm with PDI of 0.210 to 0.618 and percentage loading between 70- 81% indication the good % Drug loading but resulted in polydisperse
The zeta potential was found to be in the range -4 to -11.48 mV indicating that good stability
In the case of phosphate buffer 7.2 pH (up to 24 hours), The Cumulative % drug release from GECN1 to GECN9 ranges from 82±1.22, 72±1.32, 75±1.87, 70±1.98, 80±1.34, 78±1.34, 791.34, 73±1.41, 84±1.32 respectively.
The formulation GECN1, GECN5, and GECN9 showed the highest and controlled manner, and all formulations the release followed a controlled manner
The best fit model with the highest correlation coefficient values (R2) for the formulation codes GNS1 To GNS6 is between 0.980 to 0.989 and formulation GECN1 to GECN9 is between 0.991 to 0.998 indicating that the release is best fits to zero-order kinetics release model.
Central composite design with independent and dependent variable are used Final Equation in Terms of Actual Factors: % Drug loading= +67.75572 +0.031154 * EC -5.51777 * PVA and

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Final Equation in Terms of Actual Factors: % CDR = +72.79453 +0.031297 * EC -12.80330 * PVA. The predicted and actual are in good correlation and the model fitted is the linear model

Numerical optimization with constraints of goal maximizes the response of the suggested optimized formulation The first solution (OPGECN1) with EC (400 mg) and PVA (0.20%) with Actual % CDR is 82.55 % and % Drug loading80.12%. The second solution (OPGECN2). Actual % CDR is 82.01 % and % Drug loading79.12% were observed shows less than 2 % errors Stability study resulted in good stability less 1 % deviation from zero-time values FTIR spectra of pure Glibenclamide,

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