Title:

Full Title: Formulation and Evaluation of Nanosponges Loaded Metformin Hydrochloride Tablets.

Short Title: Formulation and Evaluation of Nanosponges Loaded Metformin Hydrochloride Tablets.

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Abstract

The objective of this study was to formulate a Nanosponges loaded Tablets of Metformin Hydrochloride for the treatment of Type-II diabetes. Metformin Hydrochloride belongs to BCS class III drug having high solubility, low permeability. This research aims to improve bioavailability of drug by increasing the retention time by entrap drug molecule in nanosponges incorporated in to a tablets. Nanosponges were prepared by an Emulsion Solvent Diffusion method by using 3^2 Full Factorial Design. Metformin Hydrochloride Loaded Nanosponges prepared by Emulsion Solvent Diffusion Method by using Ethyl cellulose and polyvinyl alcohol.

One factor was evaluated at three levels and concentration of Ethyl Cellulose (X1) and Concentration of Polyvinyl alcohol (X2) were selected as independent variables. The F8 Batch of Nanosponges out of nine formulations was found to be optimized. The average particle size of the nanosponges F8 Batch was measured at 219.1 nm with a zeta potential -24 mv. The Entrapment efficiency across 9 batches varied from 87.61%. To 92.98% the entrapment efficiency of optimized batch was found to be 95.47%. *In-Vitro* drug release for these batch was observed Between 61.17 % to 74.53 %. *In-vitro* drug release of optimized batch was found to be 74.53%. Thus, the application of nanosponges proved the potential for Nanosponges drug delivery of Metformin Hydrochloride. Nanosponge's drug delivery for Metformin Hydrochloride has been successfully developed.

Keywords: Nanosponges, Drug delivery, Oral delivery, Solubility enhancement, Controlled release, Bioavailability, Metformin Hydrochloride.

Introduction:

Novel carriers developed for to reduce the side effects and increase their therapeutics benefit, they should be delivered to their respective of action, and hence a suitable carrier system becomes a mandatory requirement. And various novel carrier developed for these purpose [1].

Nanosponges are tiny structures that resemble meshes and can contain a wide range of materials, including drug compounds. Their spherical colloidal structure allows them to improve the solubilization of both lipid-soluble and water-soluble medicines. They improve the bioavailability of medications with extended release. Nanosponges can contain therapeutic compounds that are

both hydrophilic and hydrophobic because to their amphiphile nature, which consists of outside hydrophilic branching and internal hydrophobic chambers [2]. With a void space of 5–300 μm and a diameter of 10–25 μm, NSs are superior than microsponge since the latter have a diameter of less than 1 μm. Micro sponges are stiff but break down once the temperature reaches 130°C. In contrast, these NSs are robust up to 300°C [3]. they improve the bioavailability of medications with extended release. Nanosponges can contain therapeutic compounds that are both hydrophilic and hydrophobic because to their amphiphile nature, which consists of outside hydrophilic branching and internal hydrophobic chambers. With cross linkers connecting various polymer components and a backbone of long-chain polyesters in the solution, they resemble a three-dimensional network. It has been discovered that by treating cyclodextrins (cyclic oligosaccharides) with the right crosslinking agents, a novel nanostructured material composed of hyper-cross-connected cyclodextrins can be created: nanosponges. Both neutral and acidic materials can be used to create nanosponges, and they can swell depending on the cross-linking agent used. As a result, hollow spheres containing voids that are created and may contain therapeutic compounds. To improve drug loading and provide a personalized solution, the ratio of cross-linking to cyclodextrin can be altered during preparation release profile. Their highly permeable monomeric structure, when compared to the parent cyclodextrin molecules, allows for medication [2]. Diabetes mellitus is a worldwide public health challenge due to its high morbidity and mortality rate. It is one of the most prevailing and advancing disease in the world having affected 6.6% of the world population. Metformin hydrochloride is the most widely used oral anti diabetic drug in the world. Metformin improves glucose tolerance by lowering both basal and postprandial glucose by decreasing intestinal absorption of glucose, decreasing hepatic gluconeogenesis, increasing glycogenesis, lipogenesis and glucose uptake by Adipocytes and muscle cell. Metformin is a highly water soluble drug. Metformin is an oral anti diabetic drug. The oral route is considered to be one of the most acceptable routes used for the drug administration. Tablets are mostly preferred formulations by patients for the treatment of diseases, and it is beneficial particularly when the long term therapy is require [4].

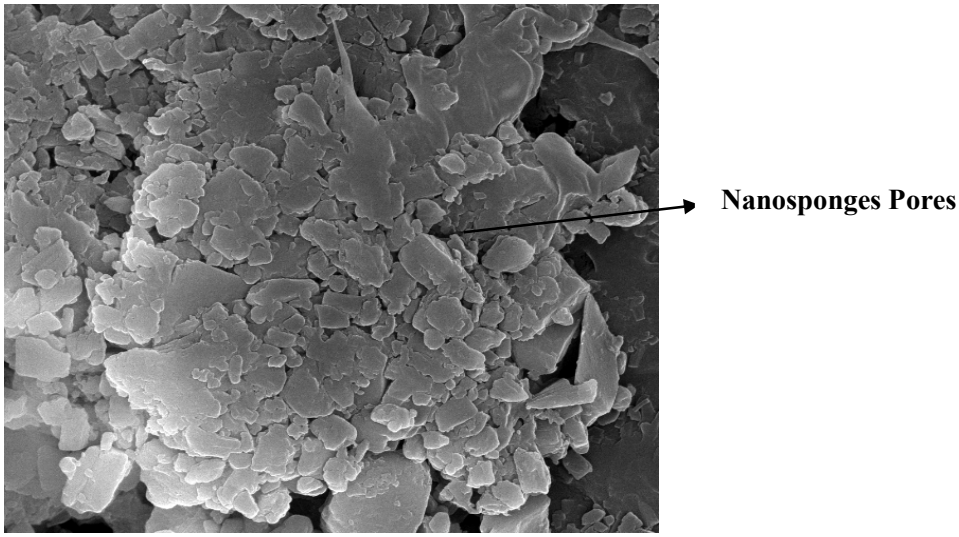

Fig.1 Structure of Nanosponges.

Pre-Formulation Studies:

The Physicochemical properties of the drug play a very important role in performance of the drug and the development of dosage form. Hence, in order to develop a safe, stable and effective dosage form the performulaion study of Metformin Hydrochloride was carried effectively.

Solubility:

The solubility of Metformin Hydrochloride was tested in different solvents like Distilled water, Ethanol, Dichloromethane, & Methanol at ambient temperature by dissolving a specific amount of API (10 mg) in (10 ml) of each solvent. The solubility was detected visually and also using UV-visible spectroscopy [5].

Melting Point:

For determination of melting point USP method was followed. Small quantity of Metformin HCl was placed into a sealed capillary tube. The tube was placed in the melting point apparatus. The temperature in the apparatus was gradually increased and the observation of temperature was noted at which Metformin HCl started to melt and the temperature when the entire drug gets melted. This method is also known as open capillary method [6]

UV-Visible Spectroscopy [7, 8]**:**

Determination of lambda max:

The structural information on Metformin hydrochloride's chromophoric component was obtained using a UV-Vis spectrophotometric approach. The solution was then analyzed in the range 200-400nm using UV/Vis Spectrophotometer to measure the Absorption maxima. 10mg of Metformin HCl was dissolved in 10ml Phosphate buffer (pH 7.4) and diluted up to 100ml using phosphate buffer.

Preparation of Calibration curve:

A series of dilutions with concentration 2, 4, 6, 8, 10 ppm were prepared from Metformin Hydrochloride 100 ppm stock solution in phosphate buffer (pH 7.4) and were scanned at the determined Absorption maximum. Absorbance vs. Concentration graph was plotted.

Fourier Transform Infra-Red Spectroscopy (FT-IR)

To demonstrate the chemical integrity and compatibility of the formulations, the IR spectra of the pure drug, Physical mixture of drug & Excipient, Optimized batch of Nanosponges formulations were obtained. The compatibility between the medicine and excipient in metformin HCL and Nanosponges was examined using a Fourier-transform infrared Analyzer [9].

FT-IR Spectroscopy was used to qualitatively identify the compound, providing adequate data on the groups present within these compound. The IR investigation was conducted using a Perkin Elmer Fourier Transform Infrared Spectrophotometer [10, 11].

Differential Scanning Calorimeter:

Differential scanning calorimeter study of Metformin hydrochloride samples was carried out on a differential scanning calorimeter. The thermal analysis was performed in a nitrogen atmosphere at a heating rate of 10°C/min over a temperature range of 30–300°C. Alumina was employed as the reference standard. The onsets of melting points and enthalpies of fusion of samples were automatically calculated by the instrument [12, 13, and 14].

Materials & Method:

Material:

Metformin hydrochloride was received as a gift sample from Sohan Healthcare, Pvt. Ltd. Ethyl Cellulose, Polyvinyl alcohol, Dichloromethane were obtained from Research lab. All the material used for formulation of nanosponges tablets.

Methods:

Preparation of Metformin Hydrochloride loaded nanosponges-

Metformin Hydrochloride loaded nanosponges were prepared by Emulsion Solvent Diffusion Method.3^2 factorial design was used to formulate F1-F9 batches indicated in Table: 1.

In this method, different proportion of Ethyl Cellulose and polyvinyl alcohol are used to prepared nanosponges. Two phases are used in this method i.e. Dispersed and Continuous phases. The dispersed phase consist of Ethyl cellulose and the drug. Which is then dissolved in 20 ml of organic solvent .i.e. Dichloromethane and Ethanol in the ratio of 1:1.The external phase (aqueous phase) is made up of a predetermined amount of polyvinyl alcohol dissolved in 100 ml distilled water. Add both the phases, then mixture is stirred on magnetic stirrer at the speed of 1000 rpm. For about 2 hours. The product i.e. nanosponges are collected by filtration. Finally the product is dried in oven at a temperature of 40℃. [15, 16]

Experimental Design:

Optimization of nanosponges preparation procedure was done by using 3^2 full factorial design. The Concentration of Ethyl cellulose (X1) and polyvinyl alcohol (X2) were selected as independent variables. % Entrapment efficiency (Y1) were selected as dependent variables.

Statistical Analysis of Data-

The effect of independent variables upon the response was checked by using Statistical tools, such as descriptive statistics and one way ANOVA by Design expert software (Version 13). A value of $p < 0.05$ was considered statistically significant.

Batch Code	Drug	Ethyl Cellulose (mg)	Polyvinyl alcohol (mg))
F1	500	100	100
F2	500	100	150
F3	500	200	200
F4	500	200	100
F5	500	100	200
F6	500	150	200
F7	500	150	100
F8	500	200	150
F9	500	150	150

***Note- Quantity expressed in milligram.**

Table 1. Composition of Metformin hydrochloride loaded Nanosponges by 3^2 factorial design [17, 18, 19]

Characterization of Drug Loaded Nanosponges:

Entrapment Efficiency:

Centrifugation method was used to determined percentage entrapment efficiency of nanosponges. [20] Freshly prepared 5 ml nanosponges suspension was taken in centrifuge tube and centrifuged at 9000 rpm for 45 minutes (REMI Instrument). 1 ml supernatant was separated which containing free drug and diluted with methanol. The concentration of free drug in supernatant layer was analyzed at wavelength of 234 nm by UV Spectrophotometer (Jasco V-530). % Entrapment Efficiency was calculated by using formula –[21]

$$\boldsymbol{Entrapment\ Efficiency} = \frac{\boldsymbol{Total\ amount\ \ of\ drug\ added - Drug\ in\ supernatant}}{\boldsymbol{Total\ amount\ of\ drug\ added}} \times \mathbf{100}$$

Production Yield: [22]

The production yield of the nanosponges was determined by calculating accurately the initial weight of the raw materials and the final weight of the nanosponges obtained. [23, 24]

The production yield was calculated accordingly:

$$\text{Production Yield} = \frac{Practical\ weight\ of\ nanosponges}{Theoretical\ weight(Drug + polymer)} \times 100$$

Particle Size: [25, 26]

Particle size must realize that any sample having the same mean diameter may have different size distribution. Particle size is crucial for drug efficacy, bioavailability and consistent performance. The average mean width and particle size of Metformin Hydrochloride nanosponges were investigated employing the Malvern Zeta sizer instrument. To obtain the correct light scattering intensity for Metformin Hydrochloride nanosponges the dried nanosponges were distributed in distilled water.

Zeta Potential:

The charge on surface of nanopsonges loaded with Metformin Hydrochloride were determined by using Zeta Potential. Zeta potential is crucial for determining the stability of nanosponges. Surface charge, Nature and composition all are determine by Zeta potential**.** [21, 27, 28]

Scanning Electron Microscopy: [29, 30, 31]

The Surface morphology of prepared Metformin Hydrochloride prepared nanosponges were evaluated by Scanning Electron Microscopy.

In Vitro drug release study of Nanosponges: [32, 33]

The *in vitro* release study of Metformin paddle apparatus. The paddle rotation speed was kept at 50 rpm and a temperature of 37.5 ± 0.5 ℃ was maintained for 8 hrs. A release study was carried out in 900 ml of phosphate buffer pH 7.4 as dissolution medium separately. 5 ml of sample was withdraw at predetermined intervals (0.5, 1, 2…8) till 8 hours and replaced by its equivalent volume of fresh dissolution medium to maintain the sink condition. The withdrawal liquids were filtered and assayed at 234 nm.

Preparation of Nanosponges Loaded Metformin Hydrochloride Tablets:

Pre-formulation Studies: [34...41]

Preformulation studies were conducted to evaluate the drug, focusing on characteristics such as the analytical techniques for the drug sample formulation. These studies included physical characterization and assessment of the powder blend for tablet formulation. Evaluations comprised calculations of bulk density, tapped density, Carr's index, Hausner's ratio, and angle of repose**.** Hydrochloride loaded nanosponges equivalent to 10 mg of drug was determined using USP Type- II.apparatus.

Pre-compression Parameters:

Pre-compression parameters in tablet manufacturing are characteristics of the powder blend assessed before the main compression stage to ensure quality and consistency, including flow properties, bulk density, and angle of repose. Main purpose of Pre-compression parameter is help evaluate the suitability of the powder blend for tablet production and identify potential issues that could lead to defects like capping or lamination.

Bulk Density:

A large funnel was used to transfer the powder into a graduated cylinder to measure its volume. The bulk density is expressed in g/cc. was then calculated using appropriate formula:

Bulk Density = Mass of Powder/Bulk Volume

Tapped density:

To measure tapped density of the powder, 10 g of the powder was placed in a 100 ml measuring cylinder. The cylinder was tapped 100 times from a consistent height, and the volume was recorded afterward. The tapped density, expressed in expressed in g/cc, was then calculated based on these measurements.

Tapped Density = Mass of Powder/Tapped Density

Hausner's ratio:

The relationship between the bulk density and tapped density of a powder is assessed using hausners ratio. Calculate as the tapped density divided by the bulk density. A ratio between 1.25 and 1.5 suggests that the flow properties of the powder may be improved by adding a glidant.

Hausner's ratio = Tapped Density/ Bulk Density.

Compressibility index (Carr's):

The Compressibility index, also known as car's index. Is a measured used to assess the flow ability and compressibility of a powder. It is calculated using the bulk density and tapped density of the powder. The compressibility index is determined by the following formula.

Compressibility Index (%) = [(Tapped Density-Bulk Density)] *100]/Tapped Density

Angle of Repose

The angle of repose is a measure used to determine the flowability of a granular material. It is the maximum angle between the surface of a pile of powder and the horizontal plane. This angle is determined by allowing the powder to flow through a funnel to form a cone-shaped pile and measuring the angle between the side of the pile and the horizontal surface.

$$\tan(\theta) = h/r.$$

h = height in cm r = radius in cm.

Sr. No	Type of flow	Angle of repose	Carr's index	Hausner's ratio
1	Excellent	25-20	5-10	1.00-1.11
2	Good	31-35	11-15	1.12-1.18
3	Fair	36-40	16-20	1.19-1.25
4	Passable	41-45	21-25	1.26-1.34
5	Poor	46-55	26-31	1.35-1.45
6	Very poor	56-65	32-37	1.46-1.54
7	Very very poor	>66	>40	>1.60

Table No. 2 Limitations for Powder Flow Properties:

Preparation of Nanosponges loaded Metformin Hydrochloride tablets:

Solids having good flowability, lubricating properties and cohesiveness can be compressed directly into tablets without granulation. This is two-step process, mixing of drug and additives and its direct compression into tablets**.** [42, 43]

The Metformin Hydrochloride loaded Nanosponges tablet were prepared by direct compression method. Tablet was made by mixing Starch up to 40% [44], Lactose up to 60-70% [45] and Talc up to 1%.[46]

Sr. No	Materials	Composition
1	Optimized batch of nanosponges	350 mg
2	Starch	Up to 40%,
3	Lactose	Up to 60-70%,
4	Talc	Up to 1%.

Table No: 3 Formulation of Nanosponges loaded Metformin Hydrochloride tablets

Characterization of Metformin hydrochloride Nanosponges Tablets:

Post-Compression parameters.

Weight variation test:

Ten tablets from each formulation (F1 to F9) were weighed using an electronic balance to assess weight variation. The average weight was calculated, revealing that only two of the individual weights for the 200 mg tablets deviated by more than 7.5 percent from the average, with no exceedingly twice this percentage. The detailed results are presented in the Table. [47, 48]

Hardness test:

Tablet hardness test could be defined as a tablet strength test which reflect overall tablet strength and it is measured by applying pressure to the tablet diameter. Tablet must have a certain strength and hardness and it could withstand from various mechanical shock during manufacture, packaging, and transportation and we needed hardness tester for the tools. Hardness was a parameter which describes the tablet's resistance to resist mechanical emphasis like shock, abrasion and crack to the tablet during the stages of packaging, transportation, and use. The hardness was used to measure compression pressure. We can say tablet in a good condition if the tablet has 4 to 8 kg. Tablet hardness test was a test which is conducted to know physical hardness of tablet preparation to the mechanical stress or friction. The aim was to know the resistance of tablet preparation in the face of pressure obtained several processes: packaging, distribution, and stored. The tablet's hardness was assessed using a Monsanto hardness tester. A zero measurement was noted when the lower plunger made contact with the tablet. [49, 50]

Thickness:

Tablet thickness is a crucial measurement that indicates how thick a tablet is, typically ranging from 1.97 to 2.26 mm. This measurement ensures uniformity in size and dosage, which is vital for effective manufacturing and performance. Low variability in tablet thickness relates to formulation flow and compression consistency. It also affects visual and functional properties, dissolution rates, and is essential for accurate dosing and packaging processes. Overall, maintaining appropriate tablet thickness is key to quality and practicality in pharmaceuticals.

The thickness of each tablet was measured using a digital vernier caliper. The results were expressed as deviations the mean values of ten readings along with the standard deviation. [51,52]

Friability Test:

This test is a method to determine physical strength of uncoated tablets upon exposure to mechanical shock and attrition. The apparatus was loaded with 20 pre-weighed tablets and subjected to 100 rotations. Afterward, the tablets were reweighed. The percentage of friability was then calculated using this method. [53, 54]

Percentage friability = [Initial weight - Average weight) / (Initial weight] X 100

$$\% \boldsymbol{Friability} = \frac{(\boldsymbol{Initial\ Weight - Average\ weight})}{(\boldsymbol{Initial\ weight})} \times \mathbf{100.}$$

In-Vitro Drug release study of Nanosponges Loaded Metformin Hydrochloride Tablets:

The *in vitro* release study of Nanosponges loaded Metformin Hydrochloride Tablets was determined using USP type-II paddle apparatus. The paddle rotation speed was kept at 50 rpm and a temperature of 37.5 ± 0.5 ℃ was maintained for 8 hrs. A release study was carried out in 900 ml of phosphate buffer pH 7.4 as dissolution medium separately. 5 ml of sample was withdraw at predetermined intervals (0.5, 1, 2…8) till 8 hours and replaced by its equivalent volume of fresh dissolution medium to maintain the sink condition. The withdrawal liquids were filtered and assayed at 234 nm. [32, 33]

Results and Discussion:

Pre-formulation Evaluation

Solubility:

Table No.4: Solubility Study of Metformin hydrochloride

Sr. No.	Solvent	Solubility data
1.	Water	Freely Soluble
2.	Ethanol	Soluble
3.	Dichloromethane	Sparingly Soluble

Determination of Melting Point

Table No 5: Melting Point of Metformin hydrochloride

Sr. No	Standard M.P. (℃)	Observed M.P. (℃)	Mean M.P. (℃)
1	223-226 (℃)	224 (℃)	225(℃)
2		225 (℃)	
3		226(℃)	

UV-Visible Spectroscopy:

Determination of λ max

The UV visible spectrum of Metformin hydrochloride in Phosphate buffer pH 7.4 shows λ_{max} 234 nm as shown in figure No.2.

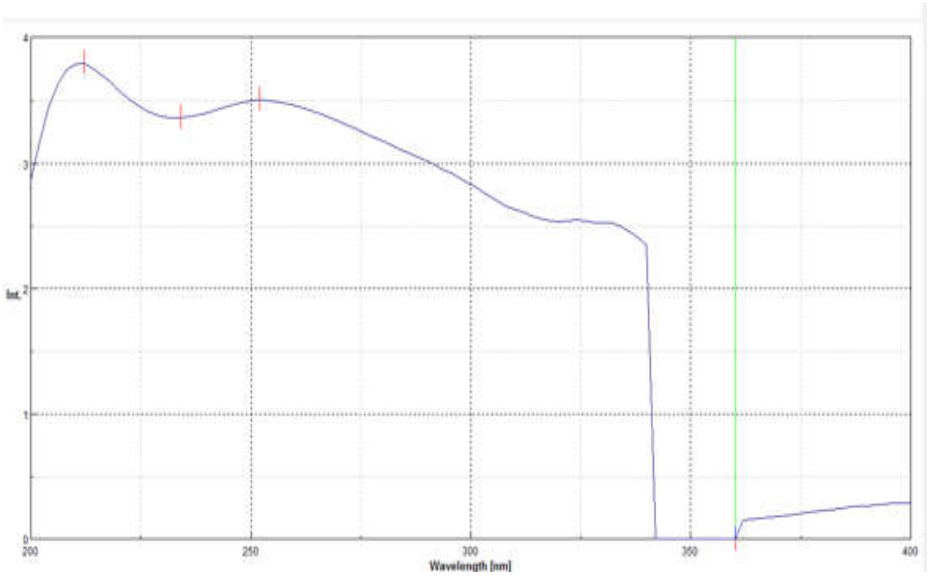

Fig. No.2: UV spectrum of Metformin Hydrochloride Calibration Curve of Metformin Hydrochloride in Phosphate Buffer pH7.4-

The following table no.3 shows the calibration curve data of different dilutions of Metformin Hydrochloride in Phosphate buffer pH 7.4 at wavelength 234 nm. The calibration curve was plotted as shown in figure no.1 in concentration range of 2-10 µg/ml. After regression of data as shown in table no. 3 value of R^2 was found to be 0.9997 which indicated linearity.

Sr. No.	Concentration (µg/ml)	Absorbance
1.	2	0.07
2.	4	0.135
3.	6	0.209
4.	7	0.273
5.	10	0.341

Table No. 6: Calibration Curve data of Metformin Hydrochloride in Phosphate buffer pH 7.4 at wavelength of 234 nm.

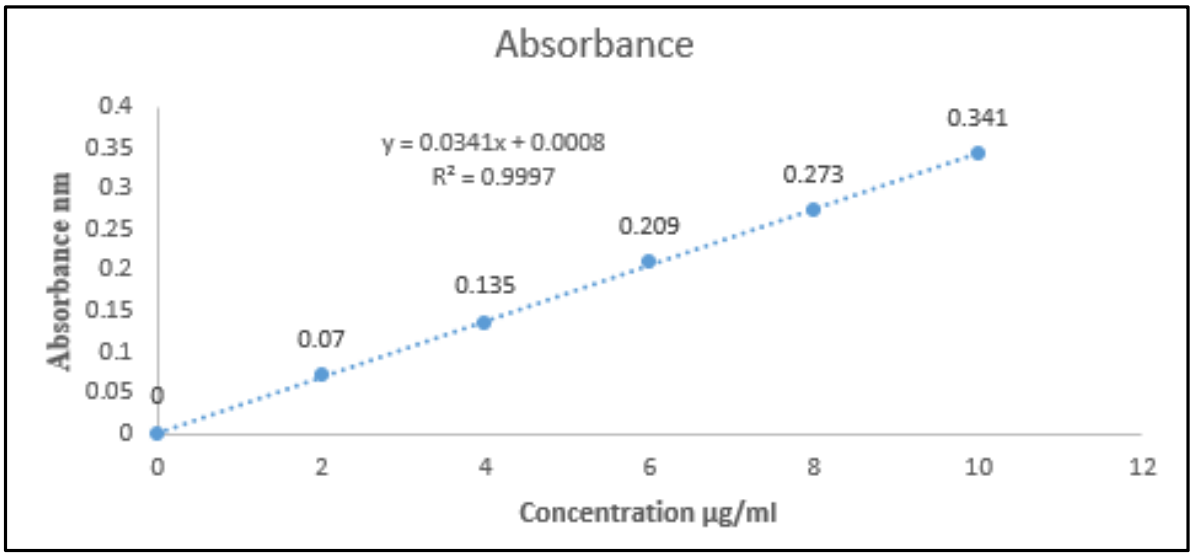

Fig No 3: Calibration Curve of Metformin Hydrochloride in Phosphate Buffer pH 7.4

Results of Calibration Curve

Sr. No	Parameters	Values
1.	Correlation coefficient (R^2)	0.9997
2.	Slope (m)	0.0341
3.	Intercept(c)	0.0008

Table No.7: Result of Calibration Curve

FTIR Spectroscopy of Metformin Hydrochloride-

The FTIR spectrum of Metformin Hydrochloride is as shown in figure No. 3 and the interpretations of FTIR spectra is as shown in table No.5

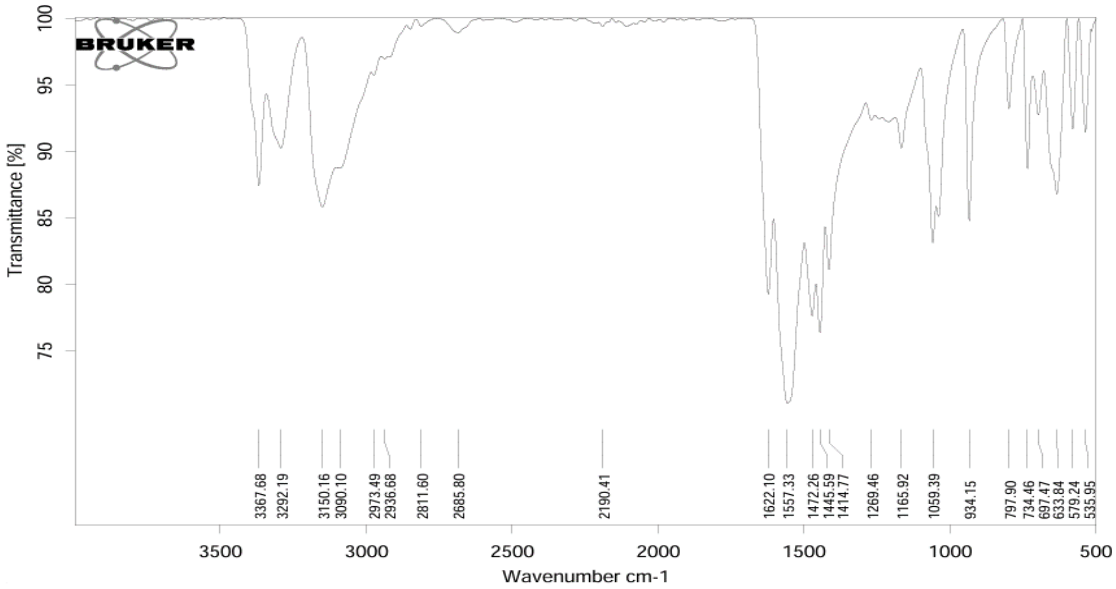

Fig. No.4: FTIR spectra of Metformin hydrochloride

Table No.8: Interpretation of FT-IR Spectra of Metformin Hydrochloride

Sr. no.	Functional group	Standard wavenumber (cm^{-1})	Obtained wavenumber (cm^{-1})
1	N-H	3300-3500(cm^{-1})	3367.80(cm^{-1})
2	C-H	1558.15(cm^{-1})	1558.33(cm^{-1})
3	N-H symmetric stretching	3296.31(cm^{-1})	3295.67(cm^{-1})
4	C-H Asymmetric bending	1419.21(cm^{-1})	1420.08(cm^{-1})

Compatibility Studies:

The FTIR spectra of physical mixture of Metformin Hydrochloride and polymer (Ethyl Cellulose, Polyvinyl alcohol) have retained characteristics peaks of pure drug and hence confirmed no significant interaction between drug and polymer mixture. Figure 5 shows that FTIR spectra of drug and physical mixture.

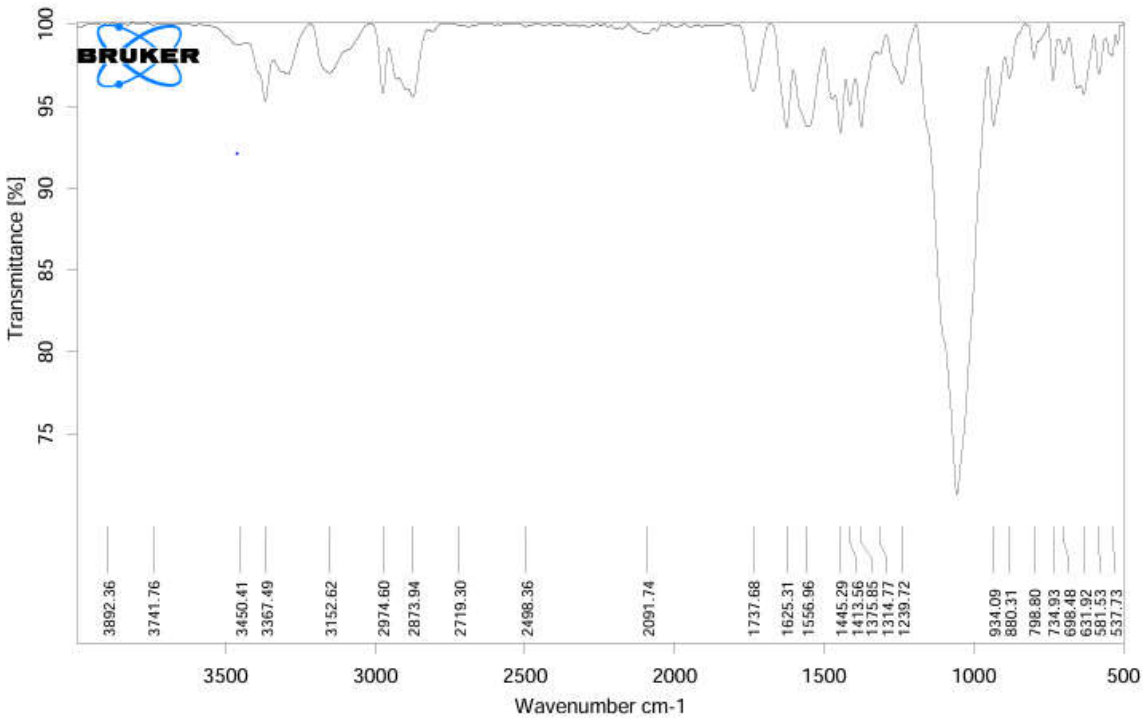

Fig. No.5: FTIR spectra of physical mixture of Metformin Hydrochloride with Ethyl Cellulose & Polyvinyl alcohol

Table No. 9: Interpretation of FT-IR Spectra of physical mixture of Metformin Hydrochloride with Ethyl Cellulose & Polyvinyl alcohol

Sr. No.	Functional Group	Wavenumber cm^{-1} (Standard range)	Wavenumber cm^{-1} (Observed value)
1.	N-H	3300-3500(cm^{-1})	3367.80(cm^{-1})
2.	C-H	1558.15(cm^{-1})	1558.33(cm^{-1})
3.	N-H symmetric stretching	3296.31(cm^{-1})	3295.67(cm^{-1})

4.	C-H Asymmetric bending	1419.21(cm^{-1})	1420.08(cm^{-1})
5.	C-O-C	1052(cm^{-1})	1051.15(cm^{-1})
6.	C-H	2880-2970(cm^{-1})	2879.33(cm^{-1})
7.	O-H	3500(cm^{-1})	3501.77(cm^{-1})
9.	-CH2	2920(cm^{-1})	2920.55(cm^{-1}
10.	CH-O-H	1425(cm^{-1})	1424.20(cm^{-1})

Data Optimization:

Generation of Polynomial Equations-

Statistical model generated interactive polynomial term for response, equation is as follows-

$$\mathbf{Y=\beta_0 + \beta_1 A + \beta_2 B + \beta_3 AB + \beta_4 A^2 + \beta_5 B^2}$$

Where, Y is the independent variable, β_0 is the arithmetic mean response of the 9 runs and β_1 is the estimated co-efficient for the factor A. The main effects of the amount of A and B signifies the average result, when the factors were changed one at a time from their lower to higher values. The interaction terms (AB) show how the response changes when two factors are concurrently changed. The data obtained from DOE strongly signifies that %EE is dependent on the selected independent variables. Conclusions can be drawn from the following polynomial equations depending on the mathematical sign it carries that is positive and negative sign, indicating synergistic and antagonistic effect.q1x

Y (%EE) =93.19+2.95A+0.3983B+0.0700AB-1.19-1.59

Where,

A= Concentration of Ethyl Cellulose

B= Concentration of Polyvinyl alcohol

Analysis of variance (ANOVA) was applied to recognize insignificant factors. Data was evaluated using Design-Expert Software (Version 13). From the data obtained it was evident that p-value was found to be less than 0.05 ($p<0.05$) for all the dependent variables. Model F value for % EE was found to be 20.09 which implies the model is significant. R-squared is a statistical measure of how close the data are to the fitted regression line. It is also known as the coefficient of determination or the coefficient of multiple determination for multiple regression. R-squared is a goodness-of-fit measure for linear regression models. This statistic indicates the percentage of the variance in the dependent variable that the independent variable.

ANOVA for Linear model of Entrapment Efficiency-

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	61.04	5	12.21	20.09	0.0163	significant
A-Ethyl cellulose	52.22	1	52.22	85.94	0.0027	
B-PVP	0.9520	1	0.9520	1.57	0.2994	
AB	0.0196	1	0.0196	0.0323	0.8689	
A^2	2.83	1	2.83	4.66	0.1197	
B^2	5.02	1	5.02	8.27	0.0638	
Residual	1.82	3	0.6076			
Cor Total	62.87	8				

Table No.10 ANOVA for Linear model indicating Model-F value of %EE

Std. Dev.	0.7795		**R^2**	0.9710
Mean	91.34		**Adjusted R^2**	0.9227
C.V. %	0.8533		**Predicted R^2**	0.6543
			Adeq Precision	12.2767

Table No.11. ANOVA for Linear model indicating R-Squared value of %EE

Generation of 3D Response Surface Plot

For the measured responses, three-dimensional plot was generated to determine the change in the response surface plot generated was found to beneficial in the study of the effect of two factors at one time.

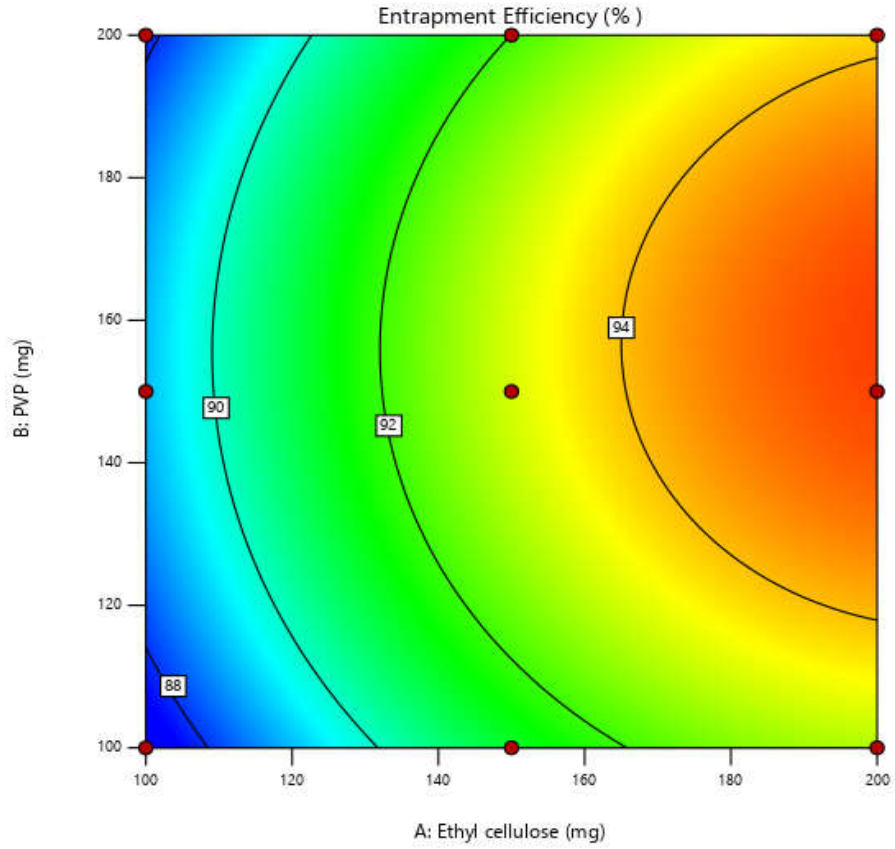

Fig no. 6: Surface plot exhibiting the effect of Ethyl Cellulose and Polyvinyl alcohol Concentration on % Entrapment efficiency

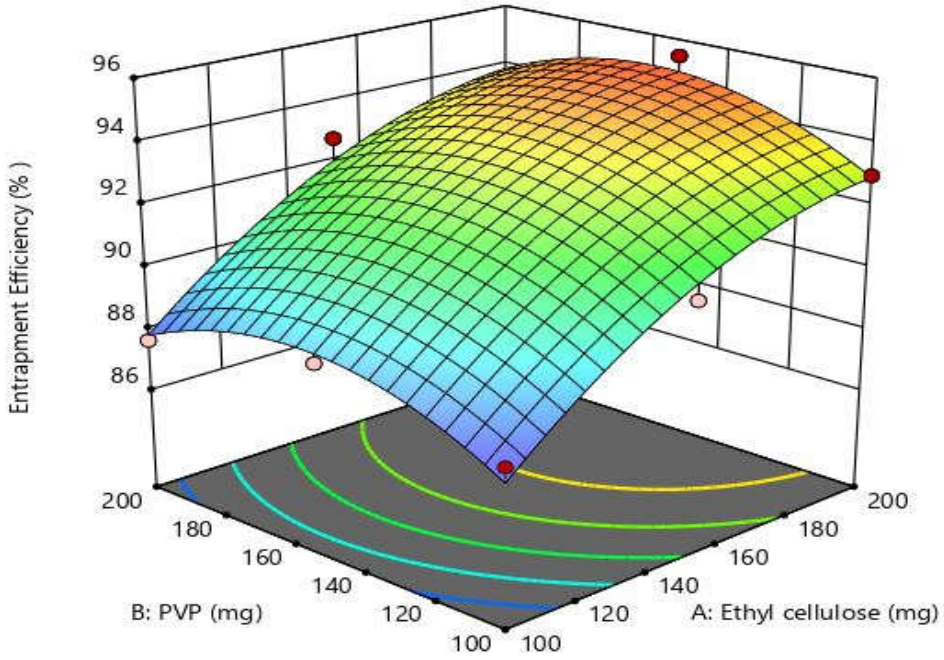

Fig. no.7 3D Response Surface Plots for % Entrapment Efficiency

Effect of Surfactant and Cholesterol Concentration on Entrapment Efficiency-

Ethyl Cellulose and Polyvinyl Alcohol is an important component in the formation of nanosponges and variation in the concentration may affect entrapment efficiency. From the observation, it was concluded that increase in concentration of Ethyl Cellulose increases the entrapment efficiency. The number of Nanosponges formed increases with initial increase in the concentration of Ethyl Cellulose consequently the volume of hydrophobic domain increases and hence increases in entrapment efficiency. An increase in the concentration of Polyvinyl Alcohol leads to provide Coating to Nanosponges & resulting an increase in entrapment efficiency. Cholesterol at a high concentration Provide Coating resulting improve Entrapment Efficiency. Thus, it has been concluded that 200 mg of Ethyl Cellulose and 100 mg of polyvinyl alcohol is the optimum quantity for Nanosponges formulation.

Evaluation of Nanosponges:

Entrapment Efficiency-

The entrapment efficiency of all nanosponges formulations batches F1 to F9 ranged between 87.61% to 92.98% which is shown in table No.7 The % entrapment efficiency of optimized batch F8 were found to be 92.98.

Production yield:

The % production yield of all nine batches of nanosponges was ranged between 91.2 ± 0.64 % to 98.5 ± 0.40 % which shows presence of drug in nanosponges. The % production yield of F8 optimized batch was found to be 37.5 ± 0.45 % and 87.3 ±0.36%. The % production yield is as shown in following table No.7

Batch Code	Drug (mg)	Ethyl Cellulose (mg)	Polyvinyl Alcohol (mg)	Entrapment Efficiency (%)	Production Yield (%)
F1	500	100	100	87.61 %	37.5%
F2	500	100	150	88.75%	42.9%
F3	500	200	200	93.26%	41.67%
F4	500	200	100	92.96%	64.6%
F5	500	100	200	87.63%	66.70%
F6	500	150	200	92.75%	72.94%
F7	500	150	100	90.68%	87.66%
F8	500	200	150	92.47%	69.41%
F9	500	150	200	92.98%	53.75%

Table No.12 - Production Yield& % Entrapment Efficiency of Nanosponges.

Mean Particle Size –

The mean particle size of nanosponges of Metformin Hydrochloride was as shown in table 7.9. The mean particle of F8 batch was found to be 219.1 nm.

Z-Average (nm)	: 219.1
Peak 1 Mean by Intensity ordered by area (nm)	: 253.1
Peak 2 Mean by Intensity ordered by area (nm)	: 46.94
Peak 3 Mean by Intensity ordered by area (nm)	:
Di (10)	: 39.89
Di (50)	: 192.9
Di (90)	: 305.1

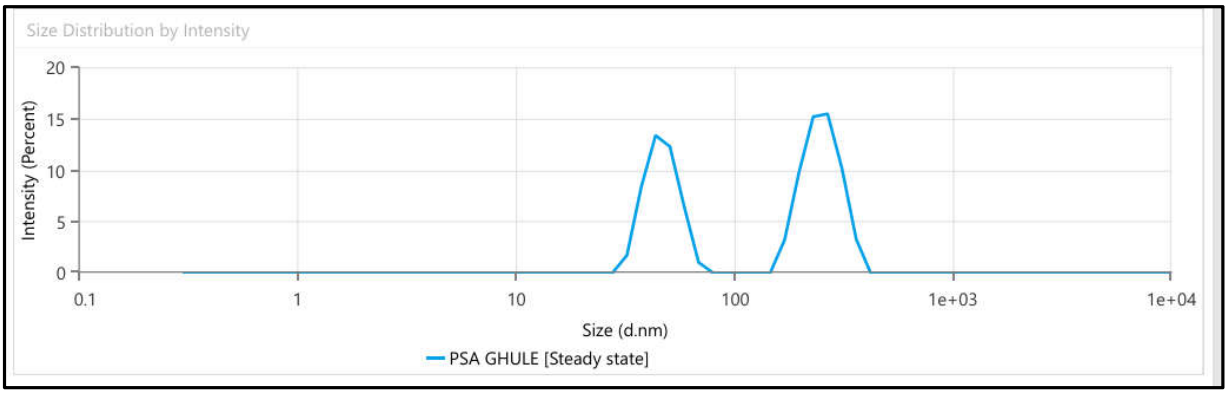

Fig. No.8: Particle size of F8 batch of Nanosponges

Zeta Potential-

The zeta potential of nanosponges formulation was as shown in table 7.9. The zeta potential of F8 batch was found to be - 0.3 mV and -31.4 mV. The zeta potential analysis showed that charge present on nanosponges vesicle surface the formulation was stable.

Statistics Table

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-24.03	-	-	-24.03	-24.03
Zeta Peak 1 Mean (mV)	-24.03	-	-	-24.03	-24.03
Conductivity (mS/cm)	0.1929	-	-	0.1929	0.1929
Wall Zeta Potential (mV)	0	-	-	0	0
Zeta Deviation (mV)	5.952	-	-	5.952	5.952
Derived Mean Count Rate (kcps)	1.022E+05	-	-	1.022E+05	1.022E+05
Reference Beam Count Rate (kcps)	2292	-	-	2292	2292
Quality Factor	2.103	-	-	2.103	2.103

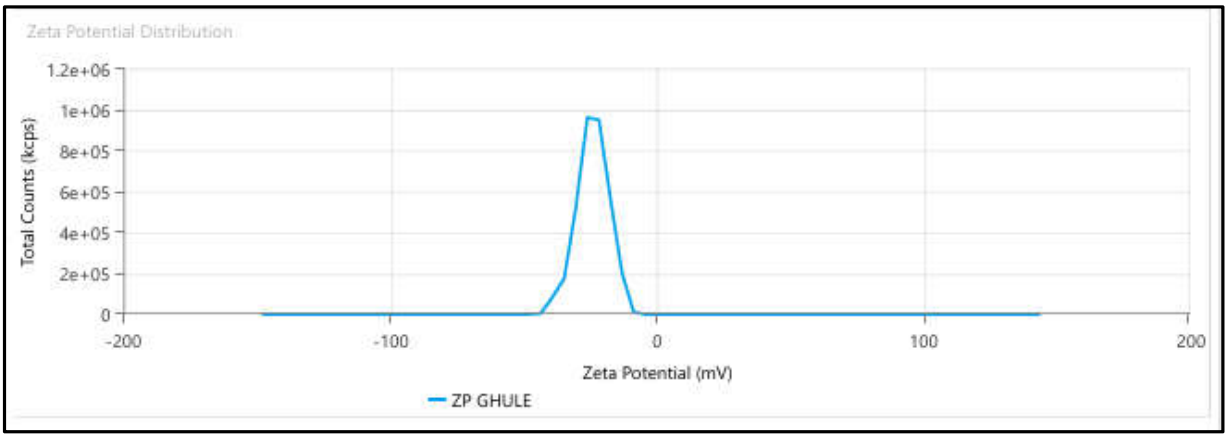

Fig. No. 9 Zeta Potential of F8 Batch of nanosponges

Differential Scanning Calorimetry-

The DSC studies was carried out for pure drug Metformin hydrochloride and nanosponges F8 optimized batch. The thermogram revealed occurrence of sharp endothermic peak at 251.19^{0}C, confirming crystalline nature of the drug. When Metformin hydrochloride is incorporated into nanosponges, a wide endothermic peak at 225.55^0 C was obtained, which might be because of conversion of crystalline Metformin hydrochloride into amorphous form.

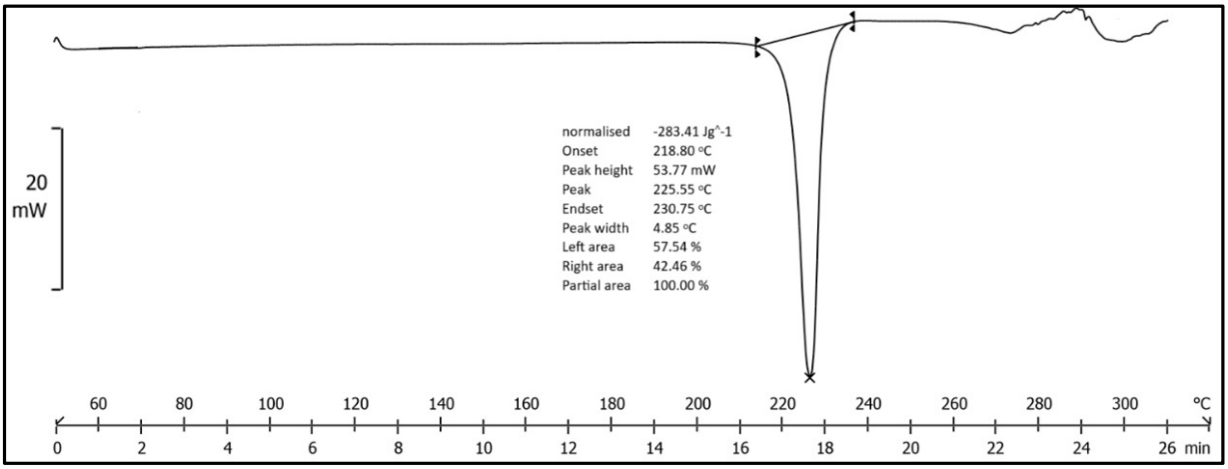

Fig. No 10. DSC thermogram of Metformin hydrochloride F8 batch

Scanning Electron Microscopy:

The SEM images of nanosponges of Metformin Hydrochloride are showing spherical structure with smooth surface and porous nature, as shown in figure No.8

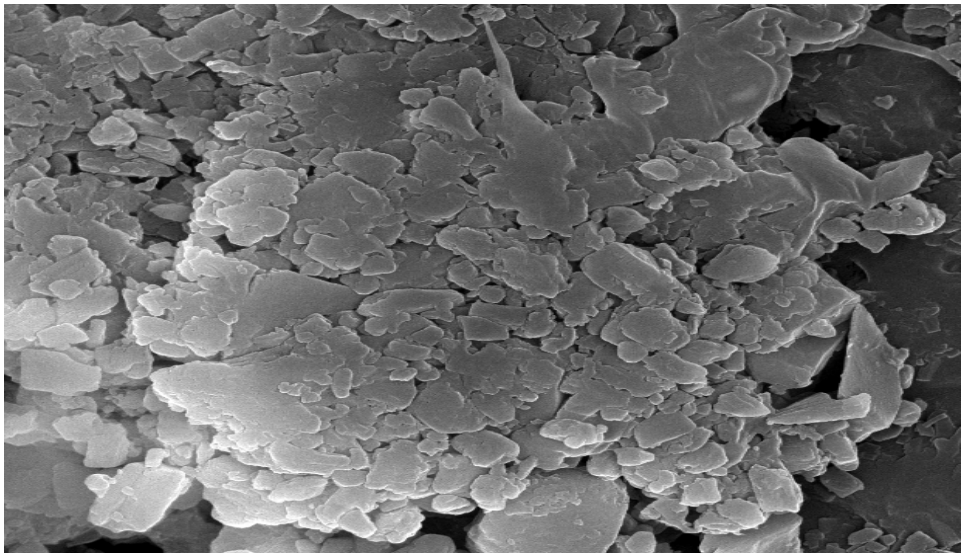

Fig. No. 11 SEM Image of Nanosponges F8 batch

In-Vitro* Drug Release*:

The in-vitro drug release of Nanosponges of Metformin Hydrochloride in Phosphate buffer pH 7.4 for 8 hours was found in the range of 21.26 % to 32.81% shown in table 7.10. The in vitro drug release of F8 batch was found to be 32.81 %

Table No. 13 *In-Vitro* Release of Nanosponges of Metformin Hydrochloride Tablets

Sr. No.	Time (min)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	30	1.45	1.47	1.55	1.6	1.53	1.55	1.68	1.82	1.75
2	60	2.95	3	3.16	3.27	1.69	1.71	1.87	2.04	1.92
3	120	5.46	5.54	5.75	5.85	4.27	4.33	4.58	4.91	4.66
4	180	7.06	7.14	7.48	7.47	5.63	5.94	6.25	10.03	8.75
5	240	10.91	11.05	12.02	11.5	9.03	10.01	10.55	13.33	11.9
6	300	14.98	15.16	16.14	14.7	12.6	13.86	14.83	18.27	16.62
7	360	17.96	18.3	18.83	17.92	16.35	17.37	19	23.18	21.32
8	420	20.97	21.87	21.89	21.16	20.07	20.96	23.11	28.01	25.99
9	480	21.26	22.25	24.98	24.46	23.74	24.63	27.2	32.81	30.63

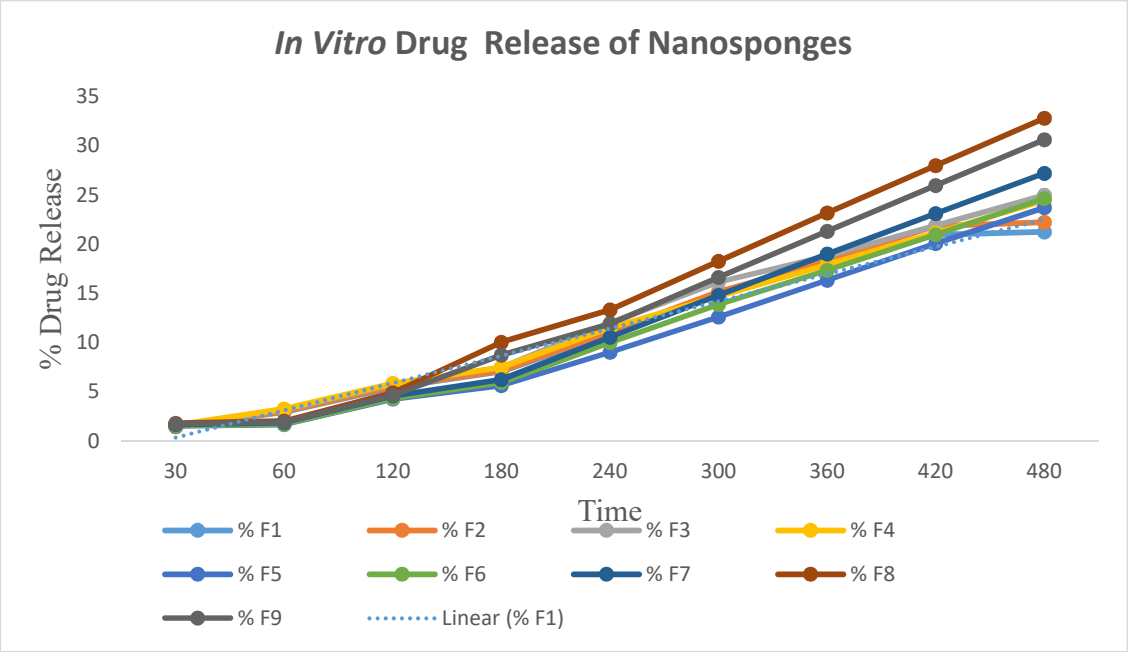

Fig No. 12 Evaluation of Nanosponges Loaded Metformin Hydrochloride Nanosponges Tablets

Flow properties:

Flow properties describe how a powder or granular material behaves under movement or flow conditions. These properties are essential across various industries, including pharmaceuticals, and material science, where powder flowability significantly affects manufacturing efficiency and product quality. Below is an overview of key flow properties.

Parameter	F8 Batch of Metformin Hydrochloride Loaded Nanosponges
Bulk Density (gm/ml)	0.40 ±0.0 153
Tapped Density (gm/ml)	0.41 ±0.0 2
Angle of Repose (gm/ml)	24.19 ±0.6 9
Carr's Index (gm/ml)	7.60 ± 0.81
Hausner's Ratio (gm/ml)	1.03 ± 0.051

Table No.13 Evaluation of flow properties of Optimize F8 Batch of Metformin Hydrochloride Loaded Nanosponges

Hardness (*N*):

The average hardness values for all formulations were measured using a Monsanto hardness tester. The results are detailed in Table no. 9. The hardness values range from 3.26±0.025 to 4.02±0.0643 kg/cm2.

Friability:

All formulations exhibited a friability percentage below 1%, confirming the tablets' mechanical strength.

Thickness (mm):

Thickness of all formulations was measured by using vernier Calipers. The Thickness value ranges from 2.25±0.010 to 2.82±0.0306

Weight variation test:

The weight variations for all formulas are presented in Table No.9. As the percentage of weight variations was within 7.5% of the average weight, all formulated optimized batch of Nanosponges loaded Metformin Hydrochloride tablets (F8) passed the weight variation test. The tablets demonstrated consistent weights and acceptable standard deviation values.

Parameter	Evaluation of Tablets of F8 Batch of Metformin Hydrochloride Laded Nanosponges
Thickness (mm) ±SD	2.25± 0.010
Hardness (Kg/cm2) ±SD	3.5± 0.37
Friability (%) ±SD	0.49± 0.026
Weight variation (mg) ±SD	200± 1.5

Table No.14 Evaluation of Post Compression parameters of Metformin Hydrochloride Loaded Nanosponges Tablets

***In vitro* drug release study of nanosponges loaded tablets, marketed formulation and Metformin Hydrochloride (API):**

The In-vitro drug release of Nanosponges Loaded of Metformin Hydrochloride tablets, Marketed Formulation and Metformin Hydrochloride in Phosphate buffer pH 7.4 for 8 hours was found in the range of 32.81 %, 69.12%, 87.78%. Shown in the following table. The *in vitro* drug release of Nanosponge loaded Metformin hydrochloride tablets was found to be 32.81 %.

Sr. No.	Time (min)	API	Marketed Tablet	Nanosponge Tablet
1	30	1.6%	5.54%	1.82%
2	60	5.17%	7.78%	2.04%
3	120	15.22%	15.57%	4.91%
4	180	25.81%	24.07%	10.03%
5	240	36.79%	32.75%	13.33%
6	300	47.85%	41.64%	18.27%
7	360	59.27%	50.75%	23.18%
8	420	73.39%	59.91%	28.01%
9	480	87.78%	69.12%	32.81%

Table no. 15: *In*-vitro drug release of Nanosponges Loaded of Metformin Hydrochloride tablets, Marketed Formulation & API

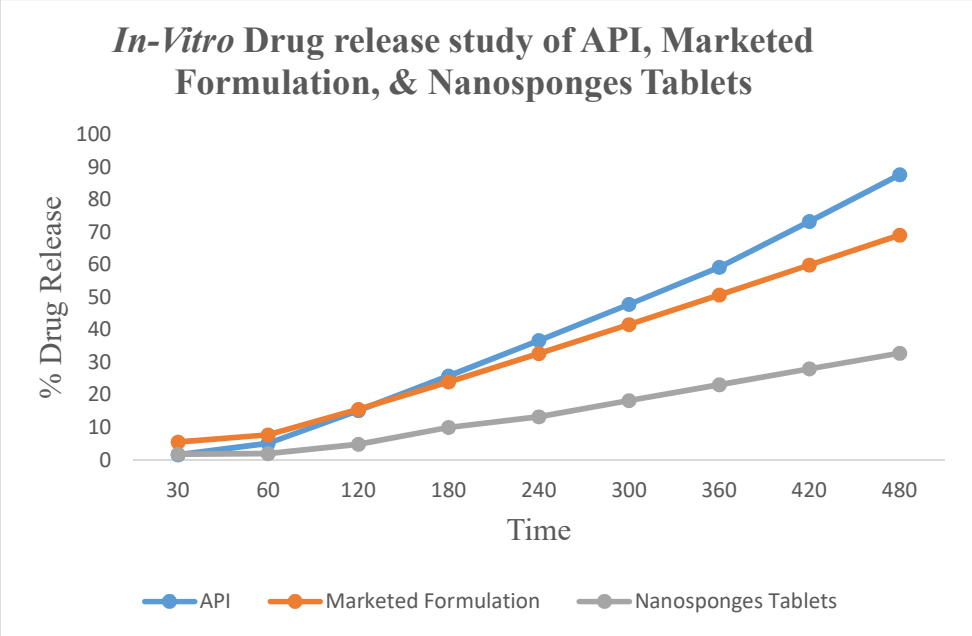

Fig no.13 *In*-vitro drug release of Nanosponges Loaded of Metformin Hydrochloride tablets, Marketed Formulation & API

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