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Formulation and In-vitro Evaluation Of Glipizide Nanosponges

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ABSTRACT

In this study B-Cyclodextrin facilitated Nanosponges were prepared by the solvent evaporation technique and subsequently formulated in a tablet form for immediate release of Glipizide. The Nanosponges formulations were prepared by solvent evaporation method employing B-Cyclodextrin as a polymer. The compatibility of the drug with formulation components was established by Fourier Transform Infra-Red (FTIR) spectroscopy. The surface morphology, particle size, production yield, and drug entrapment efficiency of Nanosponges were examined. Shape and surface morphology of the Nanosponges were examined using scanningelectron microscopy. Particle size of prepared Nanosponges was observed in the range of 428.7 to 633.5nm. Scanning electron microscopy revealed the porous, spherical nature of the Nanosponges.SEM photographs revealed the spherical nature of the Nanosponges in all variations; however, at higher ratios, drug crystals were observed on the nanosponge surface. Increase in the drug/polymer ratio (1:1 to 1:3) increased their yield (10.23 ± to 35.69), which is in increasing order due to the increase in the concentration of polymer but after certain concentration it was observed that as the ratio of drug to polymer was increased, the particle size decreased, the drug content of different formulations was found in the range 94.4to 98.6%,the entrapment efficiency of different formulations were found in the range of 82.11 to 94.40%, the drug release of the Optimized formulation was found to be 97.71%.

Keywords: Glipizide**,** B-Cyclodextrin, Dichloromethane, Nanosponges Delivery System (NDS). Scanning Electron Microscopy(SEM), UV Spectroscopy.

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INTRODUCTION

Glipizide is an oral rapid- and short-acting anti-diabetic medication from the sulfonylurea class. It is classified as a second-generation sulfonylurea, which means that it undergoes enterohepatic circulation. Second-generation sulfonylureas are both more potent and have shorter half-lives than the first-generation sulfonylureas. Glipizide acts by partially blocking potassium channels among beta cells of pancreatic islets of Langerhans. By blocking potassium channels, the cell depolarizes which results in the opening of voltage-gated calcium channels. The resulting calcium influx encourages insulin release from beta cells.

The purpose of this work is to develop a novel sustained release dosage form with to prolong the gastric residence time of Glipizide.

Various approaches for preparation of Nanosponge drug delivery system include Hyper-crosslinking with β-cyclodextrins, Emulsion solvent diffusion method[26,] Quasi-emulsion solvent diffusion method.

Nanosponges are porous polymeric delivery systems that are small spherical particles with large porous surface. Nanosponges can significantly reduce the irritation of drugs without reducing their efficacy.The size of the nanospongs ranges from 250nm-1μm in diameter[12.] Nanosponges are made up of microscopic particles with few nanometers wide cavities, in which a large variety of substances can be encapsulated. These particles are capable of carrying both lipophilic and hydrophilic substances and of improving the solubility of poorly water soluble molecules [14]. Nanosponges are encapsulating type of nanoparticles which encapsulates the drug molecules within its core [15]. As compared to other nanoparticles, nanosponges are insoluble in water and organic solvents, porous, non toxic and stable at high temperatures up to 300^0c .

Glipizide has short half life. So, In the present study, glipizide is formulated as an Nanosponge system helps to retain the drug for longer periods and increase the solubility of drug.

MATERIALS AND METHOD

Materials

Glipizide purchased from DR REDDYS LABS HYDERABAD, β-Cyclodextrin, Dichloromethane from COLORCON GOA, poly vinyl alcohol, Ethyl cellulose, HP β cyclodextrin, Water from SPECTRUM LABS HYDERABAD

Method:

Solvent Evaporation Method:

Nanosponges were prepared using different proportions of ethyl cellulose as rate retarding polymer and co-polymers like polyvinyl alcohol and β-cyclodextrin were prepared by solvent evaporation method. Disperse phase consisting of Glipizide (1gm) and requisite quantity of ethyl cellulose dissolved in 10 ml solvent (dichloromethane or ethanol) was slowly added to a definite amount of PVA in 100ml of aqueous continuous phase, prepared by using microwave oven. The reaction mixture was stirred at 1000 rpm for three hours on a magnetic stirrer. The nanosponges formed were collected by filtration through whatman filter paper and dried in oven at 50^{O}C for 2 hours. The dried nanosponges were stored in vaccum desicator to ensure the removal of residual solvent.

Table 1: Formulation table of Glipizide loaded nanosponges

S.NO	Excipients	GF1	GF2	GF3	GF4	GF5	GF6	GF7	GF8	GF9
1	Glipizide (gm)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2	Ethyl cellulose (gm)	1.0	2.0	3.0	1.0	2.0	3.0	1.0	2.0	3.0
3	Polyvinyl alcohol (gm)	1.0	1.0	1.0	--	--	--	--	--	
4	β-cyclodextrin (gm)	--	--	--	1.0	1.0	1.0	--	--	--
5	HPβ Cyclodextrin	--	--	--	--	--	--	1.0	1.0	1.0
6	Dichloromethane (ml)	10	10	10	10	10	10	10	10	
7	Water	40	40	40	40	40	40	40	40	

EVALUATION PARAMETERS:

The Nanosponges was evaluated for various parameters like Drug content uniformity, Entrapment efficiency ,Scanning electron microscopy, Particle size and shape, In-vitro drug release studies, Drug release kinetics studies.

Drug content uniformity

10 ml of each formulation was taken and dissolved in 10 ml isotonic solution and kept overnight. 10 mg (similar as in formulation) of drug was taken and dilution was made to 10 µg/ml. The dilutions were filtered and analyzed using UV for their content uniformity. The absorbance of the formulations were read using one cm cell in a UV-Vis spectrophotometer. The instrument was set at drugs wavelength (nm). The drug content in each formulation was calculated based on the absorbance values of known standard solutions.

Entrapment efficiency

The 100mg of the nanosponge suspension was analyzed by dissolving the sample in 10ml of distilled water. After the drug was dissolved 10mL of clear layer of dissolved drug is taken. Thereafter the amount of drug in the water phase was detected by a UV-spectrophotometric method at 231nm (U.V Spectrophotometer, systronics). The test was repeated with another

nanoparticulate sample. The amount of the drug in the suspension was analyzed by centrifugation at 500rpm for 5 mins and by measuring the concentration of the drug in the clear supernatant layer by the UV-spectrophotometric method. The test was again repeated with another sample. The concentration of the drug is determined with the help of calibration curve. The amount of drug inside the particles was calculated by subtracting the amount of drug in the aqueous phase of the suspension from the total amount of the drug in the nanoparticle suspension. The entrapment efficiency (%) of drug was calculated by the following equation.

$$\% \text{ of Drug entrapment} = \frac{\text{Mass of drug in nanosponge}}{\text{Mass of drug used in formulation}} \times 100$$

Scanning electron microscopy

The morphological features of prepared nanospongess are observed by scanning electron microscopy at different magnifications.

Fig: photography representation of instruments used for finding SEM analysis.

Particle size and shape

Average particle size and shape of the formulated nanospongess was determined by using Malvern Zetasizer ZS using water as dispersions medium. The sample was scanned 100 times for determination of particle size.

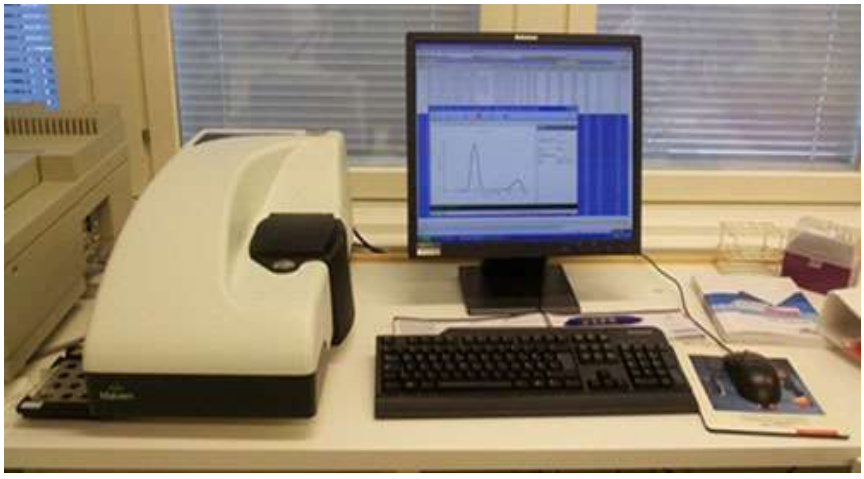

Figure: photography representation of Malvern zetasizer used for finding particle size & zeta analysis

Dissolution study:

Dissolution is pharmaceutically defined as the rate of mass transfer from a solid surface into the dissolution medium or solvent under standardized conditions of liquid/solid interface, temperature

and solvent composition. It is a dynamic property that changes with time and explains the process by which a homogenous mixture of a solid or a liquid can be obtained in a solvent. The test determines the time required for formulation to release percentage of drug under specified conditions.

Dissolution Parameters

Medium : 900ml, 0.1N HCL

Apparatus : Paddle (USP-II)

RPM : 100

Temperature : 37^{o} C±0.5

Time Points : 1,2,4,6,8,10,12, hr

Procedure:

For the oral dosage forms the in vitro dissolution study must be conducted in the dissolution medium which simulate the in-vivo conditions (actual physiological conditions). The in vitro drug release studies for the prepared formulation were conducted for a period of 12 hrs using an Electro lab model dissolution tester USP Type-2 apparatus (rotating paddle) set at 100 rpm and a temperature of 37± 0.5°C formulation was placed in the 900ml of the medium. At specified intervals 10ml samples were withdrawn from the dissolution medium and replaced with fresh medium to keep the volume constant. The absorbance of the sample solution was analyzed at 231 nm for the presence of model drug, using a UV-visible spectrophotometer.

Modeling of Dissolution Profile

In the present study, data of the in vitro release were fitted to different equations and kinetic models to explain the release kinetics of glipizide from the matrix tablets. The kinetic models used were Zero order equation, First order, Higuchi release and Korsmeyer-Peppas models.

Kinetic Studies: Mathematical models:

Different release kinetic equations (zero-order, first-order, Higuchi's equation and Korsmeyer-peppas equation) were applied to interpret the release rate of the drug from matrix systems for the optimized formulation. The best fit with higher correlation (r2) was calculated.

Zero-order model:

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation

$Qt = Q0 + K0t$

Where Qt is the amount of drug dissolved in time t, Q0 is the initial amount of drug in the solution (most times, Q0 = 0) and K0 is the zero order release constant expressed in units of

concentration/time. To study the release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug released versus time.

Application: It is used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as tablets with low soluble drugs in coated forms, osmotic systems, etc.

First Order Model:

The first order equation describes the release from systems where the dissolution rate is dependent upon the concentration of the dissolving species.

Release behavior generally follows the following first order equation:

Log C= Log C_o-kt/2.303

Where C is the amount of drug dissolved at time t,

C_o is the amount of drug dissolved at t=0 and

k is the first order rate constant.

A graph of log cumulative of % drug remaining vs time yields a straight line.

The pharmaceutical dosage forms following this dissolution profile, such as those containing water-soluble drugs in porous matrices, release the drugs in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminishes.

Higuchi model: The first example of a mathematical model aimed to describe drug release from a system was proposed by Higuchi in 1961. Initially conceived for planar systems, it was then sustained to different geometrics and porous systems. This model is based on the hypothesis that

- initial drug concentration in the is much higher than drug solubility;
- drug diffusion takes place only in one dimension (edge effect must be negligible);
- drug particles are much smaller than system thickness;
- swelling and dissolution are negligible;
- drug diffusivity is constant; and

Perfect sink conditions are always attained in the release environment.

In a general way the Higuchi model is simply expressed by following equation

$Q = K_H - t^{1/2}$

Where, K_H is the Higuchi dissolution constant.

The data obtained were plotted as cumulative percentage drug release versus square root of time.

Application: This relationship can be used to describe the drug dissolution from several types of

modified release pharmaceutical dosage forms, as in the case of some transdermal systems and tablets with water soluble drugs.

Korsmeyer-Peppas model:

Korsmeyer et al.(1983) derived a simple relationshipwhich described drug release from a polymeric system equation. To find out the mechanism of drug release, first60% drug release data were fitted in Korsmeyer-Peppas model,

$Mt / M\infty = Kt^n$

where Mt / M∞ is a fraction of drug released at time t, k is the release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices.

In this model, the value of n characterizes the release mechanism of drug as described in the following table.

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In this model, the value of n characterizes the release mechanism of drug as described in the following table.

Drug transport mechanisms suggested based on 'n' value.

S. No	Release exponent	Drug transport mechanism	Rate as a function of time
1	0.5	Fickian diffusion	$t^{-0.5}$
2	0.45 < n = 0.89	Non -Fickian transport	t^{n-1}
3	0.89	Case II transport	Zero order release
4	Higher than 0.89	Super case II transport	t^{n-1}

To find out the exponent of n the portion of the release curve, where Mt / M∞ < 0.6 should only be used. To study the release kinetics, data obtained from in vitro drug release studies were plotted as log cumulative percentage drug release versus log time.

RESULTS AND DISCUSSION

A) Particle size analysis of Nanosponges:

The particle size of the nanosponge was determined by optical microscopy and the nanosponges were found to be uniform in size. The average particle size of all formulations ranges from 428.7 nm to 645.9 nm which is in increasing order due to the increase in the concentration of polymer but after certain concentration it was observed that as the ratio of drug to polymer was increased, the particle size decreased. This could probably be due to the fact that in high drug to polymer ratio, the amount of polymer available per nanosponge was comparatively less. Probably in high drug-polymer ratios less polymer amounts surround the drug and reducing the thickness of polymer wall and nanosponges with smaller size were obtained. By performing the particle size analysis, it is concluded that the formulation has the particle size varies with the concentration of polymer drug ratio.

B) Morphology determination by scanning electron microscopy (SEM):

It was observed that the nanosponges were spherical, and uniform with no drug crystals on the surface. The shape of the nanosponges affects the surface area and surface area per unit weight of spherical nanosponges. The irregular shape of the particles may affect dissolution rate present in dissolution environment.

C) Drug content:

The drug content of the formulated Nanosponges (GF1-GF9) was found in the range of 94.4to 98.6% respectively. The percentage of drug content of formulation GF1 was found to be 96.5%,formulation GF2 was found to be 97.7%, formulation GF3 was found to be 96.1%, formulation GF4 was found to be 98.6%, formulation GF5 was found to be 95.5%, and formulation GF6 was found to be 95.8%, formulation GF7 was found to be 95.1%, formulation GF8 was found to be 94.4% and GF9 Formulation was found to be 97.2%.

Entrapment efficiency:

The entrapment efficiency of formulation GF1 was found to be 91.15%,formulation GF2 was found to be 92.03%, formulation GF3 was found to be 82.11%, formulation GF4 was found to be 94.40%, formulation GF5 was found to be 87.50%, and formulation GF6 was found to be 86.60%, formulation GF7 was found to be 86.11%, formulation GF8 was found to be 92.01%, and GF8 was found to be 78.10%. Among all the formulations GF4 shows high entrapment efficiency of 94.40.

In vitro dissolution studies of prepared nanosponges:

From the above invitro studies it was observed that the formulations containing PVA drug releases within 12 hours but it not in a sustained manner, formulations containing β-cyclodextrin with Ethyl cellulose shows drug release with in 10 hrs while that of GF4 shows drug release at 12hours in a sustained manner, formulations containing HP β-cyclodextrin drug releases within 12 hours but it not in a sustained manner. By comparing the above dissolution studies it was clearly observed that the drug was released upto 99.71% by the end of 12 hours by GF4 formulation, so it was taken as the optimized formulation.

Kinetics Analysis :

The optimized formulation **GF4** has coefficient of determination (R^2) values of 0.976, 0.953, 0.976 and 0.695 for Zero order, First order, Higuchi and Korsmeyer Peppas respectively. A good linearity was observed with the Zero order, the slope of the regression line from the Higuchi plot indicates the rate of drug release through the mode of diffusion and to further confirm the diffusion mechanism, data was fitted into the Korsmeyer Peppas equation which showed linearity with n value of 0.135 for optimized formulation. Thus n value indicates the fickian diffusion mechanism. Thus, the release kinetics of the optimized formulation was best fitted into Higuchi model and showed zero order drug release with fickian diffusion mechanism.

Table 2: Particle size of Nanosponges

S.NO	Formulation code	Particle size (nm)
1	GF1	428.7
2	GF2	446.5
3	GF3	498.9
4	GF4	532.9
5	GF5	547.9
6	GF6	559.6
7	GF7	570.2
8	GF8	633.5
9	GF9	536.4

Table 3: Table: Drug content of Formulated Nanosponges.

Formulation code	Mean % drug content
GF1	96.5
GF2	97.7
GF3	96.1
GF4	98.6
GF5	95.5
GF6	95.8
GF7	95.1
GF8	94.4
GF9	97.28

Table 4: Entrapment efficiency of Batches F1 – F9

Formulation code	Entrapment efficiency %
GF1	91.15
GF2	92.03
GF3	82.11
GF4	94.40
GF5	87.50
GF6	86.60
GF7	86.11
GF8	92.01
GF9	78.10

Table 6: Percentage of drug release of Nanosponges

TIME(HRS)	GF1	GF2	GF3	GF4	GF5	GF6	GF7	GF8	GF9
0	0	0	0	0	0	0	0	0	0
1	17.71	14.56	26.11	12.54	20.89	25.56	22.30	16.60	28.80
2	29.91	25.05	37.71	23.16	31.16	35.50	33.11	26.36	39.97
3	40.12	37.71	46.63	34.41	38.87	44.49	42.40	38.31	48.89
4	49.40	48.80	55.59	43.39	50.05	52.02	51.15	48.90	57.60
5	61.60	56.18	62.03	55.58	59.57	63.30	60.08	60.11	65.57
6	67.08	68.11	76.11	67.70	68.06	74.39	71.70	69.96	74.41
7	74.40	72.08	83.36	71.80	76.89	85.50	82.29	75.50	85.58

8	82.20	80.06	89.90	79.18	84.48	92.26	90.09	81.89	91.20
9	87.75	84.41	97.50	83.32	88.87	97.70	95.87	86.60	98.36
10	92.28	88.89		87.75	94.40		98.80	91.10	
11	98.89	95.56		94.40	97.11			97.64	
12		100.11		99.71					

CONCLUSION

The Nanosponge was prepared by solvent evaporation method and was evaluated for its different parameters which revealed many interesting results for efficient preparation of the nanosponge. The formulation GF4 has better results than other eight formulations. GF4 have its particle size 532.9nm, entrapment efficiency 94.40,Drug content 98.6**%** drug release 99.71 % in 12 hour, all these parameters are in optimized range for preparing a sustained release dosage form so showing itself as an optimized formulation in this project work.

FTIR spectroscopy analyses indicated the chemically stable, amorphous nature of the drug in these nanosponge. SEM photographs revealed the spherical nature of the nanosponge in all variations. With the revealed results by different evaluation parameters, it is concluded that nanosponge drug delivery system has become highly competitive and rapidly evolving technology and more and more research are carrying out to optimize cost-effectiveness and efficacy of the therapy. The optimized formulation GF4 has coefficient of determination (R^2) values of 0.976, 0.953, 0.976 and 0.695 for Zero order, First order, Higuchi and Korsmeyer Peppas respectively. A good linearity was observed with the Zero order, the slope of the regression line from the Higuchi plot indicates the rate of drug release through the mode of diffusion and to further confirm the diffusion mechanism, data was fitted into the Korsmeyer Peppas equation which showed linearity with n value of 0.135 for optimized formulation. Thus n value indicates the fickian diffusion mechanism. Thus, the release kinetics of the optimized formulation was best fitted into Higuchi model and showed zero order drug release with fickian diffusion mechanism.

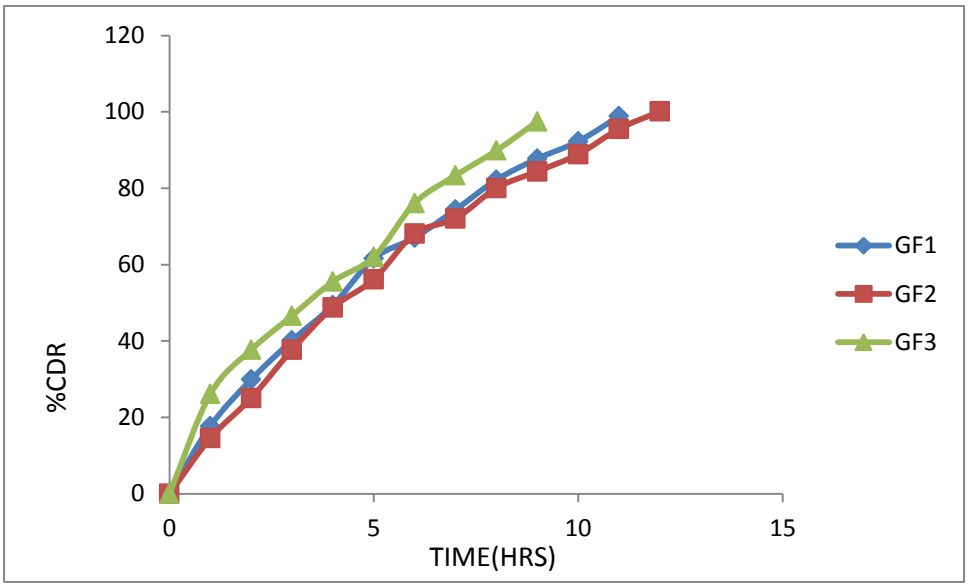

Figure 1: In Vitro Dissolution Profile For Batches F1 – F3

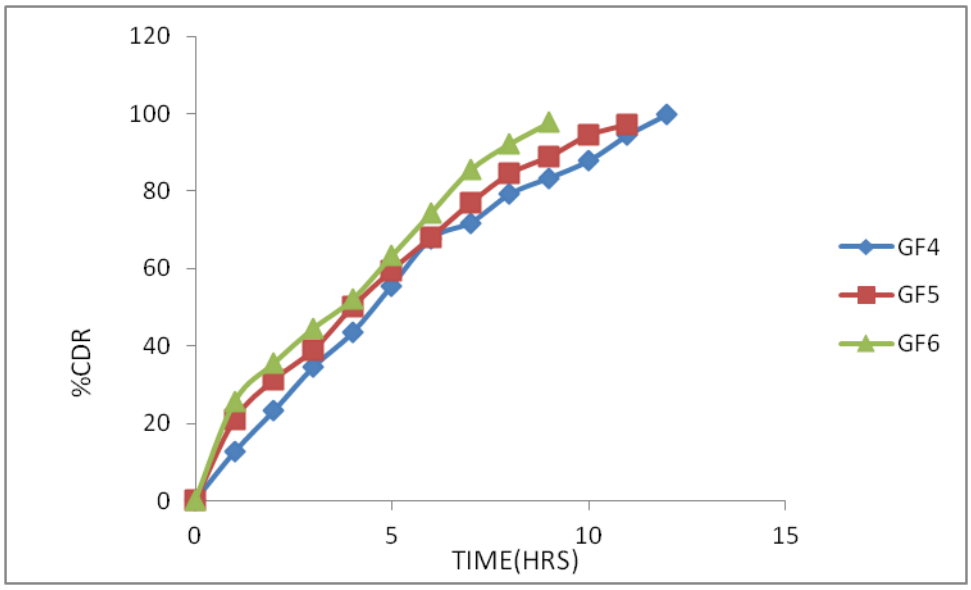

Figure 2: *In Vitro* Dissolution Profile For Batches F4-F6

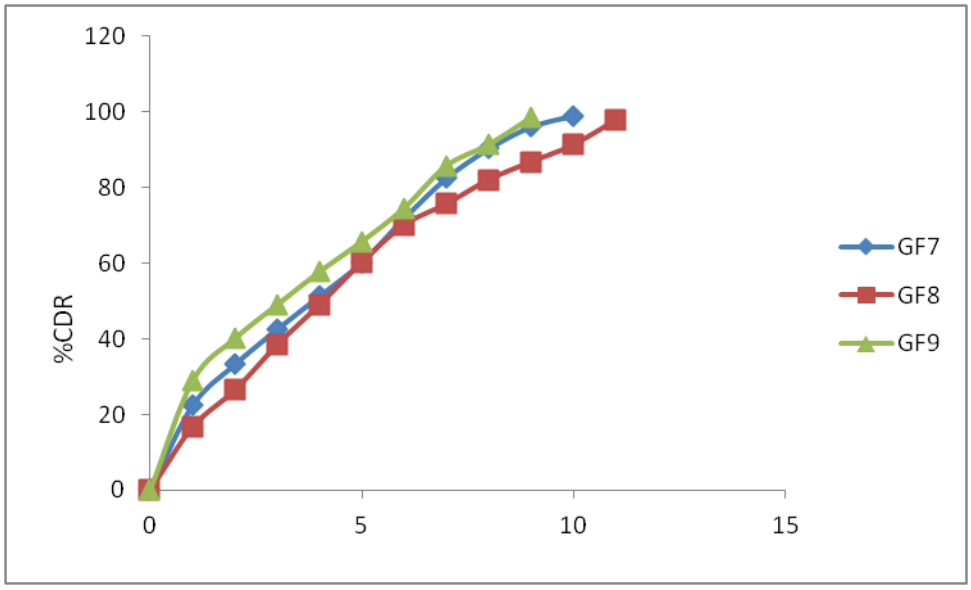

Figure 3: *In Vitro* Dissolution Profile For Batches F7-F9

DRUG RELEASE KINETICS OF Glipizide:

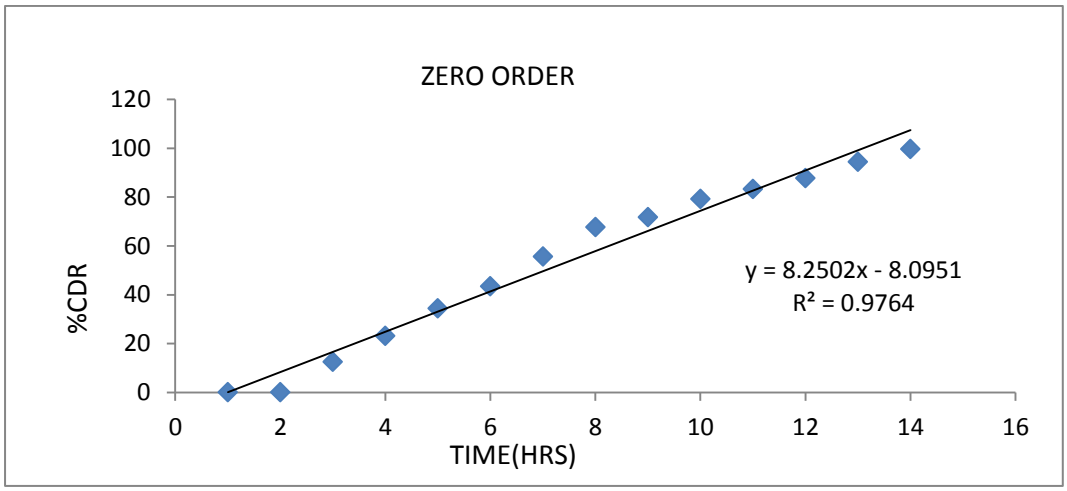

Figure 4: Zero order release profile of Glipizide Nanosponges of FT4

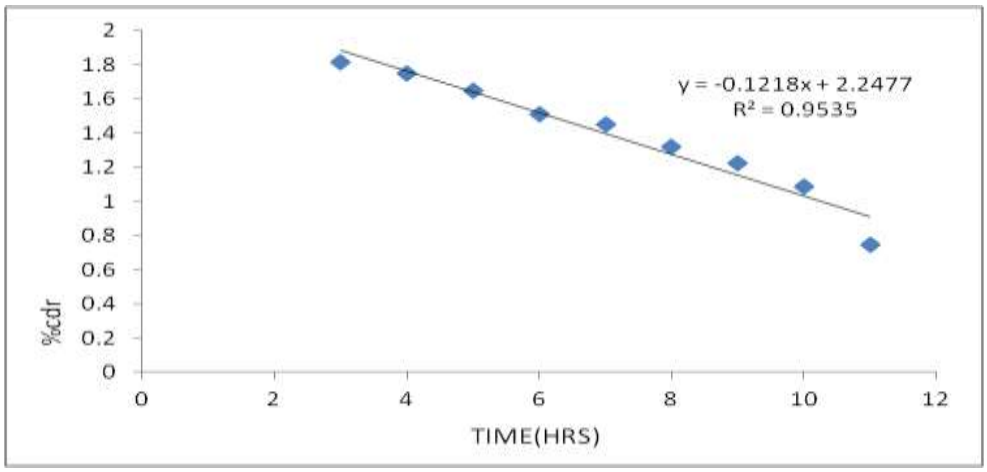

Figure: 5 first order release profile of Glipizide Nanosponges of FT4

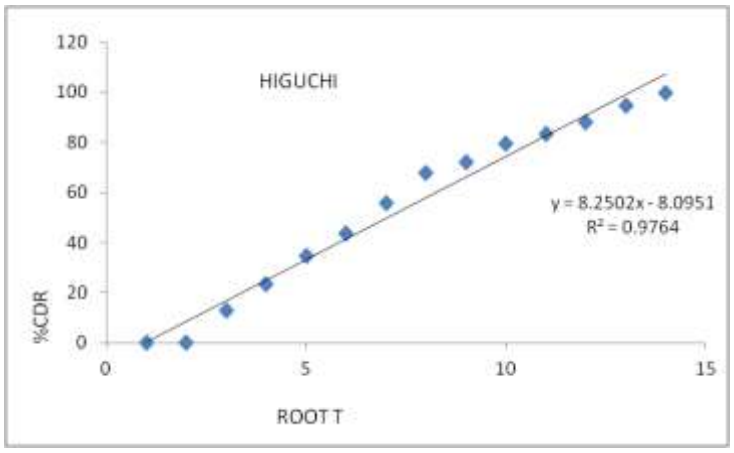

Figure : 6 Higuchi release kinetics profile of Glipizide Nanosponges of FT4.

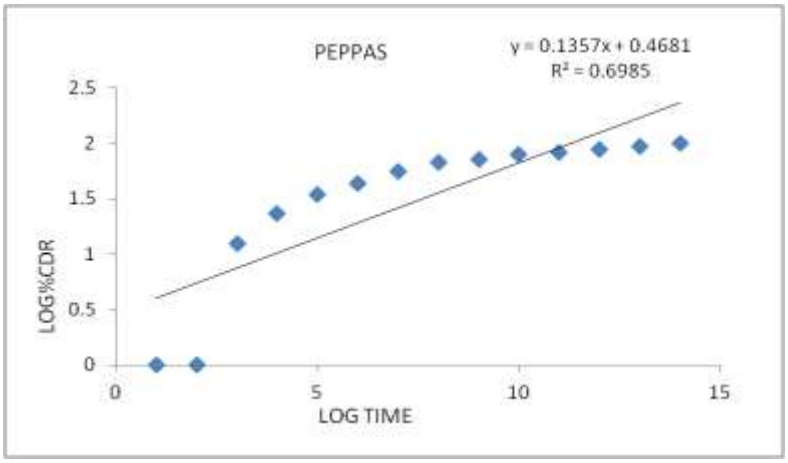

Figure: 7 Peppas release kinetics profile of Glipizide Nanosponges of FT4.

Table 7: Regression coefficients fit to different drug release kinetics models for Glipizide Nanosponges.

S.NO	Zero order	First order	Higuchi	Peppas
Code	R^2	R^2	R^2	R^2
GF4	0.976	0.953	0.976	0.698

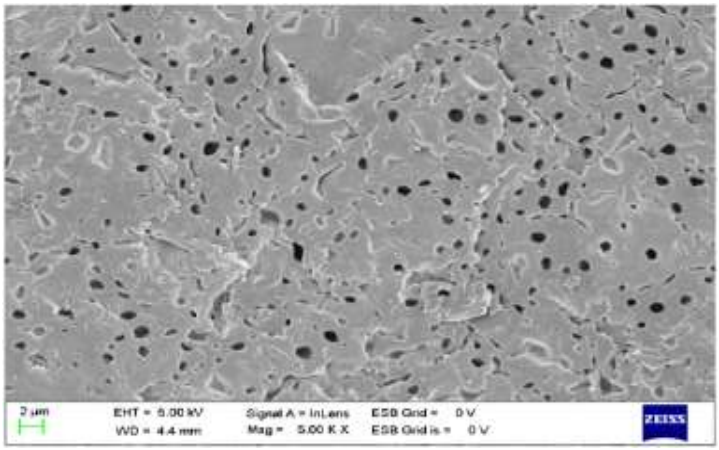

Figure 8: Nanosponges structure of optimized formulation (GF4)

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