

## Original Research Article

**Formulation and Invitro evaluation of  $\beta$ -cyclodextrin based Nanosponges**

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**Abstract:** In the present study Nanosponges were prepared by solvent evaporation method employing B-Cyclodextrin and HP  $\beta$ -cyclodextrin as a polymer. Gliplizide is taken as model drug for studying various formulations of cyclodextrin based nanosponges. The compatibility of various 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 scanning electron microscopy. Particle size of prepared Nanosponges was observed in the range of 532.9 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 ( $10.23 \pm$  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 94.4 to 98.6%, the entrapment efficiency of different formulations were found in the range of 78.10 to 94.40%, the maximum drug release of the formulation with HP  $\beta$  cyclodextrin was found to be 99.71%.

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

**INTRODUCTION:**

Cyclodextrins are produced from starch by means of enzymatic conversion. They are used in food, pharmaceutical [1] drug delivery [2] and chemical industries, as well as agriculture and environmental engineering.

Cyclodextrins are composed of 5 or more  $\alpha$ -D-glucopyranoside units linked 1- $\rightarrow$ 4, as in amylose (a fragment of starch). The 5-membered macro cycle is not natural. Recently, the largest well-characterized cyclodextrin contains 32 1, 4-anhydroglucopyranoside units, while as a poorly characterized mixture, at least 150-membered cyclic oligosaccharides are also known. Typical cyclodextrins contain a number of glucose monomers ranging from six to eight units in a ring, creating a cone shape:

- **$\alpha$  (alpha)-cyclodextrin:** 6-membered sugar ring molecule
- **$\beta$  (beta)-cyclodextrin:** 7-membered sugar ring molecule
- **$\gamma$  (gamma)-cyclodextrin:** 8-membered sugar ring molecule

CDs are able to form dynamic molecular inclusion complexes with many drugs by incorporating the drug molecule. 2-hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD) is an alternative to  $\alpha$ -,  $\beta$ - and  $\gamma$ -cyclodextrin, with improved water solubility. Gliplizide 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 entero hepatic circulation. Gliplizide 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.

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 nanosponges ranges from 250nm-1 $\mu$ m 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, nontoxic and stable at high temperatures up to 300<sup>o</sup>c. In present study various formulations are prepared by using different concentration of cyclodextrins to determine the drug release capacity of polymer.

## MATERIALS AND METHODS

### Materials

Glipizide purchased from DR REDDYS LABS HYDERABAD,  $\beta$ -Cyclodextrin, Dichloromethane from COLORCON GOA, Ethyl cellulose, HP  $\beta$  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  $\beta$ -cyclodextrin and HP  $\beta$ -cyclodextrin were prepared by solvent evaporation method. Disperse phase consisting of Drug (1gm) and required 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<sup>o</sup>C for 2 hours. The dried nanosponges were stored in vacuum desiccator to ensure the removal of residual solvent.

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

#### Scanning electron microscopy

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

#### Particle size and shape

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

#### Dissolution study:

Dissolution is pharmaceutically defined as the

## 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  $\mu$ g/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.

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° 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.

**Modelling 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 (r<sup>2</sup>) 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

$$Q_t = Q_0 + K_0t$$

Where  $Q_t$  is the amount of drug dissolved in time  $t$ ,  $Q_0$  is the initial amount of drug in the solution (most times,  $Q_0 = 0$ ) and  $K_0$  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:

$$\text{Log } C = \text{Log } C_0 - kt/2.303$$

Where  $C$  is the amount of drug dissolved at time  $t$ ,  $C_0$  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

$$Q = K_H \cdot t^{1/2}$$

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 relationship which described drug release from a polymeric system equation. To find out the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer-Peppas model,

$$M_t / M_\infty = Kt^n$$

Where  $M_t / M_\infty$  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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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  $M_t / M_\infty < 0.6$  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-GF6) was found in the range of 94.4 to 98.6% respectively. The percentage of drug content of formulation GF1 was found to be 98.6%, formulation GF2 was found to be 95.5%, and formulation GF3 was found to be 95.8%, formulation

GF4 was found to be 95.1%, formulation GF5 was found to be 94.4% and GF6 formulation was found to be 97.2%.

### Entrapment efficiency:

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

### In vitro dissolution studies of prepared nanosponges:

From the above invitro studies it was observed that formulations containing  $\beta$ -cyclodextrin with Ethyl cellulose shows drug release within 10 hrs while that of GF1 shows drug release at 12 hours in a sustained manner, formulations containing HP  $\beta$ -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 GF1 formulation, so it was taken as the optimized formulation.

### Kinetics Analysis:

The optimized formulation GF1 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 1.1: Formulation table of Glipizide loaded nanosponges**

S.NO	Excipients	GF1	GF2	GF3	GF4	GF5	GF6
1	Glipizide (gm)	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
3	Polyvinyl alcohol (gm)	--	--	--	--	--	
4	$\beta$ -cyclodextrin (gm)	1.0	1.0	1.0	--	--	--
5	HP $\beta$ Cyclodextrin	--	--	--	1.0	1.0	1.0
6	Dichloromethane (ml)	10	10	10	10	10	10
7	Water	40	40	40	40	40	40

**Table 1.2: Particle size of formed nanosponges**

S.NO	Formulation code	Particle size (nm)
1	GF1	532.9
2	GF2	547.9
3	GF3	559.6
4	GF4	570.2
5	GF5	633.5
6	GF6	536.4

**Table 1.3: Table: Drug content of Formulated Nanosponges.**

Formulation code	Mean % drug content
GF1	98.6
GF2	95.5
GF3	95.8
GF4	95.1
GF5	94.4
GF6	97.28

**Table 1.4: Entrapment efficiency of Formulated Nanosponges**

Formulation code	Entrapment efficiency %
GF1	94.40
GF2	87.50
GF3	86.60
GF4	86.11
GF5	92.01
GF6	78.10

**Table 1.6: Percentage of drug release of Nanosponges**

TIME(HRS)	GF1	GF2	GF3	GF4	GF5	GF6
0	0	0	0	0	0	0
1	12.54	20.89	25.56	22.30	16.60	28.80
2	23.16	31.16	35.50	33.11	26.36	39.97
3	34.41	38.87	44.49	42.40	38.31	48.89
4	43.39	50.05	52.02	51.15	48.90	57.60
5	55.58	59.57	63.30	60.08	60.11	65.57
6	67.70	68.06	74.39	71.70	69.96	74.41
7	71.80	76.89	85.50	82.29	75.50	85.58
8	79.18	84.48	92.26	90.09	81.89	91.20
9	83.32	88.87	97.70	95.87	86.60	98.36
10	87.75	94.40		98.80	91.10	
11	94.40	97.11			97.64	
12	99.71					

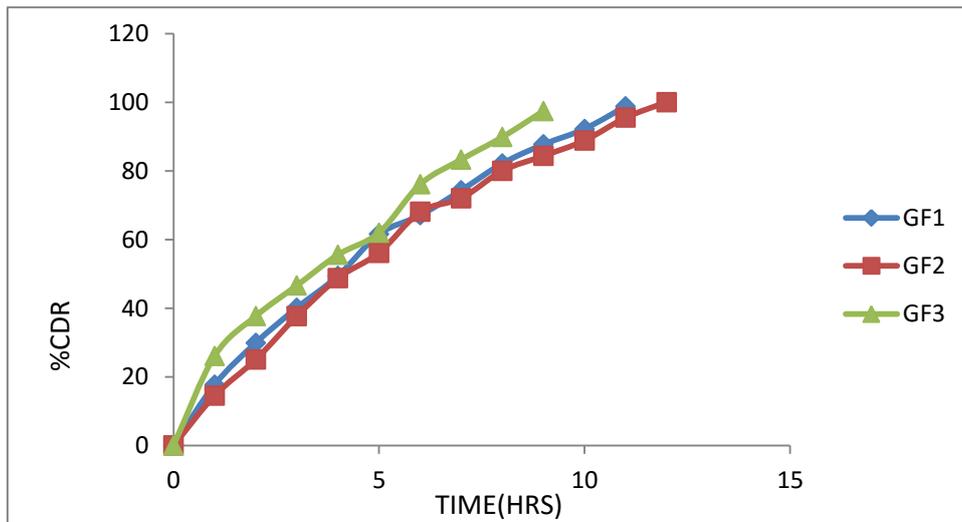


Fig 1: *In Vitro* Dissolution Profile For Batches F1 – F3

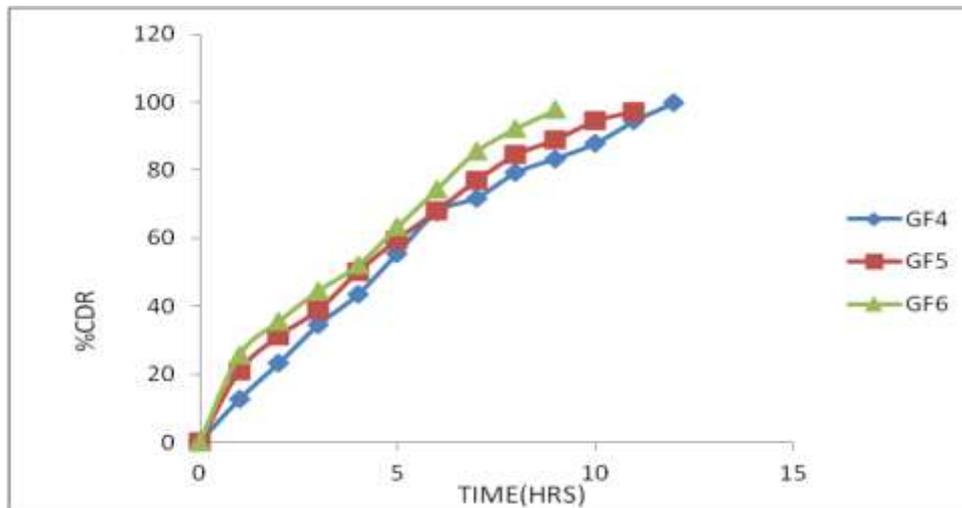


Fig 2: *In Vitro* Dissolution Profile For Batches F4-F6

**DRUG RELEASE KINETICS OF Gliplizide:**

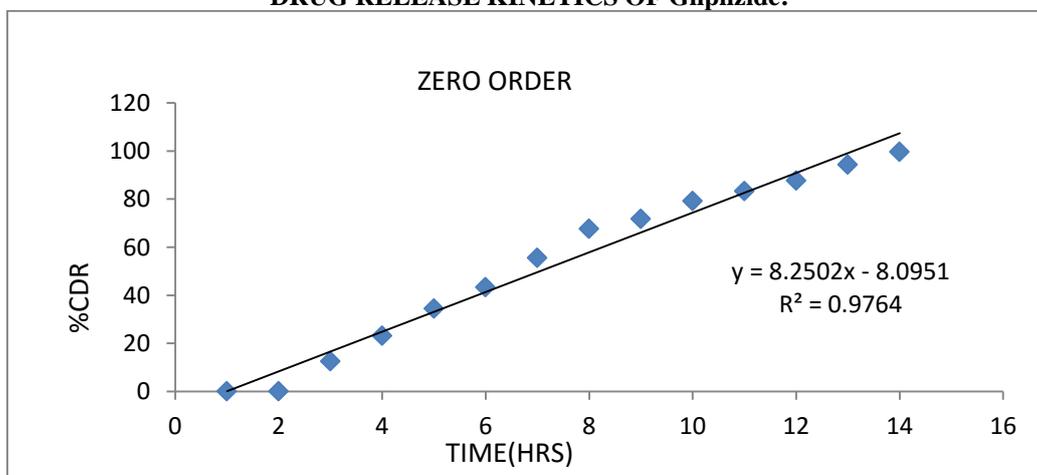


Fig 4: Zero order release profile of Gliplizide Nanosponges of FT1

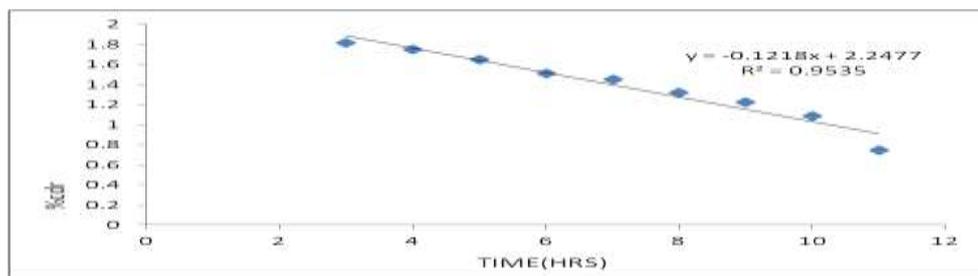


Fig 5: first order release profile of Gliplizide Nanosponges of FT1

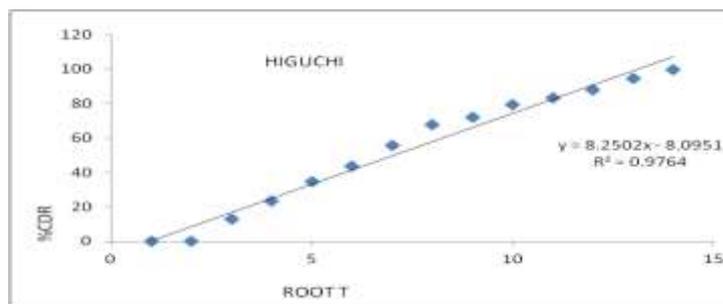


Fig 6: Higuchi release kinetics profile of Gliplizide Nanosponges of FT1.

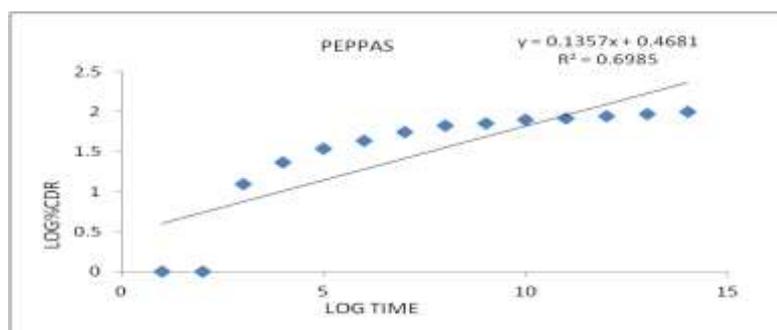


Fig 7: Peppas release kinetics profile of Gliplizide Nanosponges of FT1.

Table 1.7: Regression coefficients fit to different drug release kinetics models for Gliplizide Nanosponges.

S.NO	Zero order	First order	Higuchi	Peppas
Code	R <sup>2</sup>	R <sup>2</sup>	R <sup>2</sup>	R <sup>2</sup>
<b>GF1</b>	0.976	0.953	0.976	0.698

### CONCLUSION

The Nanosponge was prepared by solvent evaporation method by using  $\beta$ -cyclodextrin and HP  $\beta$  cyclodextrin and was evaluated for its different parameters which revealed many interesting results for efficient preparation of the nanosponge. The formulation GF1 has better results than other five formulations. With the revealed results by different evaluation parameters, it is concluded that  $\beta$ -cyclodextrin and HP  $\beta$  cyclodextrin when used as a copolymer with ethyl cellulose polymer in different concentrations nanosponge drug delivery system has showed increased drug entrapment, drug release and increased yield of dosage form. So cyclodextrins are proven to be helpful in preparation of stable and soluble Nanosponge delivery system with increased efficiency.

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