FORMULATION AND EVALUATION OF NANOPARTICLES OF GLIBENCLAMIDE WITH DIFFERENT POLYMERS BY CHEMICAL CROSS LINKING METHOD

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ABSTRACT:

Nanoparticles represent a promising drug delivery system of controlled and targeted drug release. They are specially designed to release the drug in the vicinity of target tissue. The aim of this study was to prepare and evaluate Carbopol p934 nanoparticles containing Glibenclamide in different drug to polymer ratio. SEM indicated that nanoparticles have a discrete spherical structure. FT-IR studies indicated that there was no chemical interaction between drug and polymer and stability of drug. The *in vitro* release behavior from all the drug loaded batches was found to be first order release and provided sustained release over a period of 12 h. The developed formulation overcome and alleviates the drawbacks and limitations of Glibenclamide sustained release formulations and could possibility be advantageous in terms of increased bioavailability of Glibenclamide.

KEYWORDS: - Nanoparticles, PLGA, Carbopol p934, Eudragit RL and Glibenclamide.

DOI: 10.48047/ecb/2023.12.10.994

1. INTRODUCTION:

Nanotechnology has gained huge attention over time. The fundamental component of nanotechnology is the nanoparticles. Nanoparticles are particles between 1 and 100 nanometres in size and are made up of carbon, metal, metal oxides or organic matter [1]. The nanoparticles exhibit a unique physical, chemical and biological properties at nanoscale compared to their respective particles at higher scales. This phenomena is due to a relatively larger surface area to the volume, increased reactivity or stability in a chemical process, enhanced mechanical strength, etc. [2]. These properties of nanoparticles has led to its use various applications. The nanoparticles

differs from various dimensions, to shapes and sizes apart from their material [3]. A nanoparticle can be either a zero dimensional where the length, breadth and height is fixed at a single point for example nano dots, one dimensional where it can possess only one parameter for example graphene, two dimensional where it has length and breadth for example carbon nanotubes or three dimensional where it has all the parameters such as length, breadth and height for example gold nanoparticles.

The nanoparticles are of different shape, size and structure. It be spherical, cylindrical, tubular, conical, hollow core, spiral, flat, etc. or irregular and differ from 1 nm to 100 nm in size. The surface can be a uniform or irregular with surface variations. Some nanoparticles are crystalline or amorphous with single or multi crystal solids either loose or agglomerated. Numerous synthesis methods are either being developed or improved to enhance the properties and reduce the production costs. Some methods are modified to achieve process specific nanoparticles to increase their optical, mechanical, physical and chemical properties. A vast development in the instrumentation has led to an improved nanoparticle characterisation and subsequent application. The nanoparticles are now used in every objects like from cooking vessel, electronics to renewable energy and aerospace industry. Nanotechnology is the key for a clean and sustainable **future.**[4]

MATERIALS AND METHODS:

Materials GLB was sent to me as a gift sample by Pioneer Company for Pharmaceutical Industries, . Soluplus® supplied by BASF SE, Germany. PVP K 15 was purchased from Fluka, Germany. Carbopol p934Eastman Chemical company, USA. HPMC E 5 and HPMC E15 were purchased from Baoji, China. Tween 20 and Methanol were purchased from THOMAS BAKER, India. PVA® was purchased from Alfa Aesar, German.

METHODOLOGY

Analytical Method Development

Determination of absorption maxima: Absorption maxima are the wavelength at which maximum absorption takes place. For accurate analytical work, it is important to determine the absorption maxima of the substance under study.

Procedure: For the preparation of calibration curve stock solution was prepared by dissolving 100 mg of accurately weighed drug in 100ml of Methanol (1mg/ml). Further 1ml of the stock solution was pipette out into a 100 ml volumetric flask and volume was made up with phosphate

buffer (5.5pH). From this stock solution pipette out 1ml and dilute to 10 ml with phosphate buffer and subject for UV scanning in the range of 200-400 nm using double beam UV spectrophotometer. The absorption maxima were obtained at 245 nm with a characteristic peak.

Preparation of calibration curve: It is soluble in Methanol; hence Methanol was used for solubilizing the drug. Stock solution (1 mg/mL) of Glibenclamide was prepared in Methanol and subsequent working standards (2, 4, 6, 8 and 10 μg/mL) were prepared by dilution with phosphate buffer of pH-5.5. These solutions were used for the estimation Glibenclamide by UV method. The whole procedure was repeated three times and average peak area was calculated. Calibration plot was drawn between concentrations and peak area. Calibration equation and R^2 value are reported.

Preparation of nanoparticles

Preparation of Glibenclamide loaded nanoparticles

Glibenclamide loaded Nanoparticle was prepared by previously reported Cross Linking Method. Glibenclamide was dissolved in organic solvent (20 ml, methanol and DCM 30ml). Polymers in different concentrations were dissolved in water. The organic phase was added drop wise into the polymeric solution for emulsification. Then the dispersion was sonicated (20 min) with the application of ultra-probe sonication (60 W/cm^3, Hielscher, Ultra-sonics, Germany). The formulation was stirred at 1500 rpm for 6 h using a magnetic stirrer to evaporate the organic solvent. The prepared NPs were centrifuged at 15,000 rpm for 20 min at 25 °C (Remi, Mumbai, India). NPs were separated and lyophilized using cryoprotectant (Mannitol 0.2%) and stored for further evaluation.

Table 1: Composition of nanoparticles formulations (F1 to F9)

Excipients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Glibenclamide	5	5	5	5	5	5	5	5	5
PLGA	100	200	300	-	-	-	-	-	-
Carbopol p934	-	-	-	100	200	300	-	-	-
Eudragit RL	-	-	-	-	-	-	100	200	300

Span 60 (mL)	2	4	6	2	4	6	2	4	6
Distilled water (ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Dichloromethane (ml)	30	30	30	30	30	30	30	30	30
Methanol	20	20	20	20	20	20	20	20	20

Characterization of nanoparticles:

Particle Sizes, PDI, Zeta Potential:

The mean particle length and polydispersity index (PDI), that's a degree of the distribution of nanoparticles population, was decided the usage of dynamic light scattering (Delta Nano C, Beckman counter), and Zeta capability becomes anticipated on the premise of electrophoretic mobility under an electric powered field, the use of zeta Sizer Nano ZS (Malvern Instruments, UK). Samples had been diluted with the distilled water before measurement and measure at a hard and fast angle of 1650c for the particle size and poly dispersity index (PDI) analysis. For the Zeta ability measurement, Samples have been diluted as 1;40 ratio with filtered water (v/v) before analysis. Average particle size, PDI, and zeta potential have been then measured in triplicate.

Drug content:

Glibenclamide content in solid lipid nanoparticles was assayed by an UV-visible spectrophotometer. Nanoparticles (100mg) were dissolved in 10ml methanol by shaking the mixture for 5 mins. One ml of the resultant solution was taken and diluted to 10ml with methanol. Then, aliquots were withdrawn and absorbance was recorded at 245 nm using UV-visible spectrophotometer (Lab India 3200).

Yield of nanoparticles:

After complete drying the nanoparticles powders were collected and weighed accurately. The yield of nanoparticles was calculated using the formula

$$Percentage\ yield = \frac{Total\ weight\ of\ nanoparticles}{\text{Total weight of drug } + \text{weight of added materials}} \times 100$$

Entrapment Efficiency:

Entrapment Efficiency (EE) of the Glibenclamide loaded changed into determined by measuring the awareness of uninterrupted drug in an aqueous medium by centrifugation method. The nanoparticles had been centrifuged during a high-space cooling Centrifuge (C-24.Remi) the usage of nano step centrifuge tubes with ultra-filter out having a relative molecular mass cutoff 100KD (Pall existence sciences-India) at 5000rpm for 15min at 4oc, and therefore the supernatant was separated. The amount of Glibenclamide inside the supernatant changed into determining the usage of a UV-Visible spectrophotometer (U-1800, Hitachi) at lambda max 245nm after suitable dilution.

The percent entrapment efficiency (%) changed into calculated by means of the usage of the subsequent formula:

$$\%EE=\frac{\text{Total drug content-Free drug x100}}{\text{Total drug content}}$$

Percent amount of drug release from semi permeable membrane

Franz diffusion cell was used for the in vitro drug release studies. Semi permeable membrane was placed between donar and receptor chamber of diffusion cell. Receptor chamber was filled with freshly prepared 30ml 5.5 PH phosphate buffer. SLN gel equivalent to 1gm was placed on semi permeable membrane. The Franz diffusion cell was placed over magnetic stirrer (REMI 1ML) with 500rpm and temperature was maintained at 37 ± 1^0C. 5ml of samples were withdrawn periodically and replaced with fresh buffer. The withdrawn samples were periodically diluted and analysed for drug content using UV visible spectrophotometer (Lab India 3200) at 245 nm.

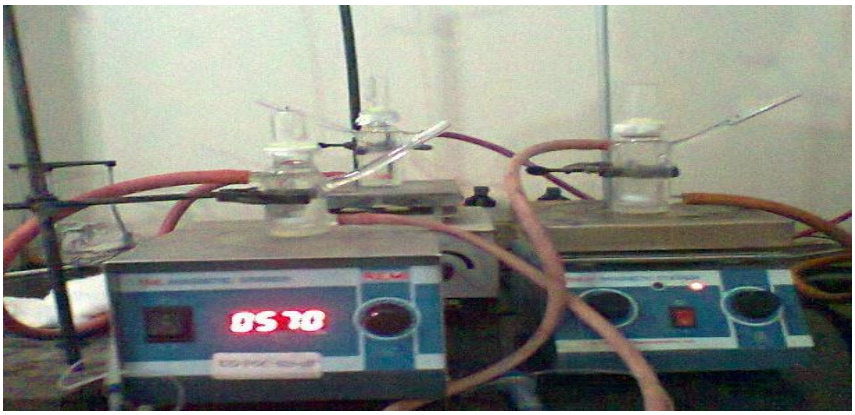

Fig 1 : Drug release from semi permeable membrane

Powder X-ray Diffraction (PXRD) Studies

The prepared mixtures were also analyzed using X-ray powder diffractometer (PXRD) which confirms the formation of the new solid phases. The difference in the 2 theta lines confirms the formation of the new solid phases as no two solids have same 2 theta lines, thus revealing the formation of new solid phases. It also reveals the information about the crystal structure, chemical composition, and physical properties of the material and also helps in structural characterization. This technique detects changes in the crystal lattice and is therefore a powerful tool for studying polymorphism, pharmaceutical salts, and cocrystalline phases. Spectra of PXRD were taken on a sample stage Spinner PW3064. The samples were exposed to nickel filtrate Cukœ radiations (40 KV, 30 mA) and were scanned from 10° to 40°, 2Ɵ at a step size of 0.045° and step time of 0.5 s.

***In Vitro* Release Studies**

Drug release was determined by dialysis method; two ml of each formulation (test and control) were poured into dialysis bags and put into 25 ml phosphate buffer (pH 6.8) and stirred (100 rpm, room temperature). At predetermined time intervals, 2 ml of phosphate buffer was taken and then substituted by fresh phosphate buffer. Finally, the amounts of released Simvastatin in phosphate buffer were measured by spectrophotometer at 245 nm. Aliquots withdrawn were assayed at each time interval for the drug released at λ max of 245 nm using UV-Visible spectrophotometer by keeping phosphate buffer pH 6.8 as blank and the amount of released drug was estimated by the standard curve.

Fourier Transform Infrared (FTIR) spectroscopy:

The formulations were subjected to FTIR studies to find out the possible interaction between the drug and the excipients during the time of preparation. FT IR analysis of the pure drug and optimized formulation were carried out using an FT IR spectrophotometer (Bruker FT-IR - GERMANY).

Differential Scanning Calorimetry:

The possibility of any interaction between the drug and the Excipients during preparation of SLN was assessed by carrying out thermal analysis of optimised formulation using DSC. DSC

analysis was performed using Hitachi DSC 7020, on 5 to 15 mg samples. Samples were heated in sealed aluminum pan at a rate of 10°C/min conducted over a temperature range of 30 to 350°C under a nitrogen flow of 50 mL/min.

SEM (Scanning Electron microscope) studies

The surface morphology of the layered sample was examined by using SEM (Hitachi, Japan). The small amount of powder was manually dispersed onto a carbon tab (double adhesive carbon coated tape) adhered to an aluminum stubs. These sample stubs were coated with a thin layer (30Å) of gold by employing POLARON-E 3000 sputter coater. The samples were examined by SEM and photographed under various magnifications with direct data capture of the images onto a computer.

RESULTS AND DISCUSSION:

Preparation of Standard Graph:

a. Determination of absorption maxima

The standard curve is based on the spectrophotometry. The maximum absorption was observed at 245 nm.

b. Calibration curve

Graphs of Glibenclamide was taken in 7.4 Phosphate buffer

Table 2 : Calibration curve data for Glibenclamide at 245 nm

Concentrations [µg/mL]	Absorbance
0	0
2	0.128
4	0.265
6	0.381
8	0.487

10	0.619

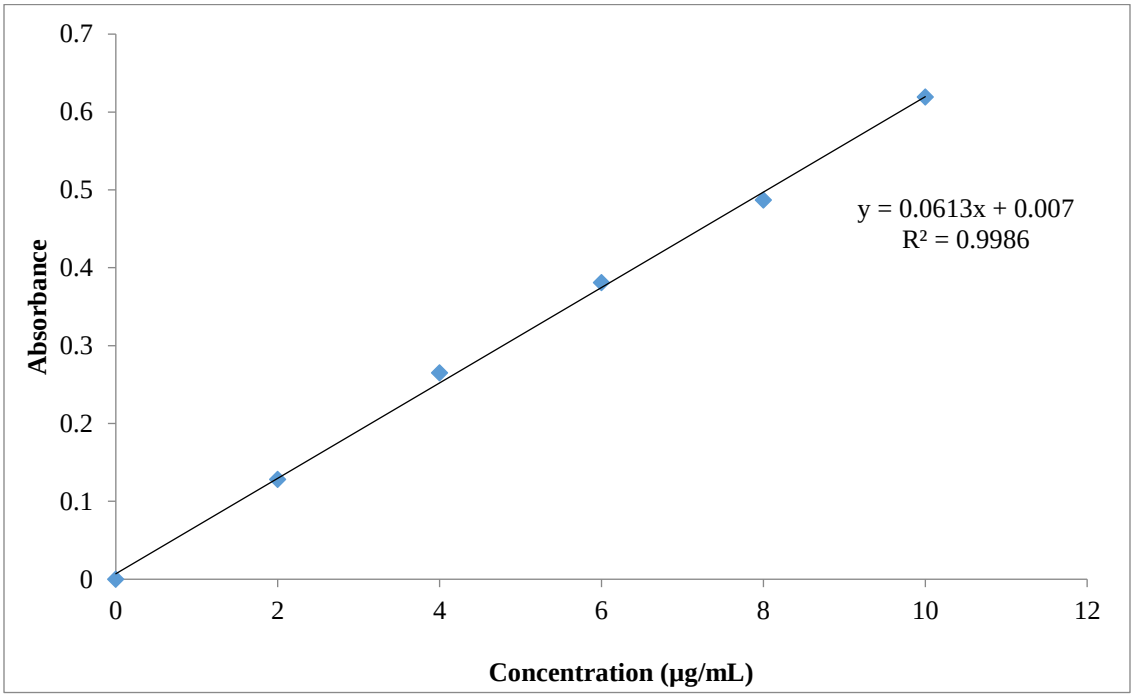

Fig 2 : Standard graph of Glibenclamide in 7.4 Phosphate buffer

Standard graph of Glibenclamide was plotted as per the procedure in experimental method and its linearity is shown in Table 8.1 and Fig 8.1. The standard graph of Glibenclamide showed good linearity with R^2 of 0.998, which indicates that it obeys "Beer- Lamberts" law.

EVALUATION OF GLIBENCLAMIDELOADED NANOPARTICLES:

Table 3 : Evaluation of Nanoparticles

Batch No	Mean Particle size(nm)	%Yield	Drug Content	Drug encapsulation efficiency	PDI	Zeta Potential (mV)
F1	286.12 ± 18	68.14	93.51	63.92	0.668	-26.12 ± 1.8

F2	292.22 ± 19	71.54	95.81	72.29	1.268	-28.22 ± 1.9
F3	305.19 ± 16	75.92	97.65	80.41	1.153	-30.19 ± 1.6
F4	267.22 ± 20	75.20	91.54	76.91	0.868	-27.22 ± 2.0
F5	278.56± 18	79.81	94.82	82.83	0.577	-28.56± 1.8
F6	281.72± 23	86.34	98.84	87.92	0.309	-32.61± 2.3
F7	351.72± 23	73.92	95.14	62.79	0.498	-25.72± 2.3
F8	368.32± 42	77.69	97.14	70.30	0.385	-26.32± 2.2
F9	371.52± 32	83.44	97.82	76.98	0.325	-27.52± 2.4

Percentage yield of formulations F1 to F9 by varying drug was determined and is presented in Table. Highest drug content, Highest Entrapment efficiency observed for F6 formulation.

PDI observed in the F6 formulation i.e., 0.309 respectively. The Zeta potential range from -25.72 mV to -32.61 mV to all the formulations.

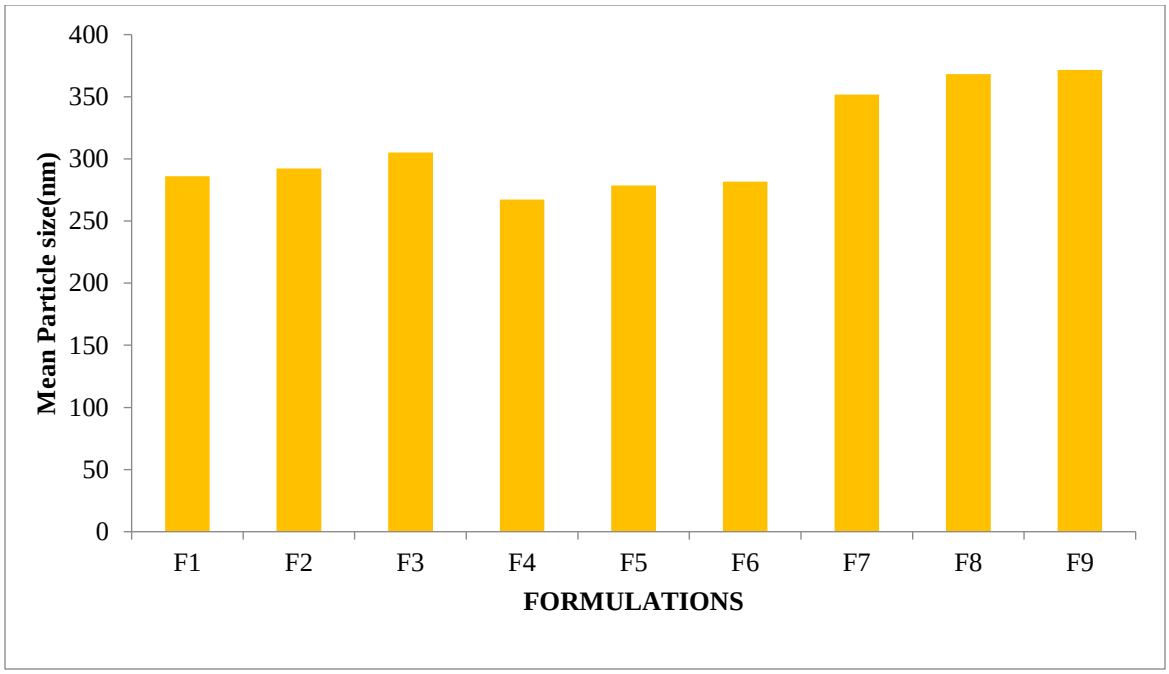

Fig 3: Mean Particle size (nm)

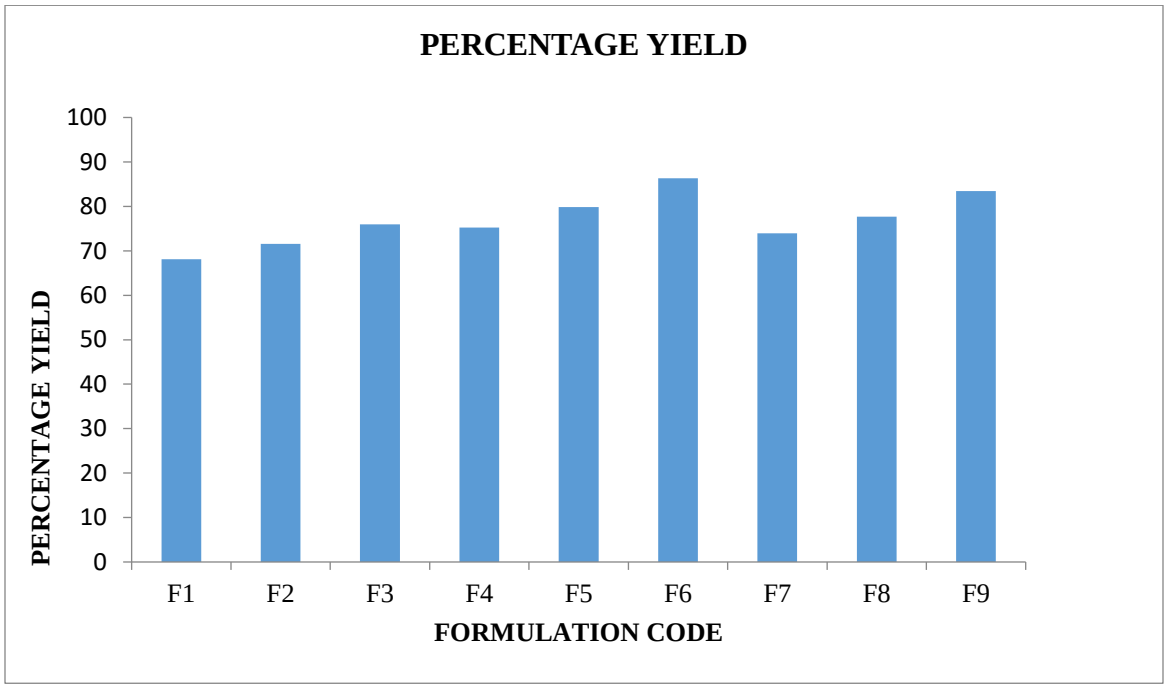

Fig 4: %Yield

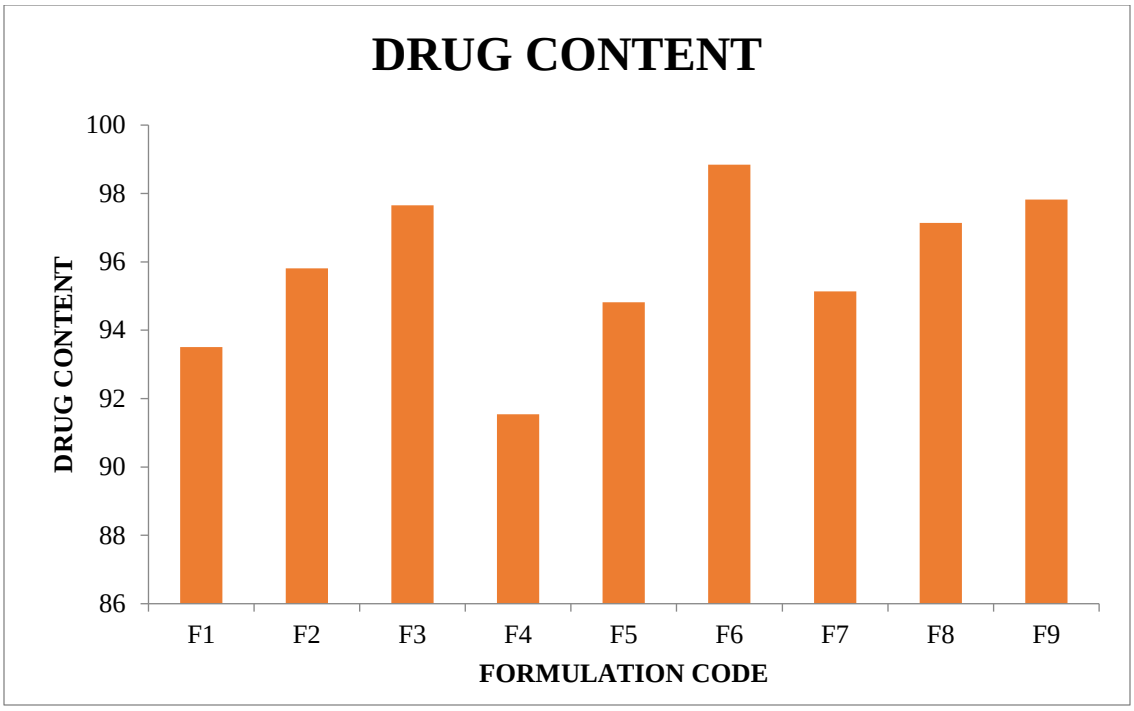

Fig 5: Drug content

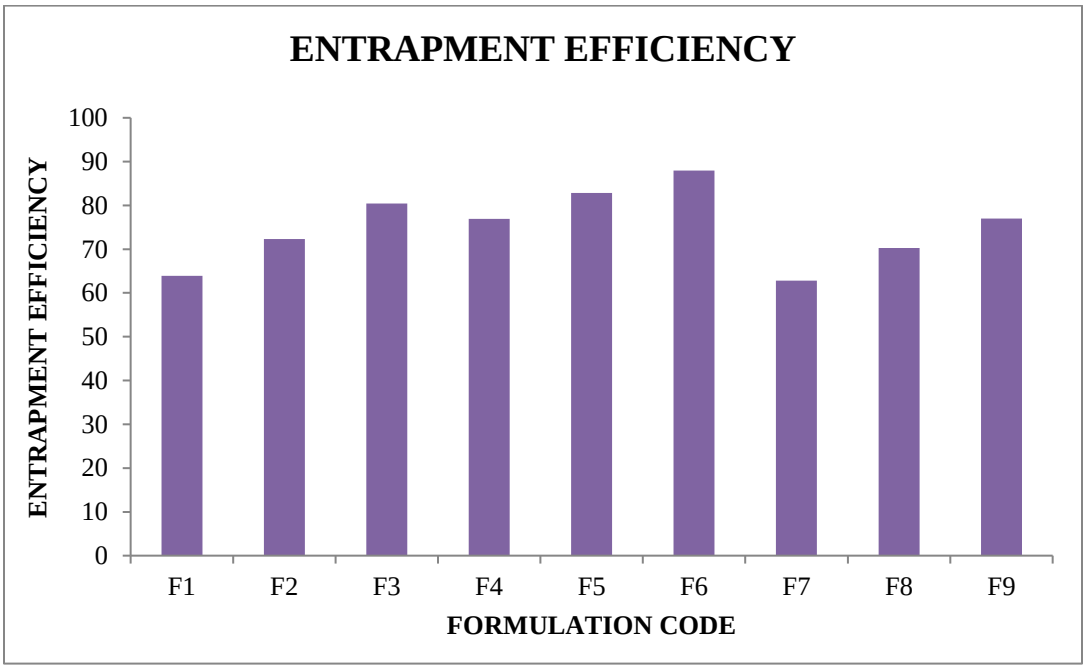

Fig 6: Drug encapsulation efficiency

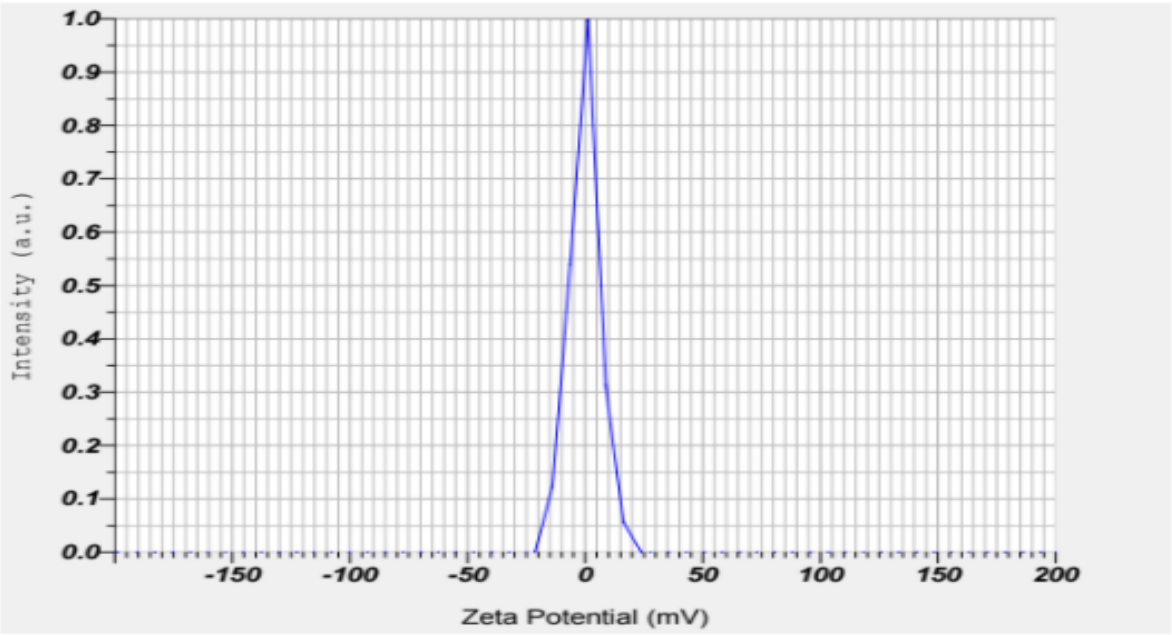

Fig 7: Zeta Potential of F6 Formulation

***In vitro* Drug release studies:**

Table 4 : *In vitro* Drug release studies of Glibenclamide

TIME (hr)	CUMULATIVE PERCENT OF DRUG RELEASED								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	27.42	29.69	32.41	27.93	16.85	22.26	20.92	17.92	14.01
2	34.39	40.09	47.69	41.62	22.76	28.78	34.36	22.65	20.08
3	47.60	46.16	58.34	48.02	30.50	35.36	42.61	33.89	31.51
4	56.51	57.65	64.61	60.47	49.11	57.23	54.53	44.32	43.98
5	67.62	65.19	70.08	66.85	61.78	66.98	61.88	52.87	50.31
6	78.37	78.67	78.39	78.68	76.89	77.46	72.46	65.90	62.57
7	85.26	81.76	84.56	87.39	83.43	85.68	81.87	73.36	67.04
8	96.78	89.54	87.98	98.77	97.14	93.14	89.29	79.77	75.91
10	99.82	95.34	93.18			98.13	98.14	90.53	83.09
12		97.54	97.14			99.37		96.91	94.91

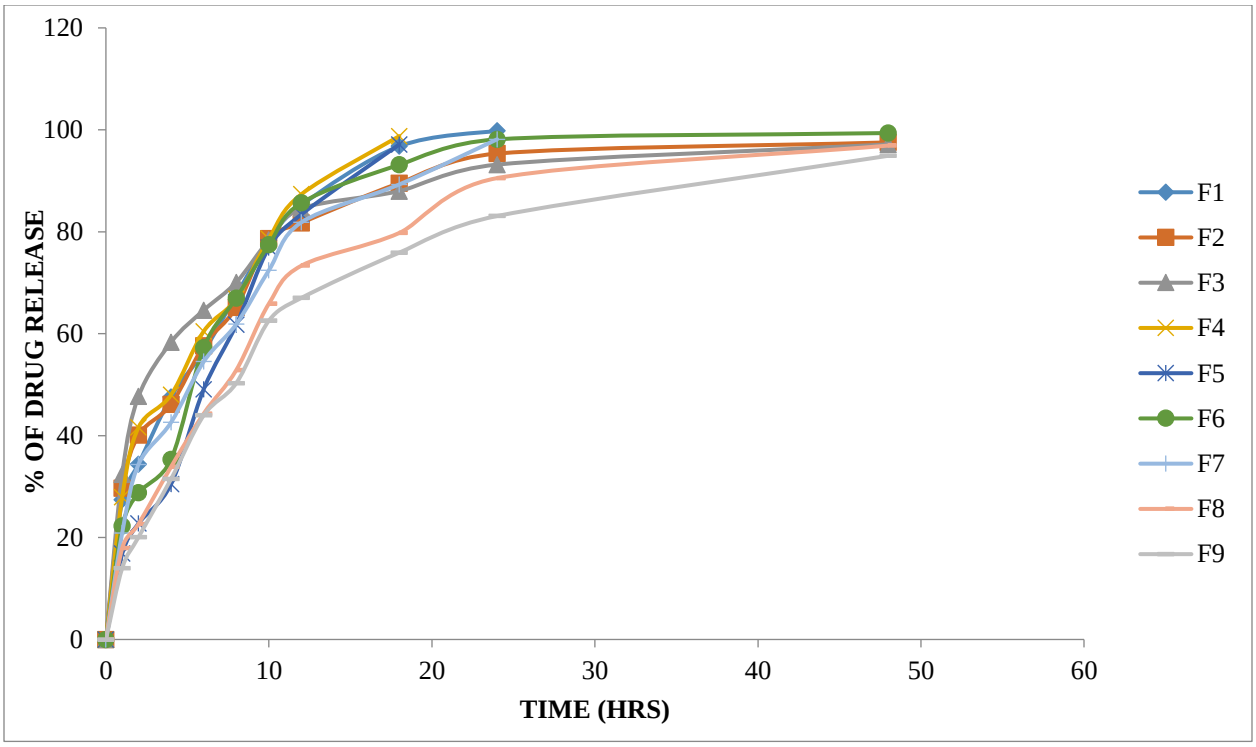

Figure No 8 : Dissolution study of Glibenclamide Nanoparticles

Hence based on dissolution data of 9 formulations, F6 **Carbopol p934 (1:3) (**300**mg)** formulation showed better release (99.37%) up to 12 hours. So F6 formulation is optimised formulation.

Application of Release Rate Kinetics to Dissolution Data

Data of *in vitro* release studies of formulations which were showing better drug release were fit into different equations to explain the release kinetics of drug release from Nanoparticles. The data was fitted into various kinetic models such as zero, first order kinetics; higuchi and korsmeyer peppas mechanisms and the results were shown in below table it follows the zero order kinetics.

Table 5: Release kinetics data for optimized formulation (F6)

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE / t)	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
22.26	1	1.000	1.348	0.000	1.891	22.260	0.0449	-0.652	77.74	4.642	4.268	0.374
28.78	2	1.414	1.459	0.301	1.853	14.390	0.0347	-0.541	71.22	4.642	4.145	0.496
35.36	4	2.000	1.549	0.602	1.811	8.840	0.0283	-0.451	64.64	4.642	4.013	0.628
57.23	6	2.449	1.758	0.778	1.631	9.538	0.0175	-0.242	42.77	4.642	3.497	1.144
66.98	8	2.828	1.826	0.903	1.519	8.373	0.0149	-0.174	33.02	4.642	3.208	1.433
77.46	10	3.162	1.889	1.000	1.353	7.746	0.0129	-0.111	22.54	4.642	2.825	1.817
85.68	12	3.464	1.933	1.079	1.156	7.140	0.0117	-0.067	14.32	4.642	2.428	2.213
93.14	18	4.243	1.969	1.255	0.836	5.174	0.0107	-0.031	6.86	4.642	1.900	2.741
98.13	24	4.899	1.992	1.380	0.272	4.089	0.0102	-0.008	1.87	4.642	1.232	3.410
99.37	48	6.928	1.997	1.681	-0.201	2.070	0.0101	-0.003	0.63	4.642	0.857	3.784

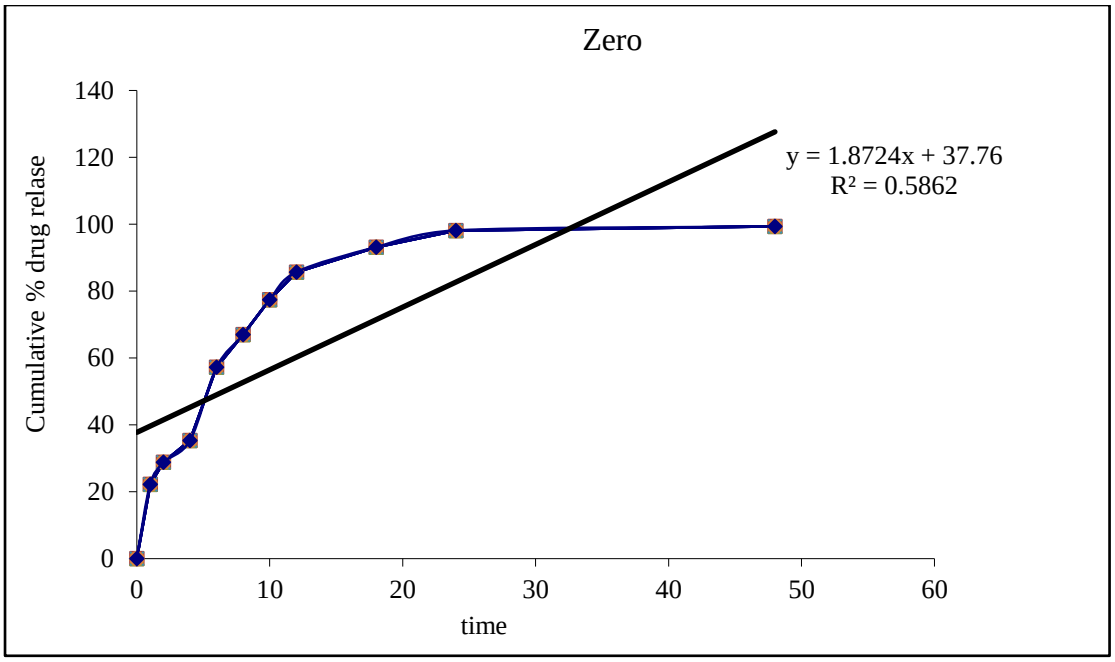

Figure 8: Graph of zero order kinetics

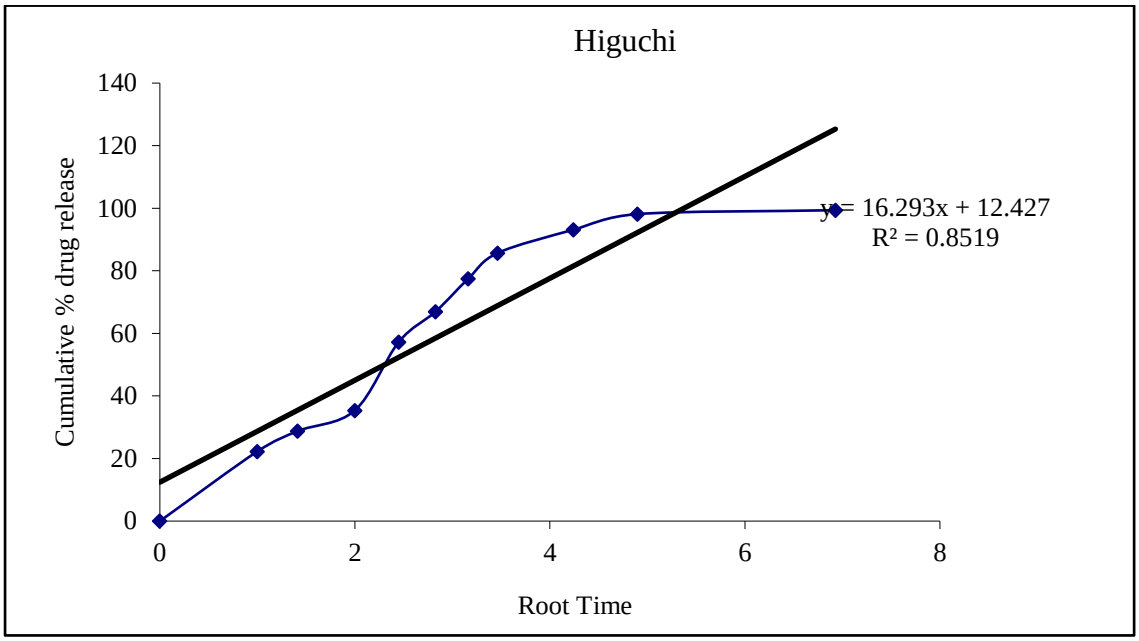

Figure 9: Graph of higuchi release kinetics

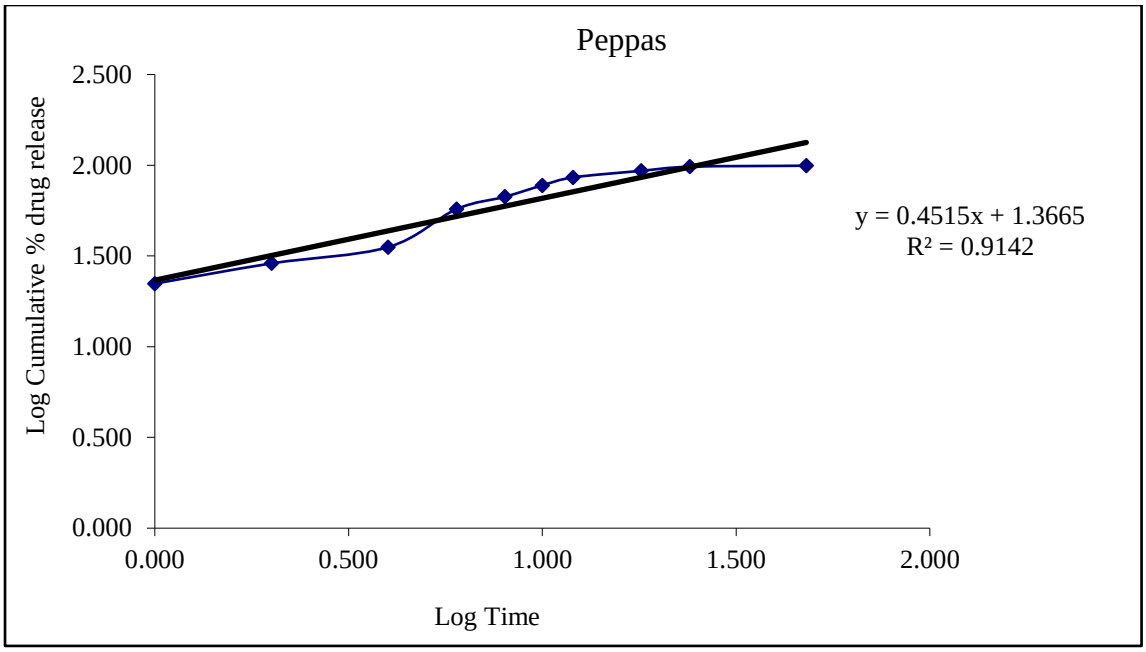

Figure 10: Graph of peppas release kinetics

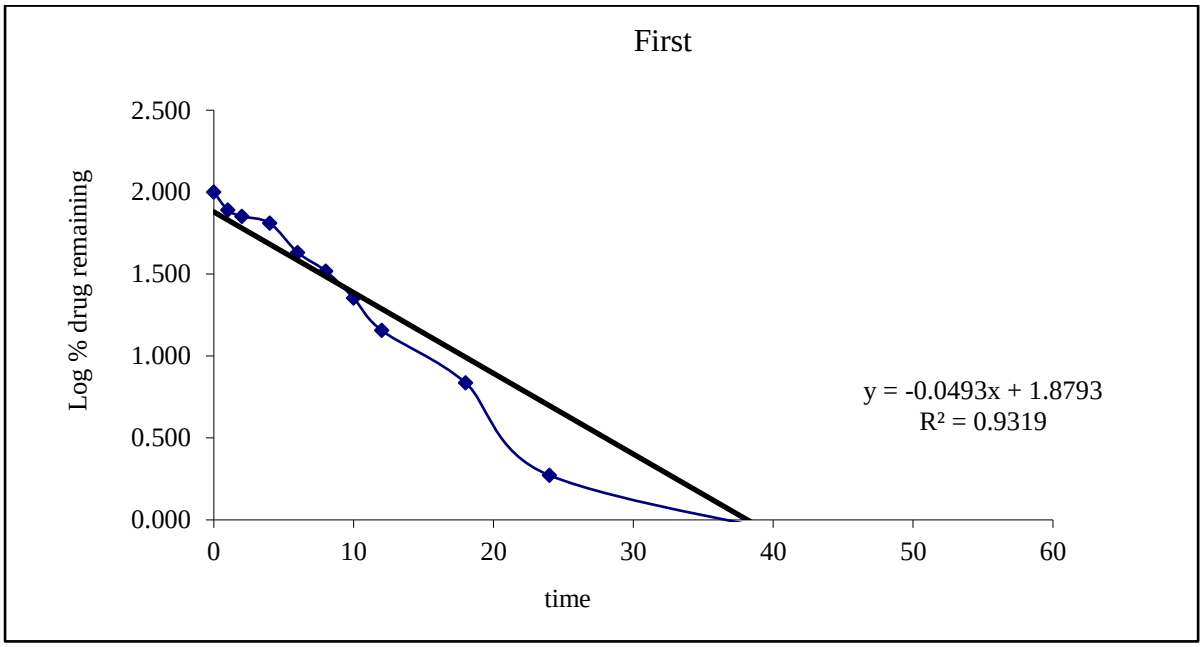

Figure 11: Graph of first order release kinetics

Based on the data above results the optimized formulation followed **first order** release kinetics.

Drug – Excipient compatibility studies

Fourier Transform-Infrared Spectroscopy:

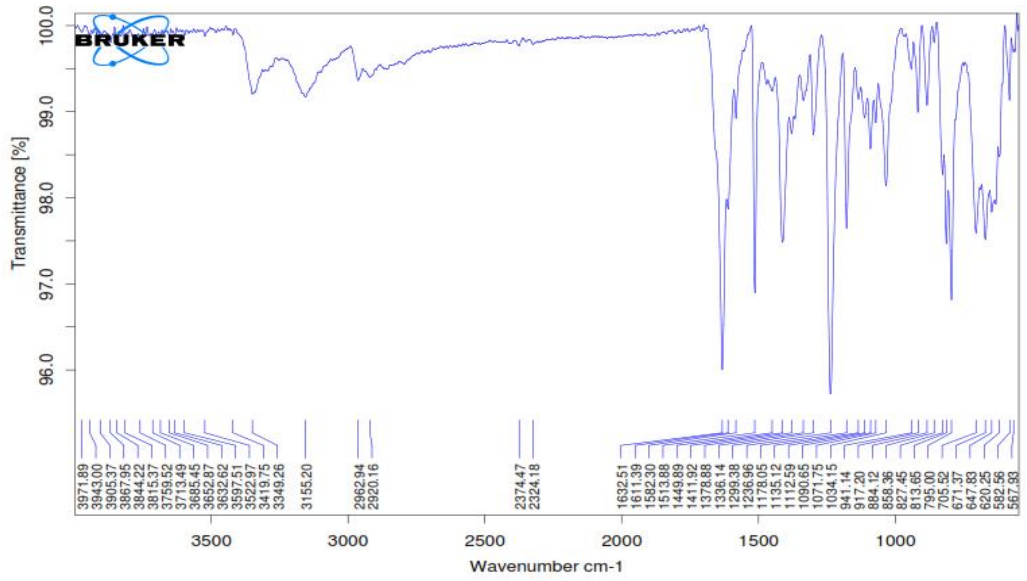

Figure 12: FT-TR Spectrum of Glibenclamide pure drug.

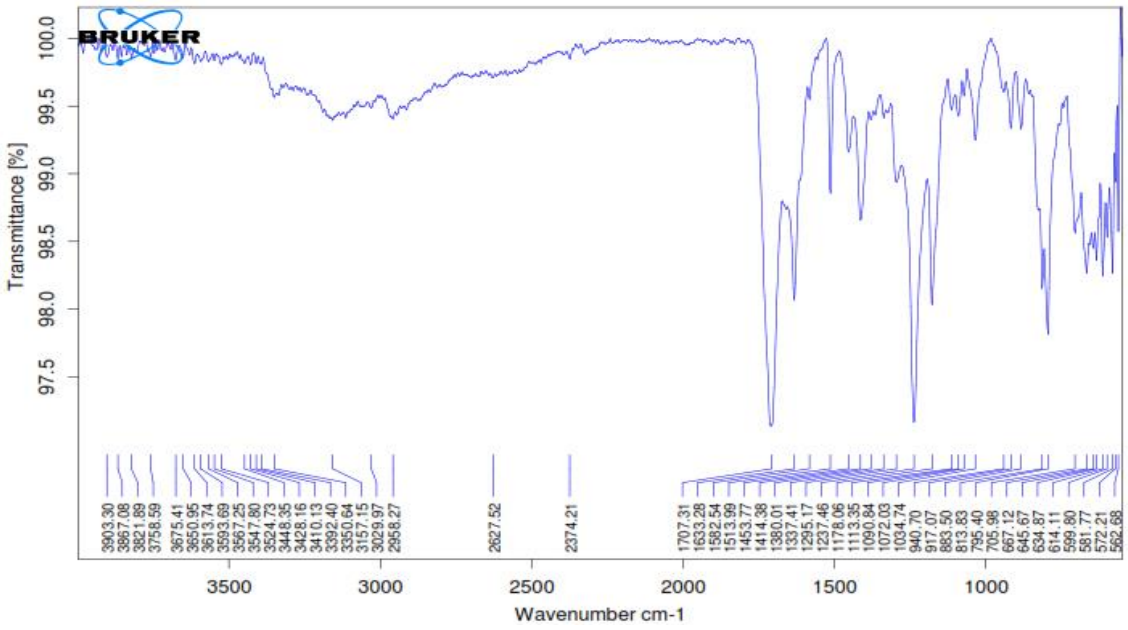

Figure 13: FT-IR Spectrum of Optimised Formulation

There is no incompatibility of pure drug and excipients. There is no disappearance of peaks of pure drug and in optimised formulation.

SEM

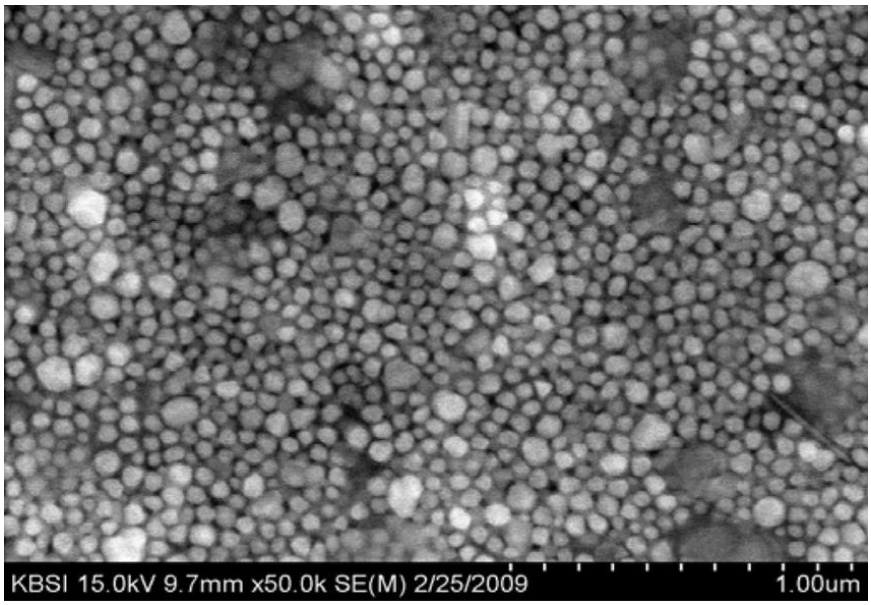

Figure 14: SEM graph of optimized formulation

SEM studies showed that the Glibenclamide- loaded nanoparticles had a spherical shape with a smooth surface as shown in Figure.

XRD

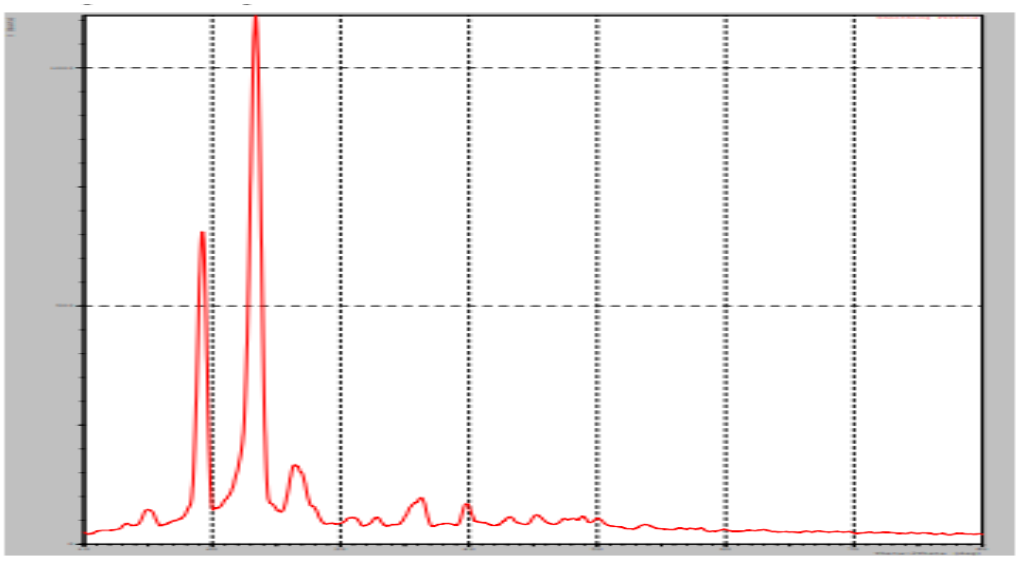

Figure 15: GlibenclamideF6 optimised formulation

9. CONCLUSION

Nanoparticles have a special place in nanoscience and nanotechnology, not only because of their particular properties resulting from their reduced dimensions, but also because they are promising building blocks for more complex nanostructures.

In our current work, we have prepared Glibenclamide nanoparticles. Cross Linking Method is a simple, fast and reproducible method which is widely used for the preparation of both nanospheres and nanocapsules and its superior advantage is obtaining small particles size and narrow size distribution. The optimized Glibenclamide loaded Carbopol p934 nanoparticles formulations (F6) were in nano size range (281.72±23nm) with high drug release (99.37%) adequate encapsulating efficiency exhibiting a homogenous, stable and effective.

REFERENCES

1. Hasan S 2015 A Review on Nanoparticles : Their Synthesis and Types Biosynthesis : Mechanism 4 9–11
2. Assessment R 2007 Nanoparticles in the Environment
3. Cho E J, Holback H, Liu K C, Abouelmagd S A, Park J and Yeo Y 2013 Nanoparticle characterization : State of the art , challenges , and emerging technologies.
4. Machado S, Pacheco J G, Nouws H P A, Albergaria J T and Delerue-Matos C 2015 Characterization of green zero-valent iron nanoparticles produced with tree leaf extracts Sci. Total Environ. 533 76–81.
5. Renu Tiruwa. A review on nanoparticles – preparation and evaluation parameters. Indian J. Pharm. Biol. Res. 2015; 4(2):27-31
6. Abhilash M., Potential applications of Nanoparticles, International Journal of Pharma and Bio Sciences 2010; 1:1: 1-12.
7. Nagavarma B. V. N., Hemant K. S. Yadav, Ayuz A., Vasudha L.S., Shivakumar H.G, Different techniques for preparation of polymeric nanoparticles – A Review, Asian Journal of Pharmaceutical and Clinical Research 2012; 5:3: 1-8.
8. A. R. Mullaicharam, Nanoparticles in drug delivery system, International Journal of Nutrition, Pharmacology Neurological Diseases 2011; 1:2: 103-121.

9. Langer R. Biomaterials in drug delivery and tissue engineering; one labortory's experience. Acc ChemRes.2000;33:94-101.
10. Bhadia D, Bhadra S, Jain P and Jain NK. Pegnology; a review of PEGylated systems; Pharmazin. 2002;57:5-20.
11. Tiwari D K, Behari J and Sen P 2008 Application of Nanoparticles in Waste Water Treatment 3 417–33
12. Salavati-niasari M, Davar F and Mir N 2008 Synthesis and characterization of metallic copper nanoparticles via thermal decomposition Polyhedron 27 3514–8
13. Nagaraju, B.; Ramu, B.; Saibaba, S.V.; Rajkamal, B. Formulation and evaluation of floating bioadhesiveDoxofylline tablets. Int. J. Drug Deliv. 2016, 8, 134–141.
14. Gopikrishna, A.; Ramu, B.; Srikanth, G.; Rajkamal, B. Formulation of isoniazide sustained release formulation by using carbopol 934 P. *Int. J. Appl. Pharm. Sci. Res.* **2016**, *1*, 60–69.
15. Ramu Bandameedi*, Provenance of Computers in Pharmacy, Clin Pharmacol Biopharm 2016; 5:1:2-7.