WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL RESEARCH

Research Article

2024
Volume: 01
Issue: 01
Page: 26-33

FABRICATION OF β- CYCLODEXTRIN LOADED GLIMEPIRIDE NANOPARTICLES AND ITS EVALUATION

Kumar Raja Jayavarapu*, Praveen Kumar Dassari and G. Sandepthi

Department of Pharmaceutical Analysis, Mother Teresa Pharmacy College, Sathupally, Telangana – 507303, India.

Received: 26 September 2024 **Revised: 18 October2024** **Accepted: 07 November 2024**

Corresponding Author: Kumar Raja Jayavarapu

Address: Department of Pharmaceutical Analysis, Mother Teresa Pharmacy College, Sathupally, Telangana – 507303, India.

ABSTRACT

This study focuses on the fabrication & evaluation of Beta-Cyclodextrin loaded Glimepride nano particles aimed at enhancing the drugs solubility &bio- availability. To obtain a desirable therapeutic response, the correct amount of the drug should be transported and delivered to the site of action. Hesperidin (Hsd), a bioactive phytomedicine, experienced an antidiabetic activity versus both Type 1 and Type 2 Diabetes mellitus. However, its intrinsic poor solubility and bioavailability is a key challenging obstacle reflecting its oral delivery. From such perspective, the purpose of the current study was to prepare and evaluate Hsd-loaded sulfobutylether-β-cyclodextrin/chitosan nanoparticles (Hsd/CD/CS NPs) for improving the hypoglycemic activity of the orally administered Hsd. Hsd was first complexed with sulfobutylether-β-cyclodextrin (SBE-β-CD) and the complex (CX) was found to be formed with percent complexation efficiency and percent process efficiency of 50.53 ± 1.46 and 84.52 ± 3.16%, respectively. Also, solid state characterization of the complex ensured the inclusion of Hsd inside the cavity of SBE-β-CD. Then, Hsd/CD/CS NPs were prepared using the ionic gelation technique. The prepared NPs were fully characterized to select the most promising one (F1) with a homogenous particle size of 455.7 ± 9.04 nm, a positive zeta potential of + 32.28 ± 1.12 mV, and an entrapment efficiency of 77.46 ± 0.39%. The optimal formula (F1) was subjected to further investigation of in vitro release, ex vivo intestinal permeation, stability, cytotoxicity, and in vivo hypoglycemic activity. The results of the release and permeation studies of F1 manifested a modulated pattern between Hsd and

CX. The preferential stability of F1 was observed at 4 ± 1 °C. Also, the biocompatibility of F1 with oral epithelial cell line (OEC) was retained up to a concentration of 100 µg/mL. After oral administration of F1, a noteworthy synergistic hypoglycemic effect was recorded with decreased blood glucose level until the end of the experiment. In conclusion, Hsd/CD/CS NPs could be regarded as a hopeful oral delivery system of Hsd with enhanced antidiabetic activity.

KEYWORDS: Beta –Cyclodextrin, Glimepride, Fabrication method Scanning electron microscopy (SEM)

INTRODUCTION TO DIABETES MELLITUS

Diabetes has emerged as a major health problem in India. The prevalence of diagnosed diabetes has risen dramatically over the past several decades. Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar because the pancreas does not produce enough insulin or because cells do not respond to the insulin that is produced.

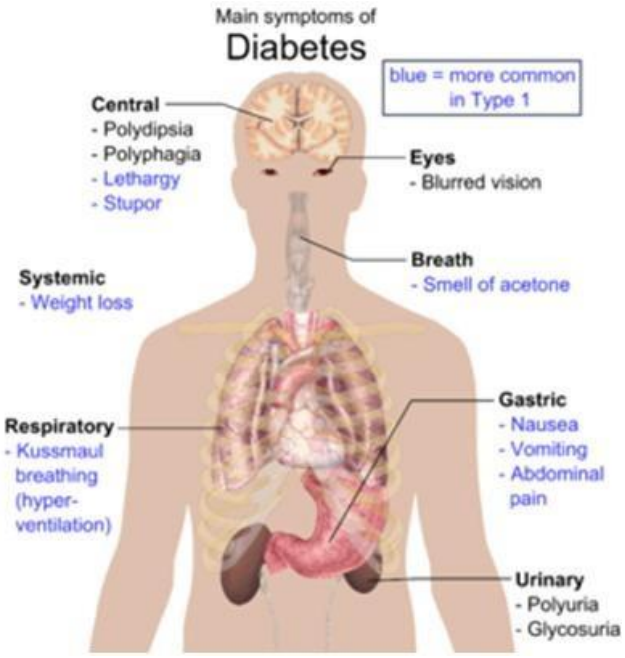

Figure 1: SIGNS AND SYMPTOMS.

The symptoms of untreated diabetes are:

1. Loss of weight,
2. Polyuria (frequent urination),
3. Polydipsia (increased thirst) and
4. Polyphagia (increased hunger).

Symptoms may develop rapid in type 1 diabetes, while they usually develop much more slowly or absent in type 2 diabetes.

These are colloidal particles ranging from 10-1000 nm which consist of macromolecular materials. This can be used therapeutically, e.g. as adjuvant in vaccines, drug carriers in which the active principle (drug or biological active material) is dissolved, entrapped or encapsulated. The active principle is adsorbed or attached. Polymeric nanoparticles are composed of biodegradable or bio stable polymers and copolymers. The active agents can be;

I. Entrapped or encapsulated within the particles

II. Physically adsorbed on surface (or)

III. Chemically linked to the surface of the nanoparticles.

GLIMEPIRIDE

1-[[p- [2-(3- ethyl-4-methyl-2-oxo-3-pyrroline1- carboxamido) ethyl] phenyl] sulphonyl]-3-(trans-4-methyl cyclohexyl) urea.

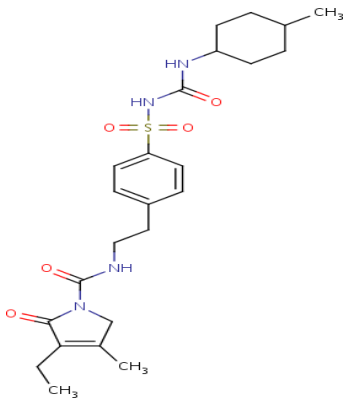

Fig. 2: Chemical Structure.

POLYMER PROFILE

β-CYCLODEXTRIN

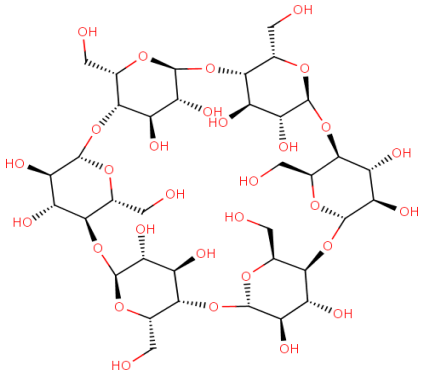

Fig. 3: Chemical Structure.

β- Cyclodextrin is the most commonly used cyclodextrin, although it is the least soluble. It is the least expensive cyclodextrin. It is commercially available from a number of sources and it is able to form inclusion complexes with a number of molecules of pharmaceutical interest. However, β- cyclodextrin is nephrotoxic and should not be used in parental formulations.

POLY EHYLENE GLYCOL 6000

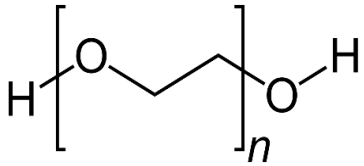

Fig. 4: Chemical Structure.

Generally PEG6000 isused in chewable tablets and tablet formulation that require water solubility. It is a non-reactive and can be used in pH sensitive (stable) drugs such as aspirin and vitamins. PEG solutions can be sprayed in aqueous or hydroalcoholic solvents into powders in twin- shell or double- cone blenders. However, they are not available in small particle size. Also used if food and food packaging. It may also be used in cosmetics and in ointments.

Methodology: Construction of Standard Curve of Glimepiride:

By UV spectroscopy method

Glimepiride is estimated spectrophotometrically at 228nm and it obeys Beer- Lambert's law in the range of 1-10µg/ml.

Determination of absorbance maximum (λ_{max})

Glimepiride was dissolved in phosphate buffer saline pH 7.4. Solution with 10µg/ml concentration was prepared by suitable dilution. The solution was scanned in UV spectrophotometer at 200-400 nm using phosphate buffer saline pH 7.4 as blank. Absorbance maximum was determined at 228nm. The drug was later quantified by measuring the absorbance at 228nm in phosphate buffer saline pH 7.4.

Preparation of pH 7.4 phosphate buffer saline

gms of potassium dihydrogen orthophosphate and 1.6gms of sodium hydroxide were accurately weighed and transferred into 1000ml volumetric flask. The volume is made upto 1000ml with distilled water. The pH was adjusted if necessary.

Preparation of stock solution:

Stock solution was prepared by dissolving 50mg of glimepiride in 50ml of the solvent medium, so as to get a solution of 1000µg/ml concentration (primary stock solution). From the primary stock solution, 1ml was taken in 100ml standard flask and it is diluted to 100ml with the solvent medium (secondary stock solution) to get a concentration of 10µg/ml.

Preparation of standard solution:

From the secondary stock solution, aliquots ranging from 1-10 ml was taken and made upto 10ml with the solvent medium to get the final concentration ranging from 1- 10µg/ml. Absorbance of the solution was measured at 228nm UV spectrophotometrically against drug free phosphate buffer pH 7.4 media as blank.

Table 1: Calibration curve of glimepiride at 228nm:

S.No	Concentration (µg/ml)	Absorbance at 228nm
1	1	0.080
2	2	0.151
3	3	0.225
4	4	0.301
5	5	0.373
6	6	0.441
7	7	0.516
8	8	0.591
9	9	0.668
10	10	0.746

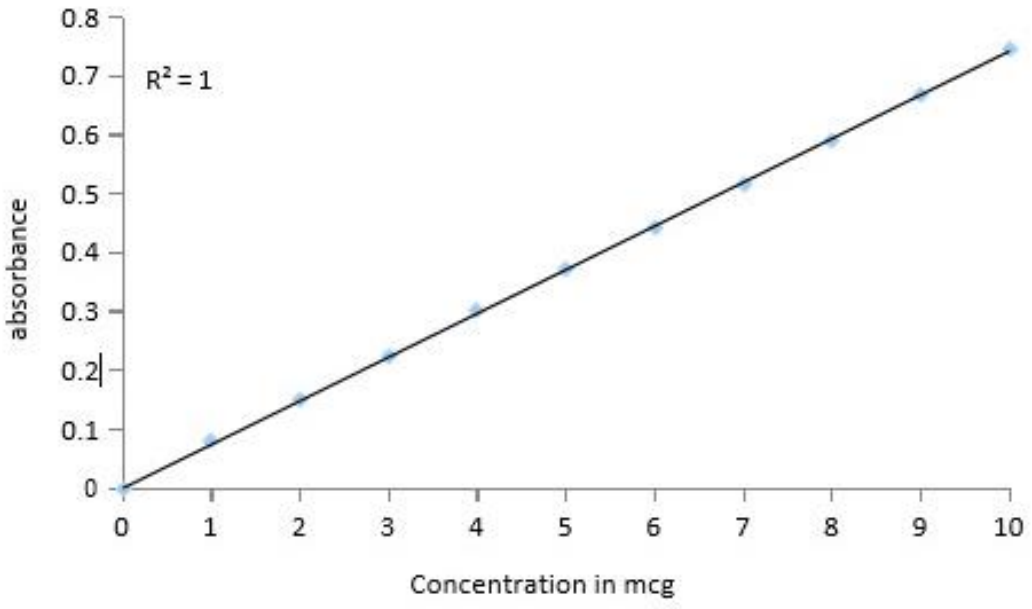

Fig. 5: Standard Curve of Glimepiride.

METHOD OF PREPARATION OF GLIMEPIRIDE- CYCLODEXTRIN COMPLEXES

Kneading Method

The glimepiride-cyclodextrin complexes were prepared by kneading method. 10mg of the drug, 4mg of β- cyclodextrin and 20mg poly ethylene glycol, 2mg of tween-80were taken. The mixture was heated and 20ml of acetone was added to it. This was then sonicated using probe sonicator set at 60W of energy output for 90 secs. This was further evaporated by flash rotatory evaporator for 60mins.

Table 2: Various compositions of formulations.

S. No	Formulation Code	Drug (mg)	β- CD (mg)	PEG (mg)	Tween 80mg	Ratio
1	CD1	4	4	20	2	1:1:5:2
2	CD2	4	8	20	2	1:2:5:2
3	CD3	4	12	20	2	1:3:5:2
4	CD4	4	16	20	2	1:4:5:2
5	CD5	4	20	20	2	1:5:5:2
6	CD6	4	24	20	2	1:6:5:2
7	CD7	4	28	20	2	1:7:5:2
8	CD8	4	32	20	2	1:8:5:2
9	CD9	4	36	20	2	1:9:5:2
10	CD10	4	40	20	2	1:10:5:2

EVALUATION OF NANOPARTICLES

Drug entrapment study

The entrapment efficiency study was determined by free drug content in the supernatant which is obtained after centrifugation at 15000rpm for 20mins at 0^0 using ultra centrifuge. The absorbance was measured by UV spectrophotometer at 228nm.

IN VITRO DRUG RELEASE STUDIES

By UV spectrophotometric method

The *in vitro* drug release study was carried out using the diffusion membrane technique. The nanoparticle preparation was placed in a dialysis membrane and it is kept in a beaker containing 100ml of diffusion medium (phosphate buffer pH 7.4). The medium was maintained at 37^0 C under magnetic stirring at constant speed. 1ml of the sample was taken from the diffusion medium at a fixed time interval. 1ml of fresh medium was replaced. This process was carried out for 24 hours. The sample was measured at 228nm using UV spectrophotometer. The percentage of drug released at various time intervals was calculated from calibration graph.

Morphology of nanoparticles by simple microscopy

The optimized formulation was morphologically characterized by microscopy. A small amount of sample was placed in a glass slide and investigated in microscopy.

Scanning electron microscopy

The formulation was morphologically characterized using scanning electron microscopy (SEM). For SEM analysis, the sample was mounted in a scotsch double adhesive tape. The sample was analysed in hitachi scanning electron microscope operated at 15 kv and photograph was taken.

Surface charge (zeta potential) determination

Zeta potential is an important parameter to evaluate an optimum condition for stability of colloidal or dispersed systems. The prepared nanoparticles were characterized by using zeta potential analyser (Malvern Zeta seizer). Zeta potential creates an electrical barrier. It is very important for drug stability. The effect of β- cyclodextrin complexes on the surface of the nanoparticles was studied.

pH and physical appearance

The pH of the formulation was measured using pH meter. It plays a role in the process of stability and formulation activity. The physical appearance of the formulation like colour and suspended foreign particulate were to be examined.

Stability studies of nanoparticles

The stability studies of nanoparticles involve observing the formulation at 45^0C/70% RH which constitutes accelerated condition at 4^0C on refrigerator and room temperature. At both the temperature the formulation was kept for 3 months. Small amount of the sample was withdrawn at periodic intervals for performing the following tests.

a) Physical appearance
b) *In vitro* drug release (dissolution)
c) pH of the solution
d) Percentage of drug entrapment.

RESULTS AND DISCUSSION

The glimepiride cyclodextrin complexes were prepared by kneading method. 4mg of glimepiride, 4mg β- cyclodextrin, 20mg poly ethylene glycol, 2mg Tween 80 were taken. The

mixture was heated and 20ml of acetone was added to it. This was then sonicated using Probe sonicator set at 60W of energy output for 90secs. This was further evaporated by flash rotatory evaporator for 60mins.

Formulations with different ratios of polymer were prepared. Several physicochemical characteristics of nanoparticles like morphology, particle size determination, drug release profile were investigated. Stability of the formulation at various temperatures was evaluated.

DRUG AND POLYMER COMPATIBILITY STUDIES BY FTIR

Infrared spectroscopy by potassium bromide pellet method was carried out on pure substance (glimepiride and β- cyclodextrin complexes) seperately and their physical mixtures. They are compressed under 15 tonnes pressure in a hydraulic press to form a transparent pellet. Then the pellet was scanned from 4000-400 cm^{-1} in a spectrophotometer.

To determine any possible molecular interactions between the drug and the polymer, the spectrum of physical mixture was compared with the original spectra. FTIR analysis measures the selective absorbance of light by the viberation modes of specific chemical bonds in the sample.

They are compressed under 15 tonnes pressure in a hydraulic press to form a transparent pellet. The pellet was scanned from 4000-400 cm^{-1} in a spectrophotometer.

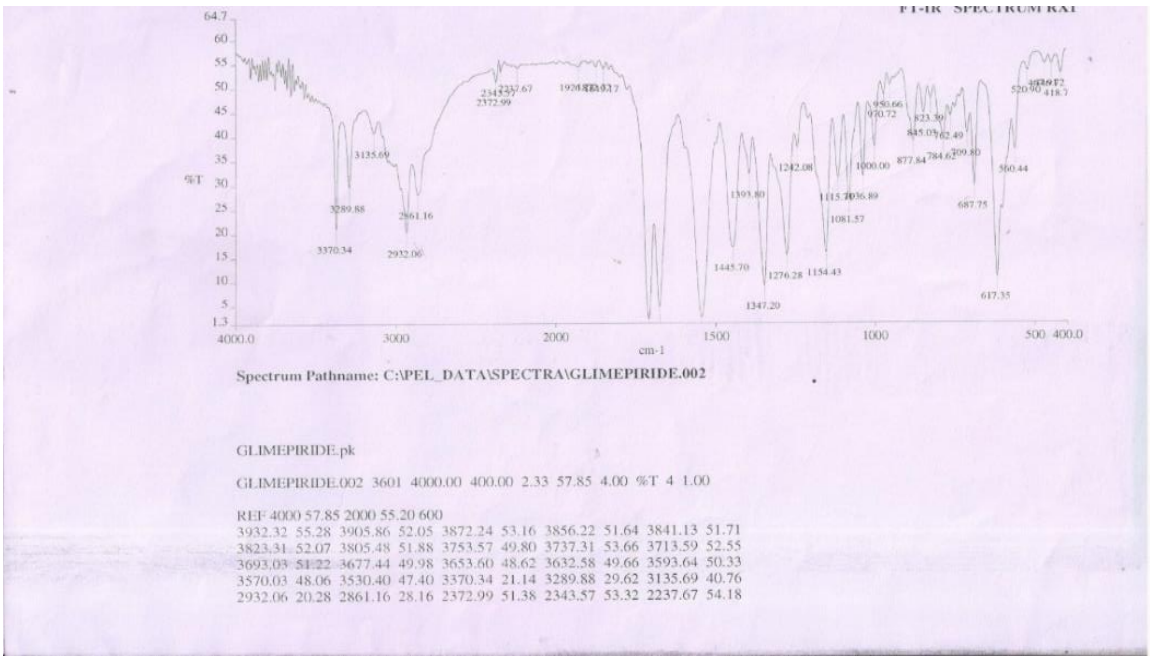

Fig. 6: FTIR spectra for pure drug glimepiride.

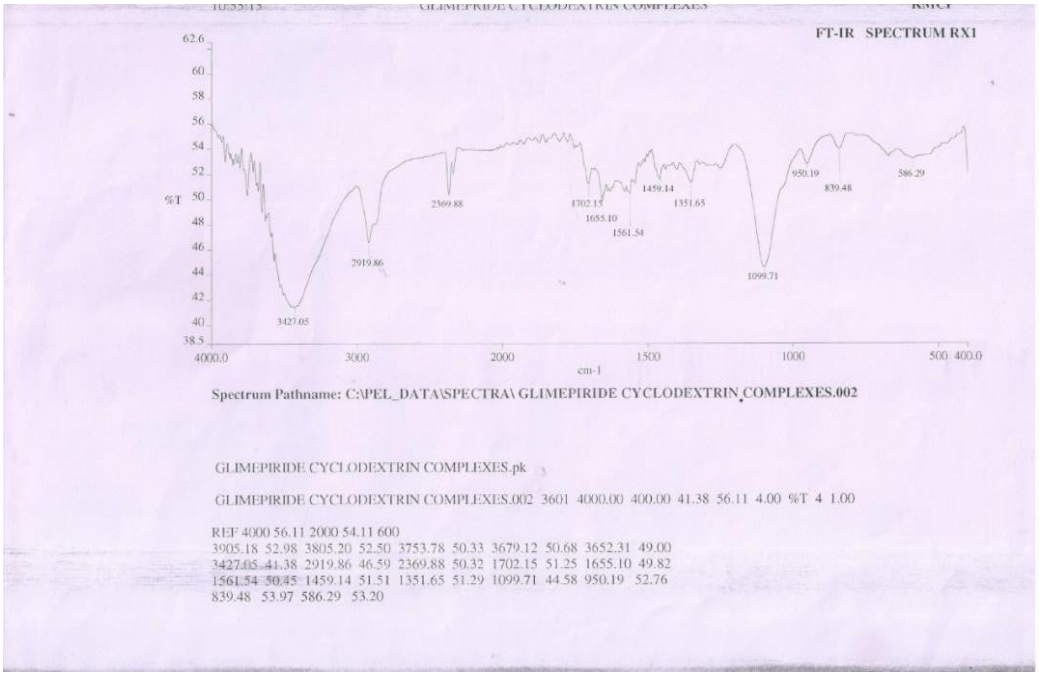

Fig. 7: FTIR spectra for β- cyclodextrin complexes.

Table 3: IR spectra data for pure drug glimepiride.

Frequency cm^{-1}	Groups assigned
3370	N-H stretching
3135	Aromatic C-H stretching
2932	C-H stretching
1700	C=O stretching
1115	C-N stretching

Table 4: IR spectra data for β- cyclodextrin complexes.

Frequency cm-1	Groups assigned
2919	Aliphatic C-H stretching
1702	C=O stretching
1099	C-N stretching

In FTIR spectra the peaks of physical mixture was compared with the original spectra. Same peaks were observed, there is no possible molecular interactions between the drug and the polymer.

ENTRAPMENT EFFICIENCY OF NANOPARTICLES

The entrapment efficiency of glimepiride loaded nanoparticles was prepared by kneading method. The formulation CD1 (GMP 4mg, β- CD 4mg, PEG 20mg, tween 80 2mg) showed entrapment efficiency value of 49%. This is due to the repulsive force between drug and the polymer.

Increase the cyclodextrin concentration in formulation CD2, (GMP 4mg, β- CD 8mg, PEG 20mg, tween 80 2mg) was used. The entrapment value was 56%. The cyclodextrin concentration was increased to 12, 16 and 20mg in the formulation CD3, CD4, CD5 and showed the entrapment efficiency value of 60%, 61% and 68%.

There was a steady increased in the entrapment efficiency from 72%, 73%, 83%, 85% in the formulation CD6, CD7, CD8 and CD9.

The concentration of β- CD was further increased to 40mg in CD10 formulation. PEG and tween 80 were kept constant in all the formulations. The E.E value showed 94%. This is due to the high repulsive force between drug and the polymer.

Table 5: Entrapment efficiency formulations with drug and polymer.

S. No	Formulation Code	Drug(mg)	β-CD(mg)	PEG(mg)	Tween 80 (mg)	E.E (%)
1	CD1	4	4	20	2	49±0.14
2	CD2	4	8	20	2	56±0.11
3	CD3	4	12	20	2	60±0.08
4	CD4	4	16	20	2	61±0.12
5	CD5	4	20	20	2	68±0.09
6	CD6	4	24	20	2	72±0.17
7	CD7	4	28	20	2	73±0.12
8	CD8	4	32	20	2	83±0.17
9	CD9	4	36	20	2	85±0.08
10	CD10	4	40	20	2	94±0.05

INVITRO DRUG RELEASE PROFILE ON NANOPARTICLES:

- The in vitro drug release of glimepiride nanoparticles was carried out by membrane diffusion method. It was carried out for 24hours.
- In all the formulations, pure drug glimepiride 4mg was taken. PEG 20mg and tween 80 2mg were taken constant in all the ten formulations. The concentration of β- cyclodextrin was gradually increased in each formulation to achieve the maximum release rate.
- In formulation CD1, β-CD 4mg was used to control the release of Glimepiride. The release at the end oh 24 hours was not found to be 63.1% only. It is not acceptable criteria of USP limit.
- Hence, in formulation CD2, β-CD concentration was increased to 3mg. The release rate at the end of 24 hours was found to be 65.6 %.

- To increase the release rate further, β-CD concentration was increased to12, 16,20mg in the formulations CD3, CD4, CD5 respectively. The release rate was increased to 78.2%, 80.7% and 83.0% respectively.
- Then in formulations CD6, CD7, CD8 and CD9. The concentration of β – cyclodextrin was further more increased to 24, 28, 32 and 36mg. Then the development in the release rate was observed with consequent increase in the release rate as 85.8%, 88.3%, 90.8% and 93.4%
- For further increase in the release rate, the concentration of β-CD was increased to 40mg in the formulation CD10.The release rate was found to be maximum. The release rate was 95%.
- Hence CD10 in the optimized formulation among the rest and formulation CD10 was chosen for further studies.

Table 6: In vitro drug release for CD1.

Time (hrs)	Amount of drug release (mg)	Cumulative amount of drug release	Cumulative % drug release
1	0.1	02.50	02.50
2	0.1	02.50	02.50
3	0.3	07.51	07.55
4	0.4	10.07	10.07
5	0.6	15.04	15.12
6	0.7	17.50	17.65
7	0.9	22.51	22.70
8	1.0	25.03	25.22
9	1.0	25.03	25.22
10	1.2	30.00	30.27
11	1.3	32.58	32.80
12	1.3	32.58	32.80
13	1.5	37.51	37.85
14	1.7	42.56	42.90
15	1.8	45.00	45.42
16	1.9	47.56	47.95
17	2.0	50.07	50.47
18	2.1	52.51	53.00
19	2.1	52.51	53.00
20	2.2	55.09	55.52
21	2.3	57.55	58.05
22	2.4	60.01	60.57
23	2.4	60.60	60.57
24	2.5	62.59	63.10

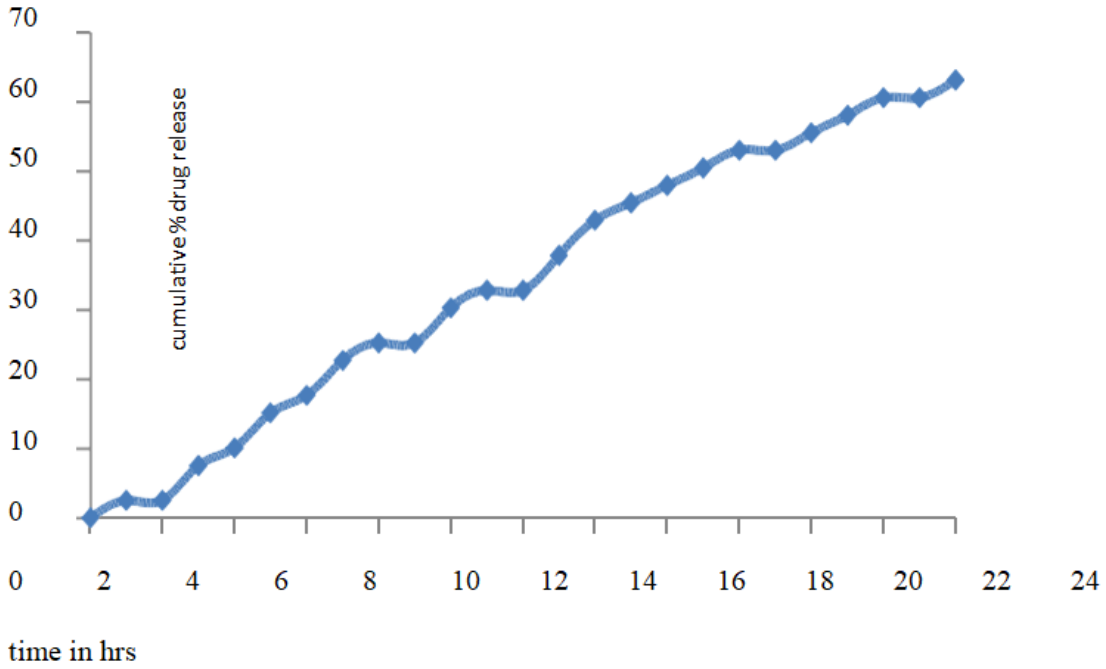

Fig. 8: In vitro drug release for formulation CD1.

Table 7: In vitro drug release for CD2:

Time (hrs)	Amount of drug release (mg)	Cumulative amount of drug release	Cumulative % drug release
1	0.1	02.50	02.50
2	0.3	07.55	07.55
3	0.4	10.05	10.07
4	0.5	12.55	12.68
5	0.6	15.05	15.12
6	0.8	20.51	20.17
7	0.9	22.59	22.78
8	1.0	25.08	25.22
9	1.0	25.08	25.22
10	1.2	30.81	30.27
11	1.3	32.55	32.81
12	1.5	37.51	37.85
13	1.6	40.05	40.37
14	1.7	42.59	42.98
15	1.9	47.55	47.95
16	2.0	50.52	50.47
17	2.0	50.52	50.47
18	2.1	52.58	53.29
19	2.2	55.04	55.52
20	2.3	57.54	58.05
21	2.3	57.54	58.05
22	2.4	60.81	60.57
23	2.5	62.51	63.18
24	2.6	65.18	65.62

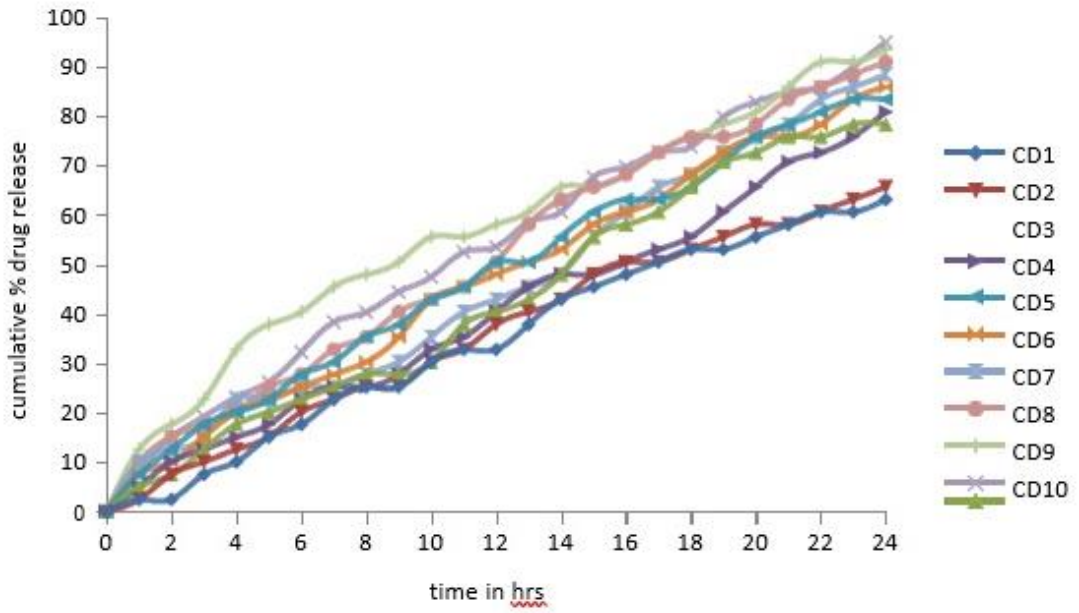

Fig. 9: Summarized in vitro drug release of nanoparticles formulation (CD1-CD10).

MORPHOLOGY OF NANOPARTICLES

The characteristics of CD10 formulation particle size was studied by simple microscopy. Small amount of the sample was placed in glass slide and viewed through simple microscope. Image of prepared nanoparticle shows the encapsulation of polymer mixture on drug particles.

SCANNING ELECTRON MICROSCOPE

The surface characteristics of optimized formulation (CD10) particle size were studied by SEM. The SEM image of prepared nanoparticle formulation shows the coating of polymer mixture on drug particles. The size distribution of nanoparticles indicates a thin and uniform coating over the drug.

SURFACE CHARGE (ZETA POTENTIAL)

Zeta potential of glimepiride cyclodextrin complexes is commonly used to characterise the surface property of nanoparticle. It reflects the electrical potential of the nanoparticle and is influenced by the composition of the particle and the medium in which it is dispersed.

The zeta potential of the formulation showed zeta potential of -4.65mV which confirmed that the particle of the formulation remains stable.

Zeta potential result shows good and the value is increased due to the fact that the surface free energy of β- cyclodextrin is increased. The size distribution report by intensity showed the average size of 170.3 nm.

STABILITY TESTING OF GLIMEPIRIDE NANOPARTICLES

The stability studies of the optimized formulation F10 was carried out for 3 months at 4^0 C, room temperature and 45^0 C/70% RH. At the time interval of 1 month the nanoparticle formulation was evaluated for entrapment efficiency. The stability of the formulation was stable at 4^0C when compared to room temperature and at 45^0C/70%RH.

Table 8: Stability studies of nanoparticles.

S. No	Storage condition	Test parameters	1st month	2nd month	3rd month
1	4^0 C	pH Colour *Invitro* % release at 24th hr	7.4 Clear and colourless 95.11	7.4 Clear and colourless 95.18	7.4 Clear and colourless 95.20
2	Room temperature	pH Colour *Invitro* % release at 24th hr	7.4 Clear and colourless 95	7.4 Clear and colourless 90.97	7.4 Clear and colourless 90.16
3	Acceleration condition at 45^0C/70% RH	pH Colour *Invitro* % release at 24th hr	7.3 Clear and colourless 95	7.3 Clear and colourless 81.66	7.3 Clear and colourless 80.65

Table 9: In vitro release for optimized formulation CD10 stability study at 4^0 C:

Time (Hrs)	Cumulative % drug release		
	1st month (%)	2nd month (%)	3rd month (%)
1	12.65	12.4	11.01
2	15.12	15.10	14.96
3	20.17	19.75	19.54
4	22.78	22.17	21.09
5	25.22	25.10	24.85
6	30.27	29.95	29.56
7	35.32	35.01	34.12
8	37.85	36.15	35.97
9	42.55	42.09	41.68
10	47.57	46.54	46.09
11	52.54	51.23	51.02
12	55.49	54.86	54.58
13	62.58	62.44	61.96
14	65.18	64.96	64.34
15	70.08	69.94	69.54
16	70.08	69.64	69.54
17	75.18	74.75	73.65
18	80.18	79.92	78.25
19	80.18	79.92	79.43
20	82.54	81.25	80.24
21	87.58	87.18	86.18
22	90.19	89.81	89.51

23	92.51	91.18	91.10
24	95.11	95.18	95.20

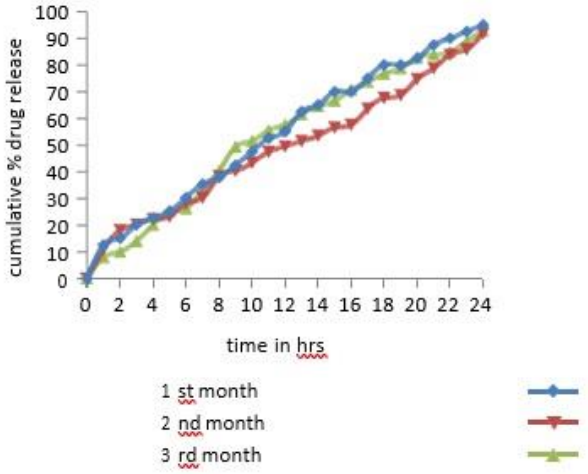

Fig. 10: Stability study release data for formulation CD10 after 3 months at 4^0C.

KINETICS OF DRUG RELEASE FOR OPTIMIZED FORMULATION CD10:

The optimized formulation CD10 was introduced into graphical treatment for kinetics of drug release.

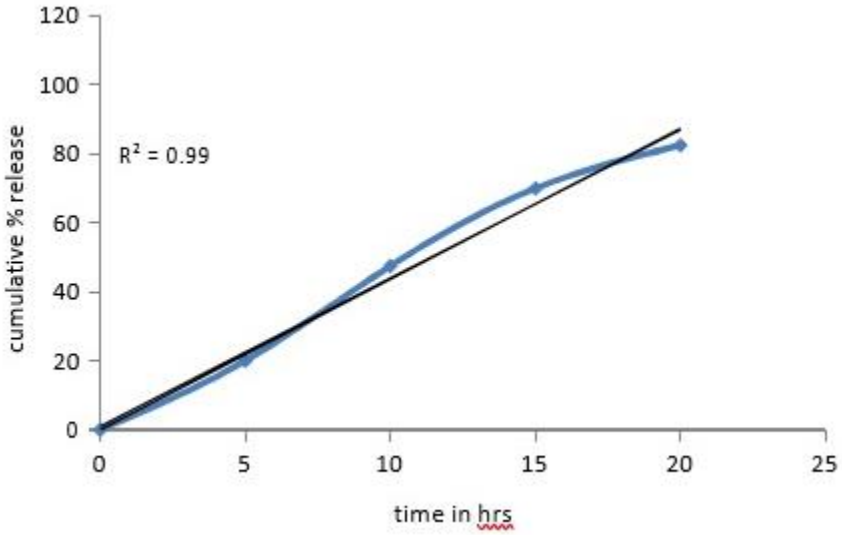

Figure 11.

The optimized formulation CD of nanoparticles is more suitable for parentral administration it shows good in the in vitro kinetic study. The zero order plots wee obtained by plotting cumulative percentage drug release versus time. The regression value is 0.987.

FIRST ORDER KINETICS OF DRUG RELEASE

The first order plot was made by plotting log time remaining cumulative % drug release against time. The regression value was found to be which indicates that drug release does not follow first order rate kinetics.

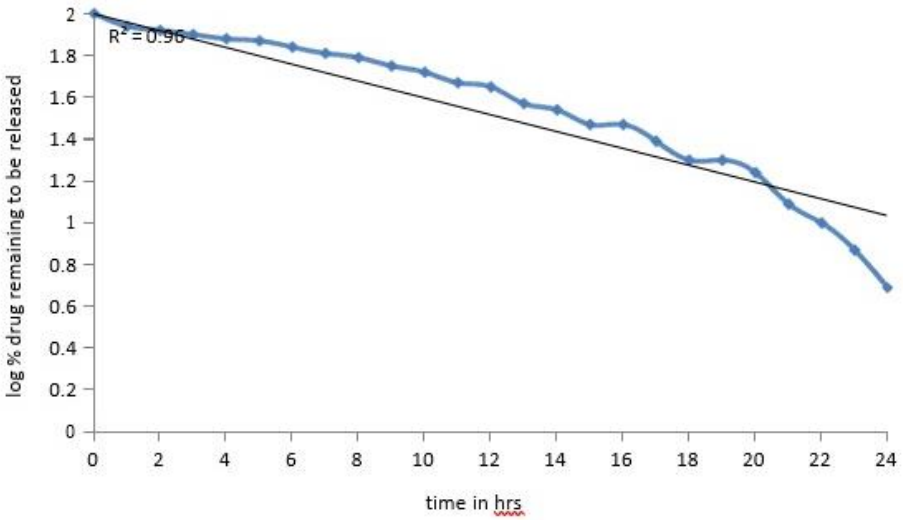

Figure 12.

HIGUCH'S PLOT

Higuchi plot was made by plotting cumulative % drug release against square root of time. The regression value was found to be 0.951. This indicates that diffusion is one of the mechanisms of drug release.

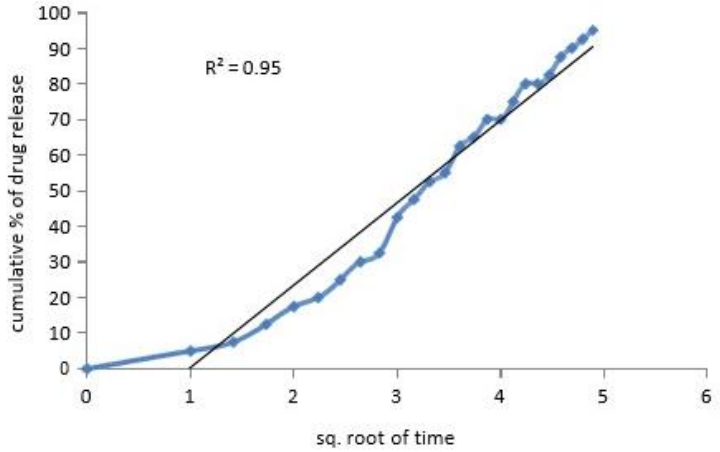

Figure 13.

KORSEMEYER PLOT:

The graph was plotted between log cumulative % of drug release and log time. [the value was found to be 0.992 anomalous (non – Fickian) diffusion.

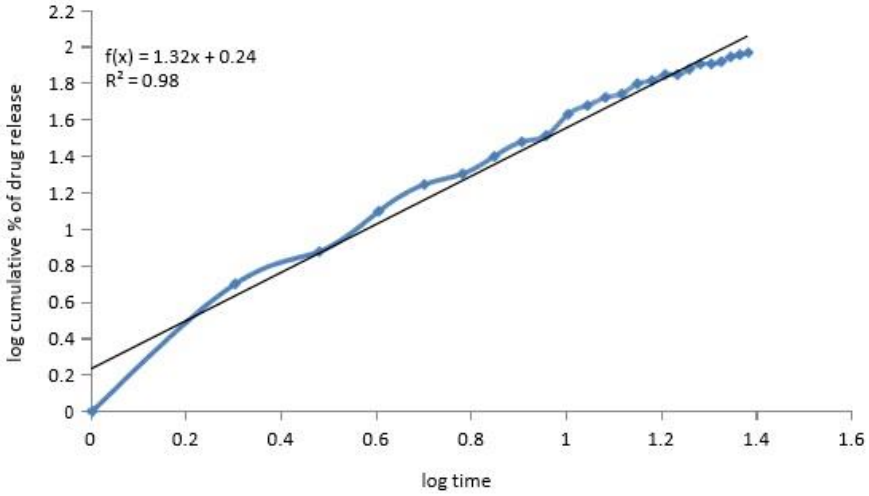

Figure 14.

SUMMARY AND CONCLUSION

The present study glimepiride nanoparticles aimed to develop a nanoparticulate drug delivery system using cyclodextrin inclusion complexes.

The polymer enhances the binding of glimepiride nanoparticles in specific or targeted site with sustained release of drug which increases the therapeutic efficacy. These nanoparticles may also reduce the dose and dose frequency with desired therapeutic response.

The preformulation studies were performed using FTIR. The spectra pure drug and the formulation were examined. The study revealed the absence of interaction between drug and the polymer.

All the batches of nanoparticles (CD1-CD10) were prepared by kneading method.

Formulation was subjected to following evaluation tests which involves;

- Entrapment efficiency
- In vitro drug release studies
- Microscopic determination
- Particle size determination

The entrapment efficiency of the optimized formulation was 94±0.05% and the in vitro drug release was 94.89% after 24 hrs. It obeys zero order, follows diffusion and erosion mechanism of release. Particle size determination by SEM shows the best formulation containing size of 170 nm. Therefore it can target the tissues and has a quick onset of action.

The optimized formulation was examined for zeta potential determination. The formulation CD10 showed maximum deviation of -4.65 mV which demonstrates that the particles are separate and highly repelling. The repelling property was more useful in decreasing opsonisation. Further studies are to be carried out to reduce the side effects.

Therefore, formulated glimepiride nanoparticles can be expected to gain considerable attention in the treatment of type 2 diabetes mellitus due to its improved therapeutic activity.

ACKNOWLEDGEMENTS

The authors are thankful to the Administration of Mother Teresa Pharmacy College, Kothuru, Sathupally-507303, and Telangana, India, for providing the essential research facilities.

BIBLIOGRAPHY

1. Rainer H. Muller, Karsten Mader and Sven Gohla, Solid lipid nanoparticles (SLN) for controlled drug delivery, review of the state of art, European Journal of Pharmaceutics and Biopharmaceutics, 2000; 50(1): 161-177.
2. Targeted delivery of drugs, Controlled and novel drug delivery by N.K. Jain, 1: 93.
3. Targeted delivery of drugs, Advances in controlled and Novel drug delivery by N.K Jain, 1: 452.
4. J. Adlin Jino Nesalin, A. Anton Smith, Nanoparticles an invisible drug delivery system, Review article, Journal of Pharmacy Research, 2011; 4: 373-377.
5. Polymers in controlled release, controlled drug delivery by Joseph R. Robinson, 2: 179.
6. Oral controlled release drug delivery system, controlled drug delivery by Joseph R. Robinson, 2: 385.
7. Hormones and hormone antagonists, the pharmacological basis of therapeutics, 11: 1613.
8. Pancreatic hormones, anti diabetic drugs, Pharmacology and pharmacotherapeutics, R.S. Satoskar, 2013; 23: 906.
9. Insulin, oral hypoglycaemic drugs and glucagon, essentials of medical pharmacology, K.D. Tripathi, 258.
10. The endocrine pancreas and the control of blood glucose, Rang and Dale's pharmacology, 6: 402.
11. Pedro Tartaj, Marıadel Puerto Morales, Sabino Veintemillas-Verdaguer, Teresita Gonzalez-Carreno and Carlos J Serna, The preparation of magnetic nanoparticles for applications in biomedicine, journal of physics and applied physics., 2003; 36(3): 182-197.

12. Mansoor Amiji and Sushma Kommareddy, Preparation and Evaluation of Thiol-Modified Gelatin Nanoparticles for Intracellular DNA Delivery in Response to Glutathione, Bioconjugate Chem., 2005; 16(6): 1423-1432.
13. Kunihiko Koshiba, Masahiro Nomura, Yutaka Nakaya and Susumu Ito, Efficacy of glimepiride on insulinresistance, adipocytokines, and atherosclerosis, The Journal of Medical Investigation, 2006; 53(1): 87-94.
14. Catarina Pinto Reis, Ronald J. Neufeld, Antonio J. Ribeiro, Francisco Veiga, nanoencapsulation methods for preparation of drug-loaded polymeric nanoparticles, nanomedicine: nanotechnology, biology and medicine, 2006; 2(1): 8-21.
15. H.O.Ammar, H.A.Salama, M. Ghorab, A.A.Mahmoud, Inclusion complexation of glimepiride in dimethyl-β-cyclodextrin, Asian Journal of Pharmaceutical Sciences, 2007; 2(2): 44-55.
16. Manivannan Rangasamy, Ayyasamy Balasubramaniam and Sandeep Gummadevelly, Design and Evaluation of β-cyclodextrin Complexes of Meloxicam Tablet, Research J. Pharm. and Tech., 2008; 1(4): 484-486.
17. V. G. Kuchake, R. D. Shimpi, P. H. Patil, P.V.Ingle, S. J. Surana, P. N. Dighore , Comparison of effect of metformin in combination with glimepiride and glibenclamide on glycaemic control in patient with type 2 diabetes mellitus, International Journal of Pharm Tech Research, 2009; 1(1): 50-61.
18. S. Tamizhrasi, A. Shukla, T. Shivkumar, V. Rathi , J. C. Rathi,formulation and evaluation of lamivudine loaded polymethacrylic acid nanoparticles, International Journal of PharmTech Research CODEN(USA): IJPRIF, 2009; 1(3): 411-415.
19. Vipul P. Patel, Natvarlal M. Patel, Evaluation of Some Methods for Preparing Glipizide-β β- Cyclodextrin Inclusion Complexes, Iranian Journal of Pharmaceutical Sciences Autumn., 2009; 5(4): 191-198.
20. N.Arunkumar, M.Deecaraman, C.Rani, K.P.Mohanraj, K.Venkates Kumar, Preparation and solid state characterization of atrovastatin nanosuspension for enhanced solubility and dissolution, International Journal of PharmTech Research, 2009; 1(4); 1725-1730.
21. L. Zhang, D. Pornpattananangkul, C.-M.J. Hu and C.-M. Huang, Development of Nanoparticles for Antimicrobial Drug Delivery, Current Medicinal Chemistry, 2010; 17(6): 585-594.
22. Carmelia Nicolescu, Ciruna Arama, Angela Nedelcu, Crina- Maria Monciu, phase solubility studies of the inclusion complexes of repaglinide with β- cyclodextrin and β-cyclodextrin derivatives, Farmacia, 2010; 58(5); 620-628.