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IN-VITRO STUDY OF FORMULATION AND EVALUATION OF GLIMEPIRIDE NANOSUSPENSION BY PRECIPITATION METHOD

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ABSTRACT

In the present study, an attempt was made to prepare oral Nanosuspensions of glimepiride is the first III generation sulphonyl urea it is a very potent sulphonyl urea with long duration of action. Glimepiride is indicated to treat type 2 diabetes mellitus; its mode of action is to increase insulin production by the pancreas in order to overcome bioavailability problems, to reduce dose dependent side effects and frequency of administration. The drug is well absorbed from gastrointestinal tract, but its bioavailability is low due to extensive first pass metabolism Nanosuspension containing the drug was prepared by precipitation method using combinations of polymers (such as PVP-k25, Urea, Acetone, SLS, and Poloxamer F127). Estimation of glimepiride was carried out spectrophotometrically at 221 nm. The Oral Nano suspension were evaluated for various physical and biological parameters, drug content uniformity, entrapment efficiency, scanning electron microscopy, in-vitro drug release, short-term stability, and drug-excipient interactions (FTIR) indicates that all were within limits. In-vitro drug release studies of all the formulations were studied of that formulation F1 shows 99.74% of drug release within 30 min and follows first order release kinetics. IR spectroscopic studies indicated that there are no drug-excipient interactions.

KEYWORDS: Glimepiride, Oral Nanosuspensions, Urea, PVP-k25, SLS, Poloxamer F127 and Acetone.

INTRODUCTION

The design of oral controlled drug delivery system (oral DDS) should be primarily aimed to achieve more predictable and increased bioavailability. Now-a-days most of the pharmaceutical scientists are involved in developing the ideal DDS. This ideal system should

have advantage of single dose for the whole duration of treatment and it should deliver the active drug directly at the specific site. Controlled release implies the predictability and reproducibility to control the drug release, drug concentration in target tissue and optimization of the therapeutic effect of a drug by controlling its release in the body with lower and less frequent dose.[1] However, this approach is be filled with several physiological difficulties such as inability to restrain and locate the controlled drug delivery system within the desired region of the Gastrointestinal tract(GIT) due to variable gastric emptying and motility. Furthermore, the relatively brief GIT in humans which normally average 2-3 hrs through the major absorption zone, i.e., stomach and upper part of the intestine can result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose. Therefore, control of placement of a DDS in a specific region of the GI tract offers advantages for a variety of important drugs characterized by a narrow absorption window in the GIT or drugs with a stability problem.[2]

Many of the new chemical entities (approximately 40% or more) being developed through drug discovery programmers are poorly water soluble. The formulation of poorly water soluble drugs has been always a challenging problem faced by pharmaceutical scientists.[3] The low saturated solubility and dissolution velocity leads to poor bioavailability. The problem is more severe for drugs belonging to Biopharmaceutics Classification System (BCS) class II, such as Itraconazole and carbamazepine as they are poorly soluble in both aqueous and organic media.[4] The performance of these drugs is dissolution-rate-limited and is affected by fed/fasted state of the patient. Dissolution rates of sparingly soluble drugs are associated to the shape as well as the particle size. Hence decrease in particle size results in increase in dissolution rate. There are number of formulation approaches that can be used to solve the problems associated with the low solubility and low bioavailability of class II drugs. Some of the approaches to increase solubility include micronization, solubilisation using cosolvents, use of permeation enhancers, surfactant dispersions, salt formation and precipitation techniques.[5],[6] Most of these techniques for solubility enhancement have advantages as well as some limitations and hence have limited utility in solubility enhancement. Other techniques used for solubility enhancement like microspheres, emulsions, micro emulsions, Liposome's, supercritical processing, solid dispersions and inclusion complexes using Cyclodextrins.[7],[8] show reasonable success but they lack in universal applicability to all drugs, which are not soluble in both aqueous and organic media.

Nanosuspensions have revealed their potential to undertake the problems associated with delivery of poorly water-soluble and lipid soluble drugs and are unique because of their simplicity and the advantages they confer over other strategies.

Nanosuspensions have revealed their potential to solve the problem associated with the delivery of poorly water soluble and lipid soluble drugs. It enhances the absorption and bioavailability and help to reduce the dose of conventional oral dosage forms.[9]

For a long duration of time micronization of poorly soluble drugs by colloid mills or jet mills was preferred. The overall particle size distribution ranges from 0.1µm to approximately 25µm, only negligible amount being below 1µm in the nanometer range.

When to go for Nano Suspensions Approach

- Preparing nano suspensions is preferred for the compounds that are insoluble in water (but are soluble in oil) with high log P value.
- Conventionally the drugs that are insoluble in water but soluble in oil phase system are formulated in liposome, emulsion systems but these lipidic formulation approaches are not applicable to all drugs.
- In these cases nano suspensions are preferred. In case of drugs that are insoluble in both water and in organic media instead of using lipidic systems Nanosuspensions are used as a formulation approach.
- Nanosuspension formulation approach is most suitable for the compounds with high log P value, high melting point and high dose.[10], [11]

Depending upon the production technique applied changes in the crystalline structure of the drug particle may occur. An increase amount of amorphous drug fraction could induce higher saturation solubility. Nanosuspension not only solves the problem of poor solubility and poor bioavailability but also alters the pharmacokinetics of the drug and improves the drug safety and efficacy.

Precipitation technique (solvent-antisolvent method): Precipitation method has been used for long years for the preparation of submicron particles. It is mainly used for the poorly soluble drugs. First drug is dissolved in a suitable solvent. This solution is then mixed with a miscible antisolvent system in the presence of surfactants. Rapid addition of drug solution in to the antisolvent leads to the sudden super saturation of drug in the mixed solution forms

ultrafine drug solids. Precipitation method involves two phases - nuclei formation & crystal growth. When preparing a stable suspension with the minimum particle size, a high nucleation rate and but low growth rate is necessary. Both rates are depending on temperature. In this technique the drug needs to be soluble in at least one solvent which is miscible with nonsolvent.[12]

CHARACTERIZATION OF NANOSUSPENSIONS

Nanosuspensions are evaluated as same as conventional suspensions such as appearance, colour, odour, assay, related impurities etc. Along with that particle size, zeta potential, morphology, dissolution study, in-vivo studies are also performed.[13]

The particle size, particle size distribution, and zeta potential affect the safety, efficacy, and stability of nanodrug delivery systems as well as dissolution performance is also altered by solid state of nanoparticles. Thus, characterization of nanoparticles plays a great role in forecasting in vitro and in vivo performance of nanodrug delivery systems. In vivo pharmacokinetic performance and biological function of Nanosuspensions strongly depends on its particle size and distribution, particle charge (zeta potential), crystalline state, and particle morphology.

Glimepiride is the only third generation sulphonyl urea, which lowers the blood glucose level in the healthy subjects as well as in patients with type II diabetes. Its biological half life is 2-3hrs. Due to its low biological half life, it requires frequent administration in order to maintain plasma concentration. This causes inconvenience to the patient and also leads fluctuations in plasma concentration. Glimepiride is used along with diet and exercise, and sometimes with other medications, to treat type 2 diabetes (condition in which the body does not use insulin normally and, therefore, cannot control the amount of sugar in the blood).

Like all sulfonylureas, glimepiride acts as an insulin secretagogue. It lowers blood sugar by stimulating the release of insulin by pancreatic beta cells and by inducing increased activity of intracellular insulin receptors. Glimepiride exhibit low PH dependent solubility. These poorly soluble drugs provide challenge to deliver them in active and absorbable form.[14]

The aim of the present work is to develop oral Nanosuspension of glimepiride by precipitation method and to evaluate it and

- To perform Drug excipient compatibility studies, carry out.

- To formulate and develop the Nanosuspensions and formulations.
- To carry out scanning electron microscopy for optimized formulation.
- To determine the drug entrapment efficiency of all the formulations.
- To evaluate the formulation by establishing drug release kinetics using various dissolution models.
- To establish *In–vitro* drug release compliance with the established criteria.
- To establish stability studies of the final formulation, fo8r the selected oral Nano suspension.

MATERIALS AND METHODS

MATERIAL

Glimepiride was purchased from Aurobindo Pharma(Hyderabad, India). Polyvinylpyrrolidone K-25(PVPK25), SLS(sodium lauryl sulphate),Urea, polaxomer, HCL (Hydrochloric acid) were obtained from Narmada chemicals. Acetone was supplied by SD fine chem.

Table 1: Equipment Used.

Equipment	Source
DISSOLUTION TEST APPARATUS	Electro lab, TDT-06N
UV- VISIBLE SPECTROPHOTOMETER	UV-1700, Shimadzu
MAGNETIC STIRRER	REMKI STIRRER
DIGITAL BALANCE	Shimadzu, (BL-220H)
MALVERN ZETASIZER	MALVERN
IR SPECTROSCOPY	1615 series

METHODS

Pre-formulation studies

Prior to the development of nanosuspension form, it is essential that certain fundamental physical and chemical properties of the drug molecule alone and when combined with excipients are determined. This first learning phase is known as pre-formulation. The overall objective of the pre-formulation is to generate information useful to the formulator in developing stable and bioavailable dosage forms which can be mass produced. The goals of pre-formulation studies are to evaluate the drug substance analytically and determine its necessary characteristics, and to establish its compatibility with different excipients.

Spectroscopic study

Identification of pure drug

Melting Point

The temperature at which the first particle of the substance completely melts is regarded as melting point of the substance. The temperature at which the first particle starts to melt and last particle completely melts is regarded as the range of melting point. Melting point of the drug was determined by capillary tube method and found to be 212-215^{o}C.

Solubility studies: Solubility of Glimepiride was carried out in different buffers. Saturated solutions were prepared by adding excess drug to the vehicles and shaking on the shaker for 24 hrs at 25°C under constant vibration. Filtered samples (1ml) were diluted appropriately with suitable buffer and solubility of Glimepiride was determined spectrophotometrically at 221nm.

Physicochemical parameters: The colour, odour and taste of the drug were recorded using descriptive terminology and found to be white to off-white crystalline powder, tasteless and odourless.

Determination of absorption maximum (λ_{max})

Accurately weighed 100mg of Glimepiride was dissolved in 0.1N HCL buffer taken in a clean 100ml volumetric flask. The volume was made up to 100ml with the same which will give stock solution-I with concentration 1000µg/ml. From the stock solution-I, 1ml was pipette out in 10ml volumetric flask. The volume was made up to 10ml using 0.1N HCL buffer to obtain stock solution-II with a concentration 100µg/ml. From stock solution-II, 1ml was pipette out in 10ml volumetric flask. The volume was made up to 10ml using 0.1N HCL buffer to get a concentration of 10µg/ml. This solution was then scanned at 200-400nm in UV-Visible double beam spectrophotometer to attain the absorption maximum (λ-max).

PREPARATION OF CALIBRATION CURVE OF GLIMEPIRIDE

Procedure for standard curve in 0.1N HCL

10mg of Glimepiride was dissolved in 10ml of 0.1N HCL by slight shaking (1000 mcg/ml). 1ml of this solution was taken and made up to 10ml with 0.1N HCL, which gives 100mcg/ ml concentration (stock solution). From the stock solution, concentrations of 5, 10, 15, 20, 25 and 30 µg/ml in 0.1N HCL were prepared. The absorbances of diluted solutions were measured at 221 nm and a standard plot was drawn using the data obtained. The correlation

coefficient was calculated, the absorbance data of the above concentrations are shown in Table.

Method of Preparation of Nanosuspension

Precipitation method

Preparation: Nanosuspension precipitation method has been employed to prepare oral Nanosuspension of Glimepiride using PVP-K25, UREA, SLS and Polaxomer F127 as polymers.

Procedure: Nanosuspension of glimepiride was prepared by precipitation method with various carriers and drug. At first the weighed amount of glimepiride was taken and dispersed into the beaker containing acetone which acts as organic solvent. This drug and acetone solution is termed as organic phase. Now the carriers Urea, PVP was dissolved in water and add surfactant (SLS) to the stabilizer solution and labelled as aqueous phase. This solution was kept on magnetic stirrer for uniform mixing. The organic phase was slowly added drop wise to aqueous phase and continues the stirring on magnetic stirrer at an rpm of about 800 until complete evaporation of solvent. After 1 hour, the solution was kept in sonicator for about 30mins. Finally the nanosuspension was formed.

Table 2: Composition of Nanosuspensions of Glimepiride precipitation method.

Ingredients	F1	F2	F3	F4	F5	F6
GLIMEPIRIDE	10	10	10	10	10	10
PVP-K25	40	-	-	20	20	-
UREA	-	40	-	20	-	20
SLS	-	-	40	-	20	20
POLAXOMER F127	10	10	10	10	10	10
ACETONE	5	5	5	5	5	5
WATER(ml)	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Evaluation parameters of Nanosuspension Glimepiride

The nanosuspension was evaluated for various parameters

1. Content uniformity
2. Entrapment efficiency
3. pH
4. Scanning electron microscope (Sem analysis)
5. Particle size analysis
6. Zeta potential

7. *In-vitro* drug release studies

Drug content uniformity

10ml of each formulation was taken and dissolved in 10ml isotonic solution and kept overnight. 10 mg (similar as in formulation) of drug was taken and dilution was made to 10µg/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 221 nm. The drug content in each formulation was calculated based on the absorbance values of known standard solutions.

Entrapment efficacy

Entrapment efficacy was calculated by following formula.

%Entrapment efficiency= Drug content *100/Drug added in each formulation

pH measurement

The pH values were measured at 25 ◦C using a pH digital meter at 20 ± 1 ◦C. The formulation was brought in contact with the electrode of pH meter and equilibrated for 1 min. This method was done in triplicate and mean was calculated along with standard deviation.

Scanning electron microscopy: Determination of surface morphology of glimepiride nanosuspension was carried out by scanning electron microscopy (sem).

Zeta potential

The particle charge is of importance in the study of the stability of the suspensions. Usually the zeta potential of more than ±40mV will be considered to be required for the stabilization of the dispersions. For electrostatically stabilized nanosuspension a minimum zeta potential of ±30mV is required and in case of combined steric and electrostatic stabilization it should be a minimum of ± 20mV of zeta potential is required. Surface charges can arise from (i) ionization of the particle surface or (ii) adsorption of ions (such as surfactants) onto the surface. Typically, the surface charge is assessed through measurements of the zeta potential. Zeta potential is the potential at the hydrodynamic shear plane and can be determined from the particle mobility under an applied electric field. The mobility will depend on the effective charge on the surface. Zeta potential is also a function of electrolyte concentration.

***In-vitro* drug release study:** This is carried out in USP dissolution test apparatus-II (Electrolab TDT-06N), employing paddle stirrer at 50 rpm and 900 ml of pH 0.1N HCL buffer as dissolution medium. The release study is performed at 37 ±0.5^{0}C. The disk is placed at the bottom of the dissolution vessel. Samples of 5 ml are withdrawn at predetermined time intervals and replaced with fresh medium. The samples were filtered through 0.22 µm membrane filter disc (Millipore Corporation) and analyzed for Glimepiride after appropriate dilution by measuring the absorbance at 221 nm.

The results of *in vitro* release profiles obtained for the formulations were fitted into four models of data treatment as follows.

1. Cumulative percent drug released versus time (zero order kinetic model).
2. Log cumulative percent drug remaining versus time (first- order kinetic model).

Table 3: Drug release kinetics.

Kinetic Model	Relation	Systems Following the Model
First order	$\ln Q_t = \ln Q_o + K_t$ release is proportional to amount of drug remaining	Water-soluble drugs in porous matrix
Zero order	$f_t = K_o t$ (independent of drug concentration)	Transdermal systems Osmotic systems

Where, f_t = fraction of dose released at time 't'

K_H, K_o, and K_s = release rate constants characteristic to respective models

Q_o = the drug amount remaining to be released at zero hour

Q_t = the drug amount remaining to be released at time 't'

W_o = initial amount of drug present in the matrix

W_t = amount of drug released at time 't'

Mechanism of Drug Release To find out the drug release mechanism due to swelling (upon hydration) along with gradual erosion of the matrix, first 60% drug release data can be fitted in Korsmeyer–Peppas model which is often used to describe the drug release behaviour from polymeric systems when the mechanism is not well-known or when more than one type of release phenomena is involved.

$$\text{Log } (M_t / M_\infty) = \text{Log } K_{KP} + n \text{ Log } t$$

Where,

M_t = is the amount of drug release at time t,

M_∞ = is the amount of drug release after infinite time,

$\mathbf{K_{KP}}$ = is a release rate constant incorporating structural and geometrical characteristics of

n = is the release exponent indicative of the mechanism of drug release.

Drug-Excipient Interactions Studies: There is always possibility of drug- excipient interaction in any formulation due to their intimate contact. The technique employed in this study is IR spectroscopy. IR spectroscopy is one of the most powerful analytical technique, which offers possibility of chemical identification. The IR spectra of Glimepiride, PVP K-25, SLS, UREA and Polaxomer (127), formulations (F_1 and F_6) were obtained by KBr pellet method (Perkin-Elmer series 1615 FTIR Spectrometer).

RESULTS AND DISCUSSION

PREFORMULATION STUDIES

Determination of melting point

The melting point of Glimepiride was found to be in range of 212-215°C which was determined by capillary method. Fine powder of glimepiride was filled in glass capillary tube (previously sealed on one end). The capillary tube is tied to thermo meter and the thermometer was placed in fire. The powder at what temperature it will melt was noticed.

Saturation Solubility

Saturation solubility was carried out at 25^0C using 0.1N HCL, 6.8 phosphate buffer, and purified water.

Table 4: Solubility data.

Media	Solubility(mg/ml)
0.1N HCL	3.92
pH 6.8 phosphate buffer	1.11
Purified water	0.40

Discussion: From the above conducted solubility studies in various buffers we can say that 0.1N HCL has more solubility when compared to other buffer solutions.

ESTIMATION OF GLIMEPIRIDE BY UV SPECTROSCOPY

Determination of lambda max: A simple Spectrophotometric method for estimation of Glimepiride was developed in 0.1N HCL, which exhibited λ_{max} at 221 nm in Beer's range of 5-30 µg/ml as shown in figure.

Construction of calibration curve

Preparation of 0.1N HCL: Weigh accurately 8.5ml of HCL in 1000ml volumetric flask and then dissolve & diluted with purified water and then make up the volume upto 1000ml to make 0.1N HCL solution.

Preparation of drug solutions of Glimepiride

Primary stock solution: Primary Stock solution at concentration of 1mg/mL i.e., 1000µg/mL was prepared by dissolving accurately weighed quantity of pure drug Glimepiride (10 mg) in 10mL volumetric flask with 0.1N HCL and made up to 10 mL with same buffer solution.

Secondary stock solution: From above stock solution, 1 mL was accurately pippetted out from the primary stock solution and transferred into a 10ml standard volumetric flask then the volume was made up to 10mL using 0.1N HCL to make 100µg/mL.

Sample solution: Aliquots were prepared from the secondary stock solution by pippetting 0.5, 1, 1.5, 2, 2.5 and 3mL to get concentrations of 5, 10, 15, 20, 25 and 30µg/mL by diluting with same buffer solution. The absorbance of prepared drug solutions was measured at 221 nm using UV- spectrophotometer against an appropriate blank.

Spectrum Curve of Glimepiride

The standard calibration curve shown linearity, through that the drug obeys Beers and Lamberts law in the concentration range of 5 to 30µg/mL. A standard graph was plotted by keeping the known concentration on X – axis and obtained absorbance on Y – axis. The values of calibration curve of Glimepiride with 0.1N HCL were given below.

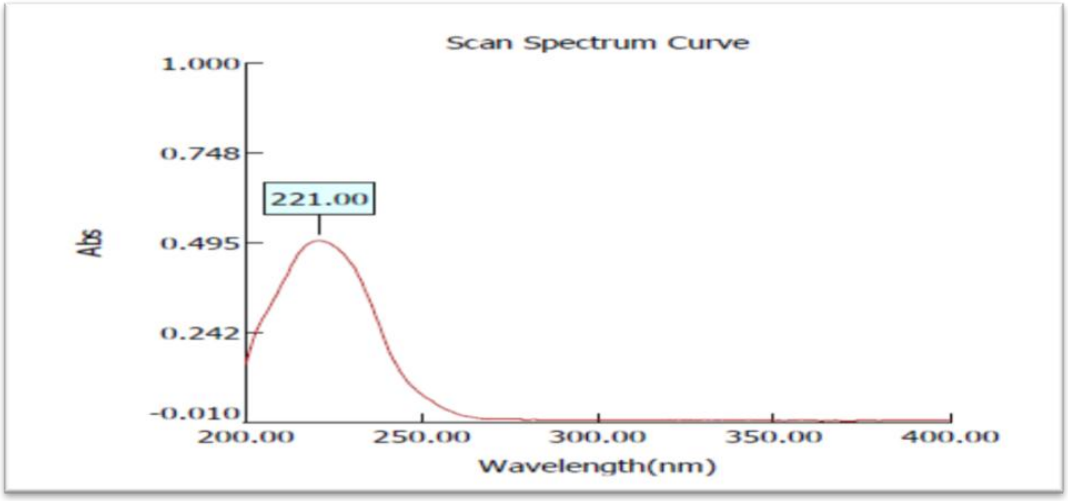


Figure 1: Spectrum Curve of Glimepride.

Discussion: The maximum absorbance of the glimepiride in 0.1N HCL was found to be 221nm. Hence the wavelength of 221nm was selected for analysis of drug in dissolution media.

Table 5: Standard graph of Glimepiride (λmax 221 nm)

Concentration	absorbance
0	**0**
5	0.140
10	0.315
15	0.452
20	0.595
25	0.746
30	0.919

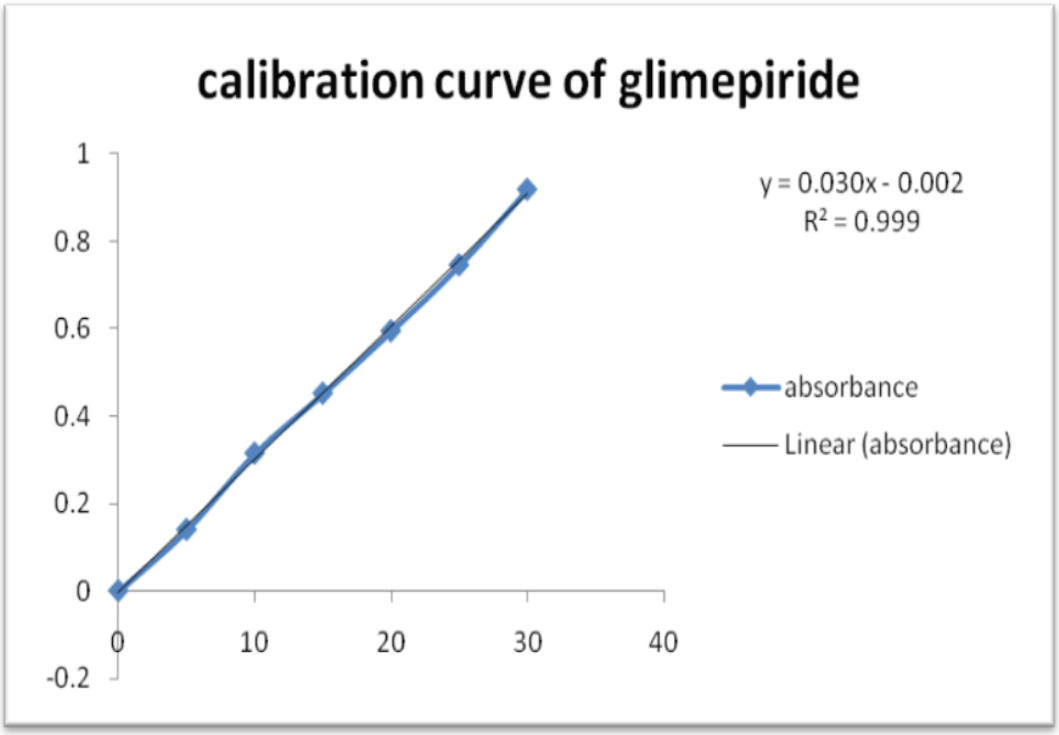


Figure 2: Standard calibration curve of Glimepiride (0.1N HCL buffer).

The linear regression analysis: The linear regression analysis was done on absorbance data points the results are as follows.

For calibration curve of glimepiride in simulated gastric fluid (0.1N HCL)

The slope = 0.030

The correlation coefficient= 0.999

A straight-line equation (Y= mX + C) was generated to facilitate the calculation of amount of drug.

Discussion: Linear relationship was observed between concentration of drug solution (5-30µg/ml) and absorbance in 0.1 N HCL The coefficient of correlation (R^2) was found to be 0.999, indicating that drug solution obeys Beer's- Lambert law in the concentration range of 5-30µg/ml. Hence it was concluded that dissolution samples can be analyzed in 0.1N HCL by measuring absorbance at 221 nm using UV-Visible Spectrophotometer.

Drug excipient compatibility

Drug and excipient compatibility was confirmed by comparing spectra of FT-IR analysis of pure drug with that of various excipients used in the formulation.

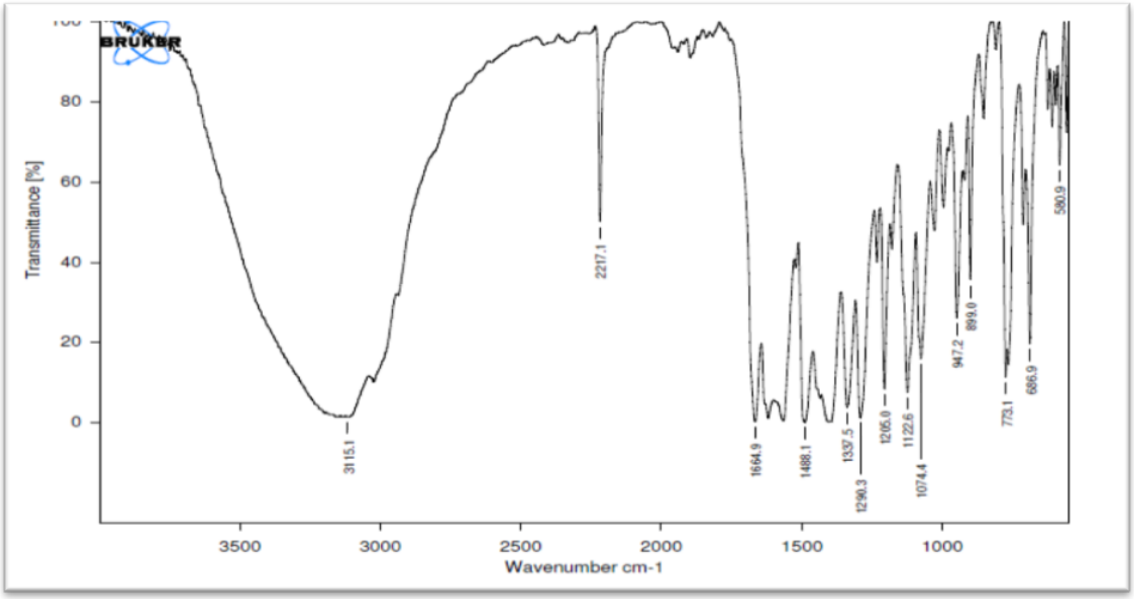


Figure 3: IR spectrum of Glimepiride pure.

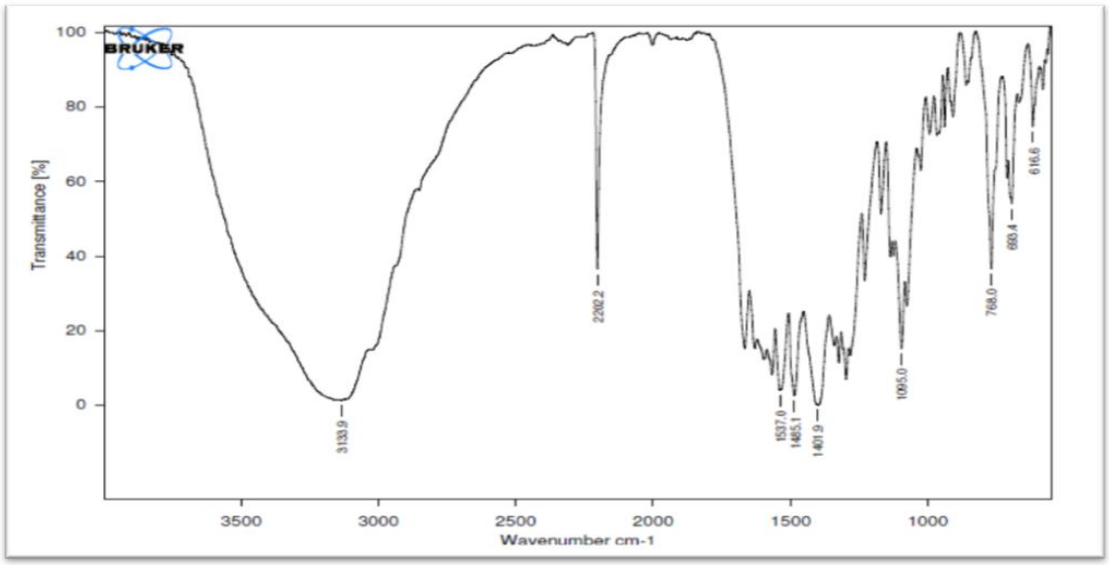


Figure 4: IR spectrum of Glimepiride best formulation.

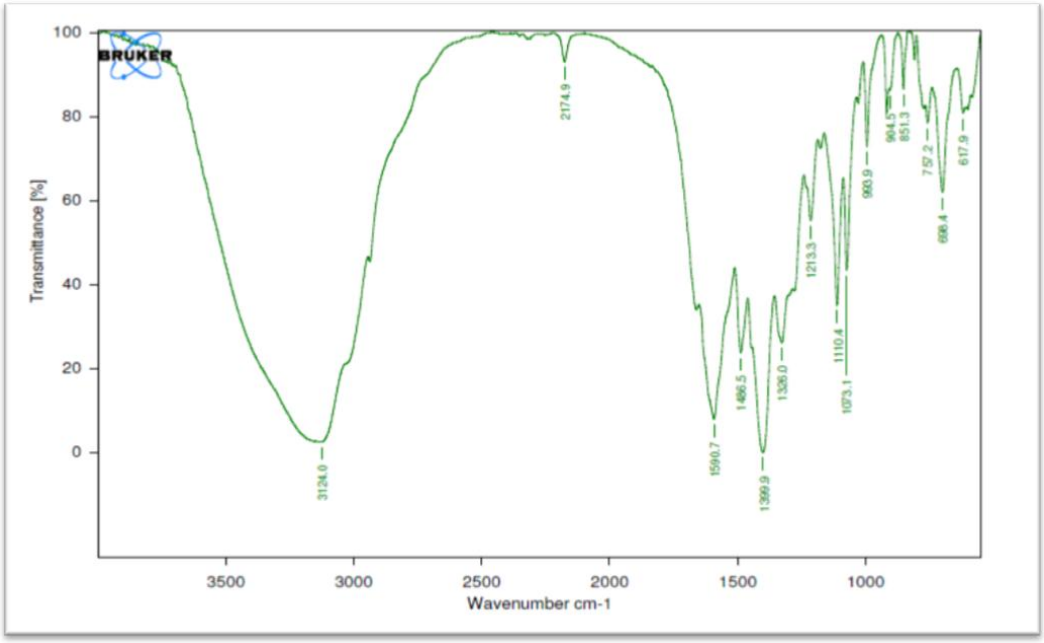


Figure 5: IR spectrum of PVP K-25.

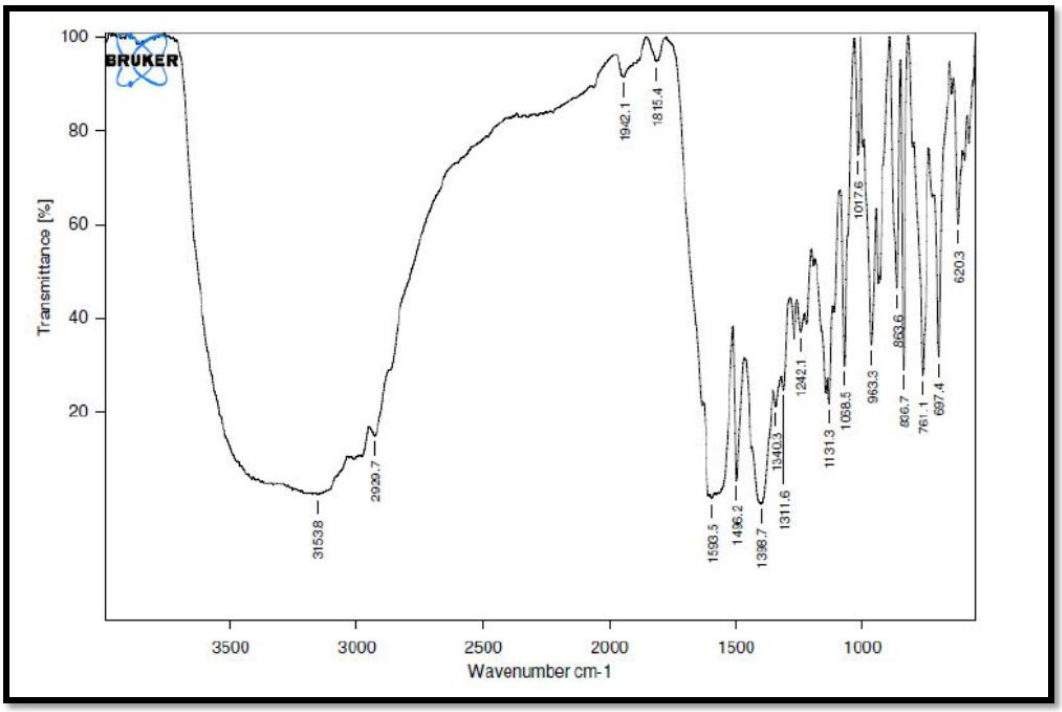


Figure 6: IR spectrum of urea.

Discussion: Form the drug excipient compatibility studies we observe that there are no interactions between the pure drug (glimepiride) and optimized formulation (glimepiride+ excipients) which indicates there are no physical changes.

Drug content: - The drug content of the formulated Nanosuspension was found in the range of 96.22 to 98.97% respectively.

Table 6: Formulated Nanosuspension of Drug content.

Formulation code	Mean % drug content* ± S.D
F1	98.97
F2	98.54
F3	96.87
F4	98.11
F5	98.36
F6	96.22

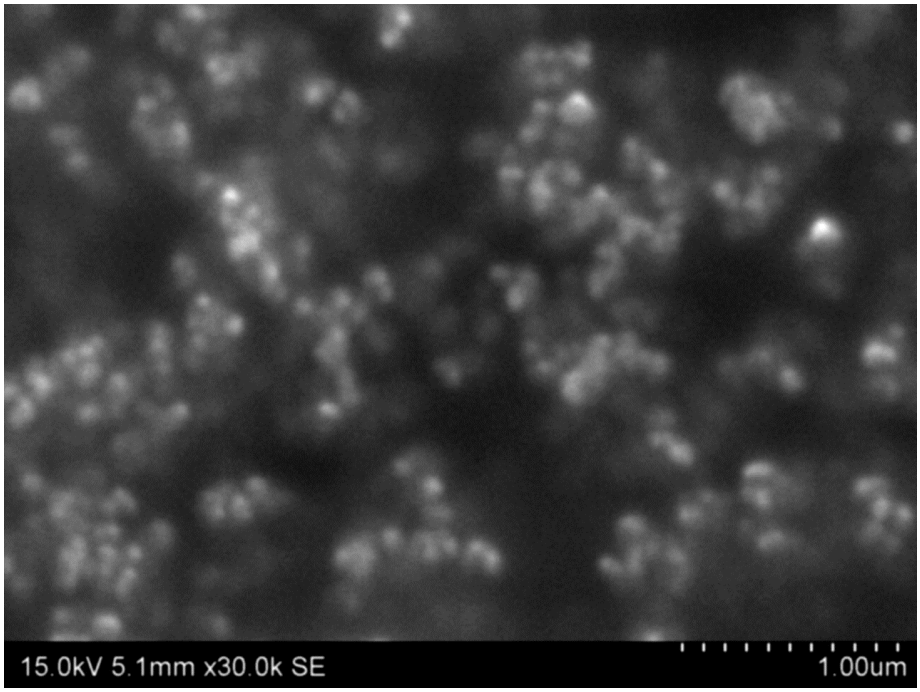
Discussion: The percentage of drug content of formulation F1 was found to be 98.97%, formulation F2 was found to be 98.54%, formulation F3 was found to be 96.87%, formulation F4 was found to be 98.11%, formulation F5 was found to be 98.36%, and finally formulation F6 was found to be 96.22%.

Entrapment efficacy: - The entrapment efficacy of the formulated Nanosuspension was found to be in the range of 87.09%-97.15% respectively.

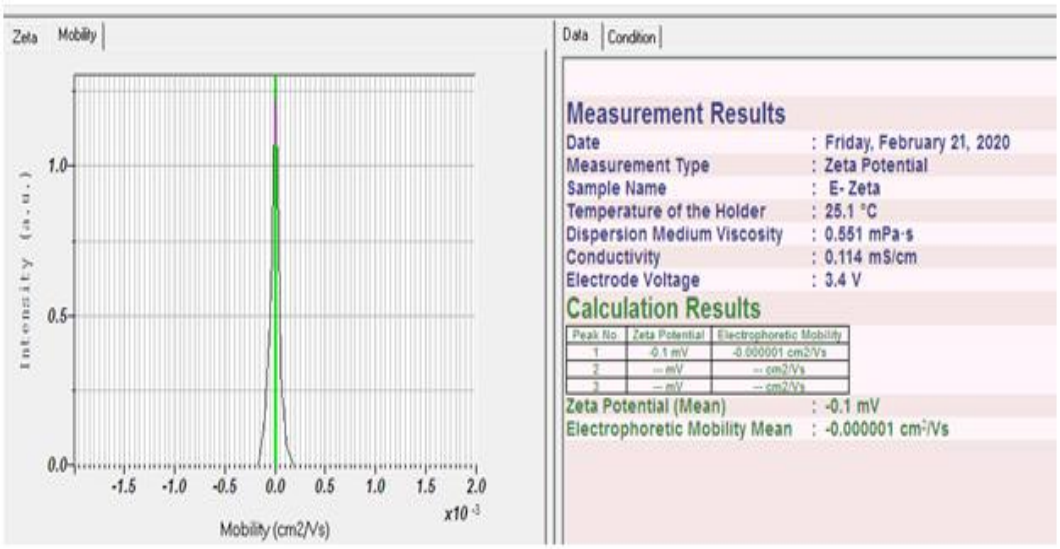
Formulation code	Mean % drug content* ± S.D
F1	97.15
F2	96.23
F3	93.08
F4	94.61
F5	92.04
F6	87.09

Discussion: The entrapment efficacy of formulation F1 was found to be 97.15%, formulation F2 was found to be 96.23%, formulation F3 was found to be 93.08%, formulation F4 was found to be 94.61%, formulation F5 was found to be 92.04%, and finally formulation F6 was found to be 87.09%.

Scanning electron microscopy: Determination of surface morphology of glimepiride nanosuspension of optimized formulation (F1) was carried out by scanning electron microscopy (sem).



Zeta potential: The measurement itself is a particle electrophoresis, the particle velocity is determined via the doppler shift of the laser light scattered by the moving particles. The field strength applied was 20 V/cm. The electrophoretic mobility was converted to the zeta potential in mV using the Helmholtz-Smoluchowski equation. At standard measuring conditions (room temperature of 25 °C, water) this equation can be simplified to the multiplication of the measured electrophoretic mobility (µm/cm per V/cm) by a factor of 12.8, yielding the ZP in mV.



Discussion: zeta potential value for the optimized formulation (F1) was found to be -0.1 Mv which indicates that it was within the acceptable limits.

Particle size analysis.

Calculation Results

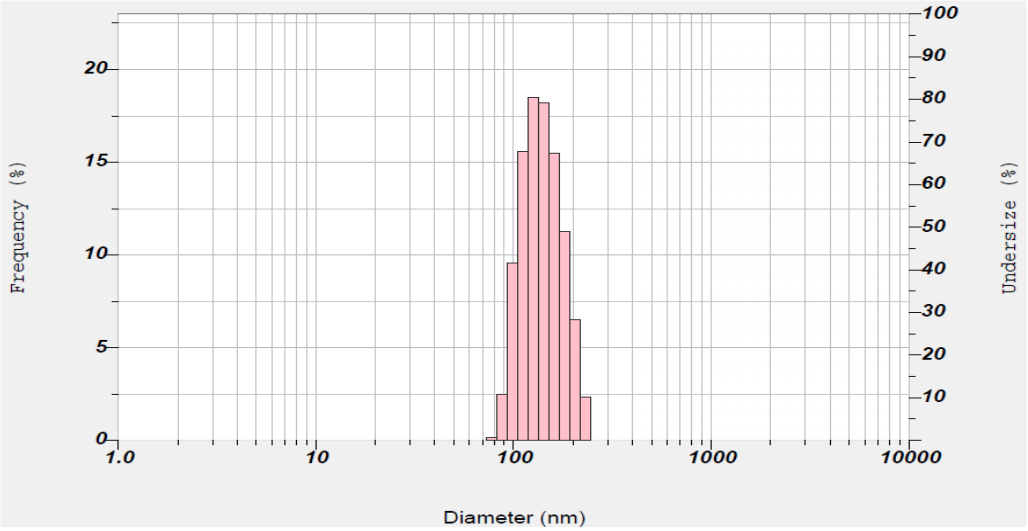
Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	142.6 nm	33.7 nm	127.3 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	142.6 nm	33.7 nm	127.3 nm

Cumulant Operations

Z-Average : 128.1 nm
PI : 3.198

Molecular weight measurement

Molecular weight : ---
Mark-Houwink-Sakurada parameters : ---

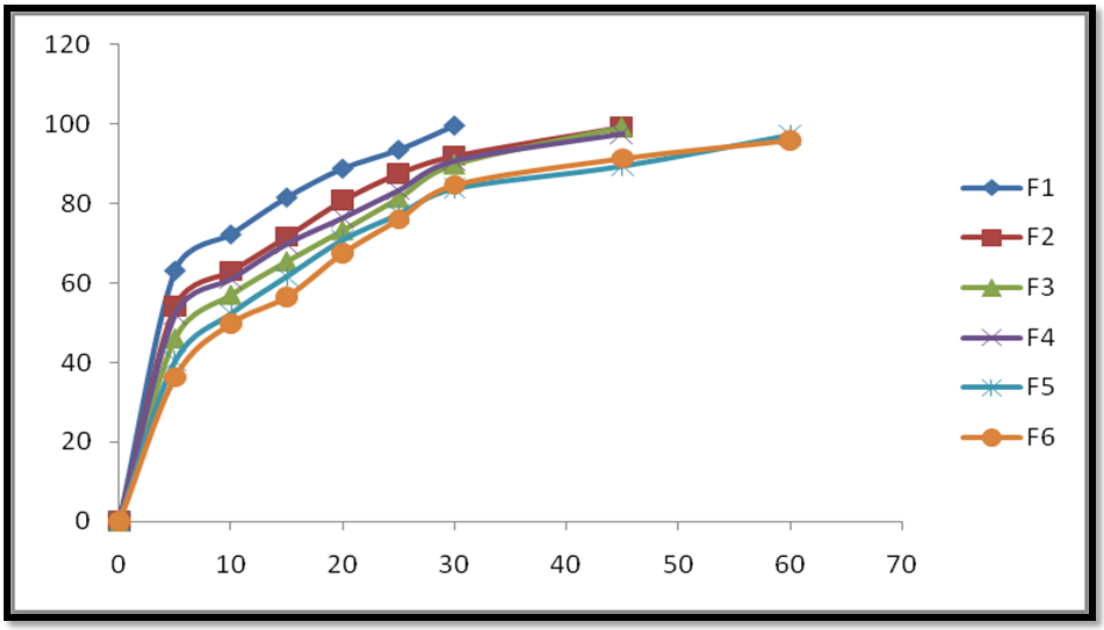


Discussion: Average particle size of Nanosuspensions of optimized formulations (F1) was found to be 128.1 nm.

Table 7: Dissolution parameters for the formulations of Nanosuspension of Glimepiride.

Time (min)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
5	63.21	54.21	46.12	52.24	40.35	36.35
10	72.32	63.02	57.06	61.08	52.28	49.83
15	81.64	71.78	65.51	69.98	61.62	56.62
20	88.91	80.87	73.32	76.54	70.83	67.58
25	93.64	87.65	81.42	83.47	77.26	76.06
30	99.74	92.08	89.93	90.92	83.69	84.72
45	--	99.40	99.31	97.72	89.39	91.42
60	--	--	--	---	97.19	96.02

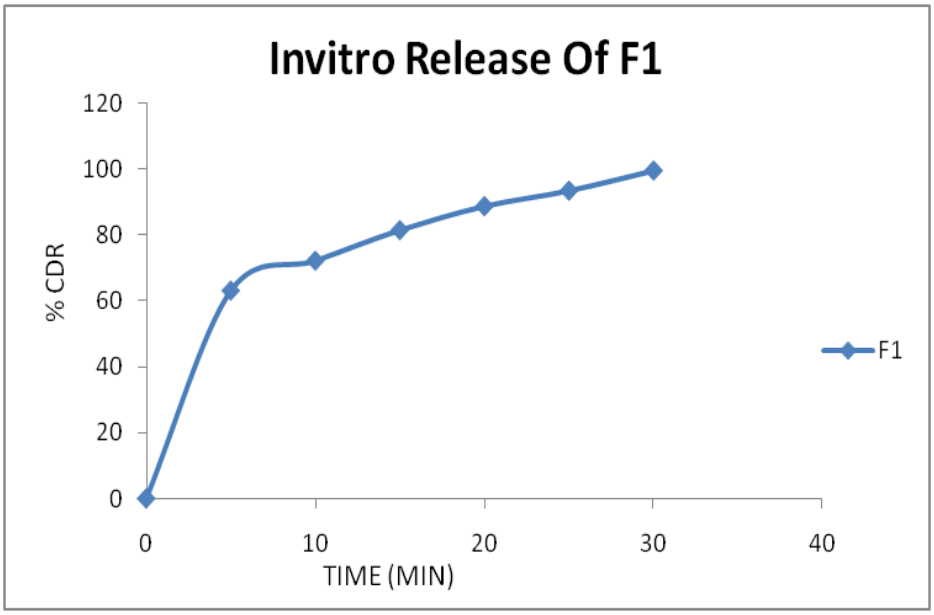
Discussion: From the above invitro studies we can say that of all the formulations (F1) containing PVP K-25 and poloxamer shows best drug release of 99.74% within 30 minutes where as all the other formulations takes about 45 to 60 minutes to release the drug.



Graph 1: Dissolution parameters for all the formulations (F1-F6)

Table 8: Invitro drug release studies of F1.

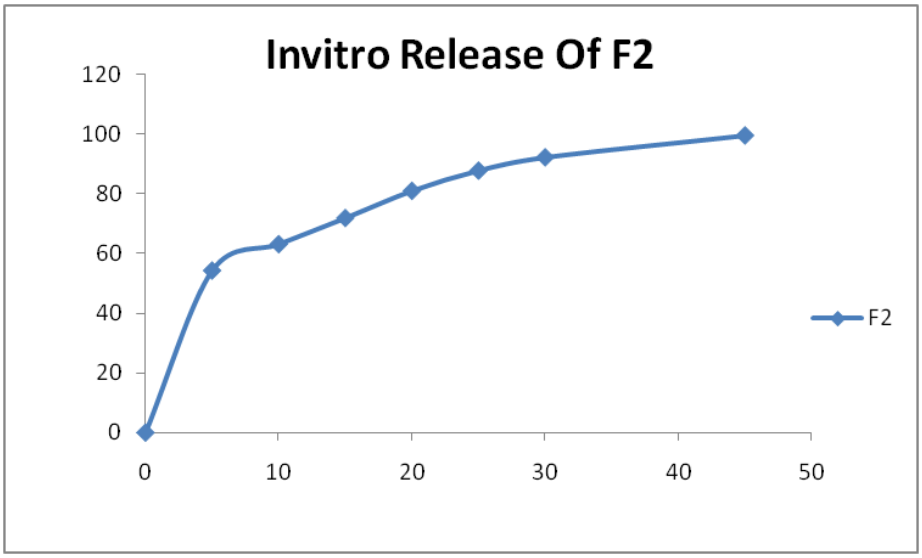
Time (min)	F1
0	0
5	63.21
10	72.32
15	81.64
20	88.91
25	93.64
30	99.74



Graph 2: Dissolution parameters for formulation F1.

Table 9: Invitro drug release studies of F2.

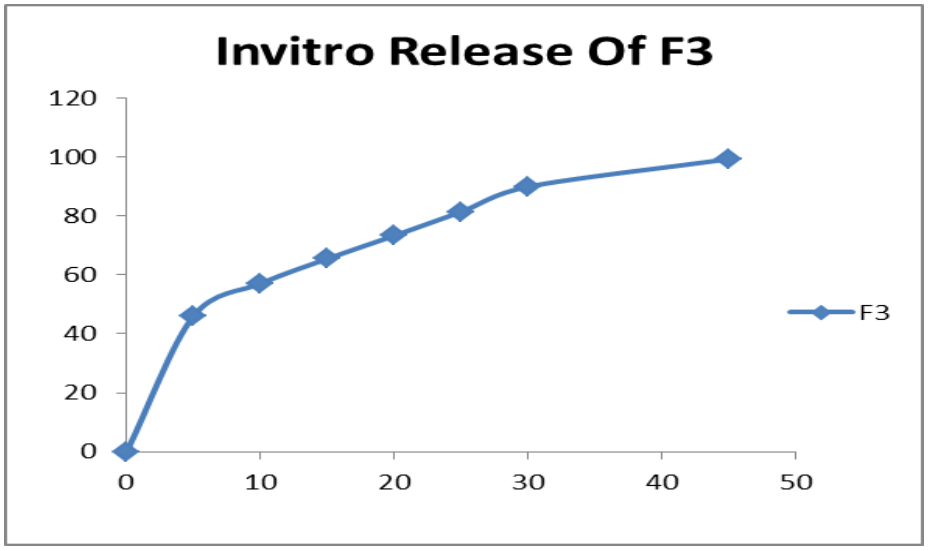
Time (min)	F2
0	0
5	54.21
10	63.02
15	71.78
20	80.87
25	87.65
30	92.08
45	99.4



Graph 3: Dissolution parameters for the formulations F2.

Table 10: Invitro drug release studies of F3.

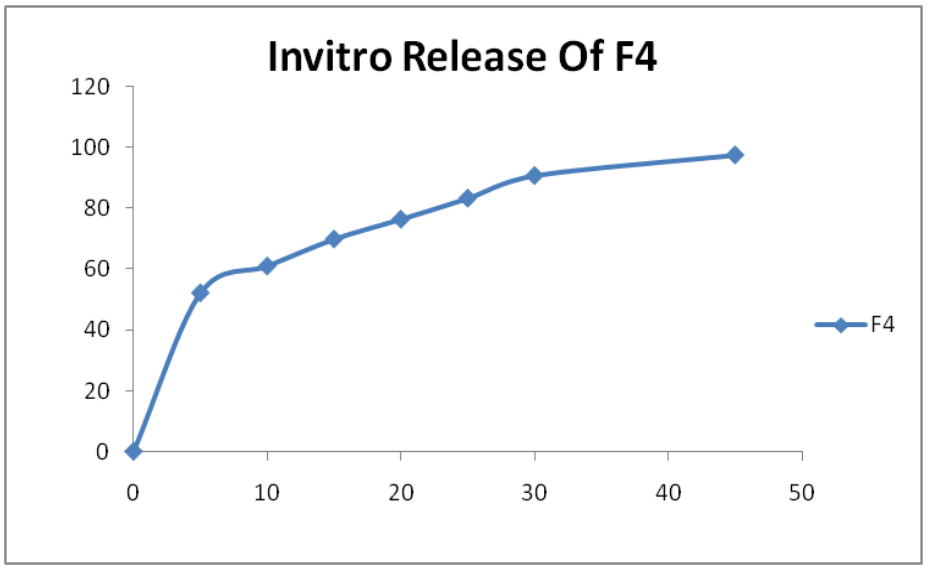
Time (min)	F3
0	0
5	46.12
10	57.06
15	65.51
20	73.32
25	81.42
30	89.93
45	99.31



Graph 4: Dissolution parameters for the formulations F3.

Table 11: Invitro drug release studies of F4.

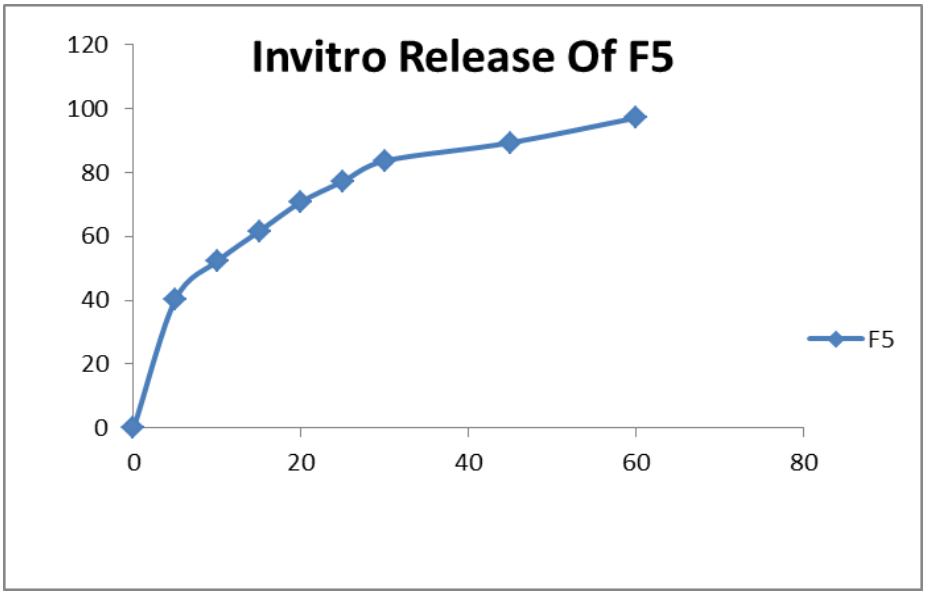
Time (min)	F4
0	0
5	52.24
10	61.08
15	69.98
20	76.54
25	83.47
30	90.92
45	97.72



Graph 5: Dissolution parameters for the formulations F4.

Table 12: Invitro drug release studies of F5.

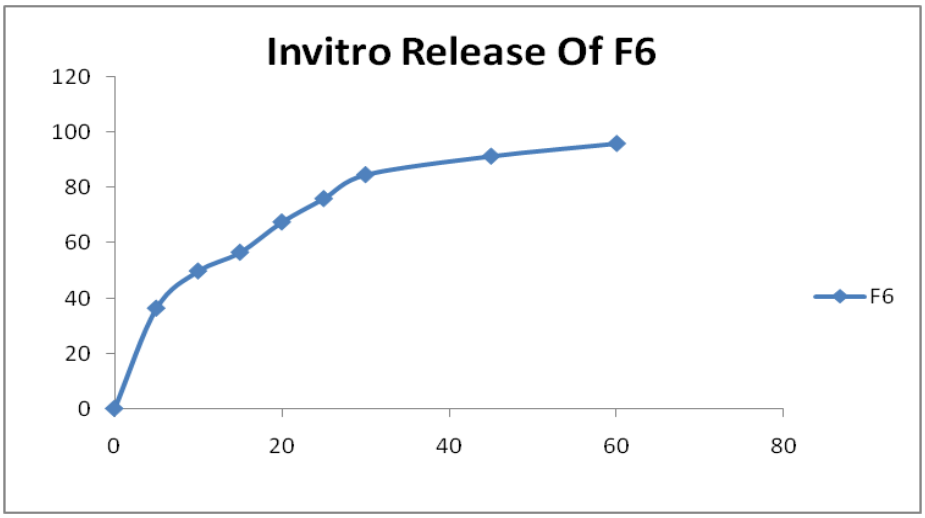
Time	F5
0	0
5	40.35
10	52.28
15	61.62
20	70.83
25	77.26
30	83.69
45	89.39
60	97.19



Graph 6: Dissolution parameters for the formulations F6.

Table 13: Invitro drug release studies of F6.

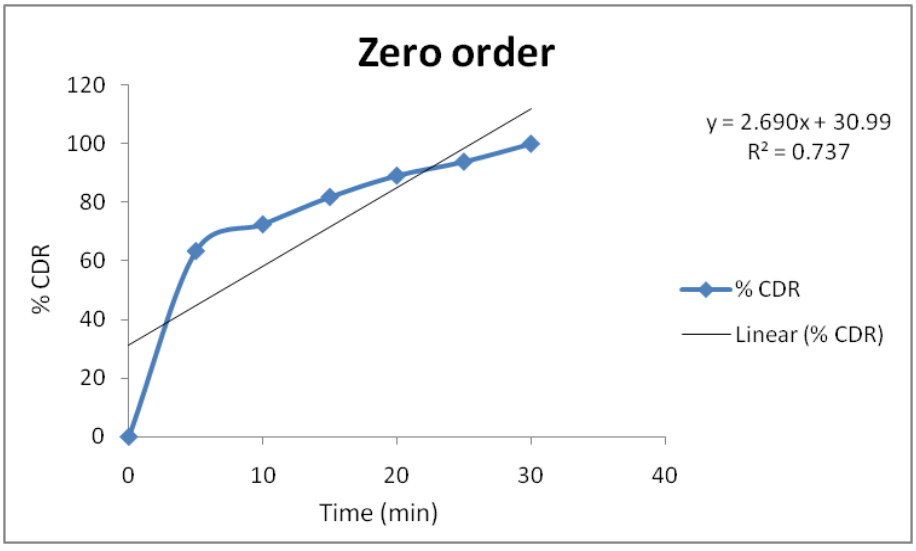
Time (min)	F6
0	0
5	36.35
10	49.83
15	56.62
20	67.58
25	76.06
30	84.72
45	91.42
60	96.02



Graph 7: Dissolution parameters for the formulations F6.

Drug release kinetics studies: Best formulation F1.

Time (min)	F1
0	0
5	63.21
10	72.32
15	81.64
20	88.91
25	93.64
30	99.74



Cumulative percent drug released vs time plots (zero order) of formulation F1

Graph 8: Zero order release kinetics studies.

Time (min)	LOG % ARA
0	2
5	1.56573
10	1.442166
15	1.263873
20	1.044932
25	0.803457
30	-0.58503

First order release kinetics studies

Log cumulative percent drug released vs time plots (First order) of formulation F9

Kinetic data of the optimized formulation F1.

ORDER OF KINETICS	ZERO ORDER	FIRST
REGRESSION	0.737	0.816

CONCLUSIONS

From the present study, the following conclusions can be drawn

Oral Nanosuspension of glimepiride can be prepared by precipitation method using combinations of polymers PVP-k25, urea, acetone, SLS, Poloxamer F127, and quantity sufficient of distilled water.

All the prepared formulations were found to be having drug content within acceptable limits in the range of 96.22 to 98.97% respectively. All the prepared formulations were found to be having entrapment efficiency within acceptable limits in the range of 87.09 to 97.15%

respectively.

As the polymer is increases, the drug release rate decreases, whereas Nanosuspension strength increases. Optimized formulations of Nanosuspension displayed first order release kinetics and drug release. IR spectroscopic studies indicated that there are no drug-excepient interactions. When compared to other all the formulations F1 is the best formulation which showed effective drug release percentage of Nanosuspension. This showed formulation F1 is 99.74% of drug released respectively with in 30 min and follows first order release kinetics.

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