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Research Article on Formulation and Evaluation of Metformin Hydrochloride Nanoparticles for the Treatment of Type 2 Diabetes

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ABSTRACT-
In the previous two decades, there has been a marked increase in the use of Novel Drug Delivery Systems, and this effort makes a little contribution to this advancement. The goal of this Design and Development of Nanoparticles, a type of Novel Medication Delivery System, is to raise the bioavailability of dosage forms, to increase the biological half-life of the drug, and to improve patient compliance. Nanoparticles are prepared by various methods, one of which is Solvent Evaporation Method which is chosen here for preparation of nanoparticles for the treatment of Type 2 diabetes. Nanoparticles are formulated using a suitable formula as mentioned further, and among all the formulations, formula F4 outperformed all other formulations. The evaluation results of F4was, found to be much promising. The formula F4 showed drug entrapment efficiency of 83.67%, percentage yield of 77.42% and In-vitro release of drug was found to be 79.64% in 14 hrs.

Keywords: Novel Drug delivery, Chitosan, Solvent evaporation method, Type2 Diabetes, Nanomedicines

I. INTRODUCTION-

Nanoparticles are among the most important materials of our time, and they have the potential to change the world. There has been various research conducted on this topic. Nanoparticles are now being employed in a variety of industries, including the electronics industry, medicinal applications, medicines, cosmetics, and environmental activities, among others.

Around the world, there is an increase in investment in nanotechnology applications and research. There is insufficient information available about the current use of nanoparticles and the rate at which they are produced. On the other hand, according to current estimates of nanoparticle production rates, around 2,000 tonnes were created in 2004 and the pace of production is predicted to climb to 58,000 tonnes by 2020. Because of the rising manufacturing and utilisation of nanoparticles, there will be an increase in environmental and human health problems as well.

Narrow-banded polymers are not simple molecules in and of themselves, and as such they consist of three layers, which are as follows:
(a) The topmost layer (which can be designed and synthesised with various of small molecules, polymers and metal ions).
(b) The shell layer, (that is a chemically different substance from the core) and
(c) The core(which is essentially the main and the central section of the nanoparticle and is commonly termed as the nanoparticle many times) are the three layers that make up the NP. Because of their remarkable features, these materials have piqued the curiosity of researchers working across a wide range of disciplines.

Nanoparticles are particles with sizes ranging between one and one hundred nanometers and are formed of carbon, metal oxides, metal and organic materials. Nanoparticles have diverse chemical, physical and biological properties as compared to their larger-scale counterparts. This is especially true at the nanoscale. The nanoparticles have a variety of shapes, sizes, and structural configurations. It can be spiral, cylindrical, spherical, conical, hollow core, tubular, flat, and the size ranges are from 1 nm to 1000 nm. It can also be irregular in shape. The surface might be homogeneous or uneven, with surface variations or without surface variations. Some nanoparticles are amorphous or crystalline in nature, containing single or multi crystal solids that areeither in a free or flocculated state, depending on the type of particle (Ealia et al., 2017, Garcia et al., 2007)

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Classification of nanoparticles:

1) Nanosphere (Nanosphere or matrix-type nanodevices, are polymeric nanoparticles (NPs) in which the entire mass is solid and is made up of a sphere-shaped polymer matrices.)

2) Nanocapsule (Nanocapsules, also known as reservoir-type nanodevices, are vesicular systems that consist of a liquid core (water or oil) into which a medicine can be put and a polymeric membrane or coating (Tatsumi et al., 2007). Diabetes mellitus, often reffered to the as type 2 diabetes, is a metabolic condition characterised by extremely high blood glucose levels (hyperglycemia). Type 1 diabetes (characterised by decreased insulin production) and type 2 diabetes (characterised by reduced insulin sensitivity and beta-cell dysfunction) are the two most frequent types of diabetes. In all cases, high blood sugar levels are associated with very excessive urine output, compensatory thirst, increased fluid consumption, unexplained weight loss, lethargy,impaired vision, and alterations in energy metabolism (Tatsumi et al., 2007, Garcia et al., 2020)

Increased levels of blood glucose as a result of impaired insulin action and/or secretion characterise type 2 diabetes, which is a complicated diverse set of metabolic disorders. Physiologically, the pancreatic beta-cells produce insulin on a continuous basis, independent of the level of blood glucose in the blood. A rise in blood glucose levels causes insulin to be stored in vacuoles, where it is then released when the glucose level rises. Among the hormones that regulate the uptaking of glucose from the blood into the most cells, insulin is the primary regulator of skeletal muscle cell and adipocyte glucose uptake. Moreover, insulin serves as the primary signal for the conversion of glucose into glycogen, which is then stored in the hepatic and skeletal muscle cells. When the blood glucose level drops, the release of insulin from the beta cells decreases, while the release of glucagon from the beta cells increases, both of which encourage the conversion of glycogen to glucose. Following an overnight fast, glycogenolysis and gluconeogenesis are the primary sources of glucose production.There are three major problems with the beginning of hyperglycemia in type 2 diabetes: The synthesis of glucose by the liver is increased, whereas insulin secretion is decreased and insulin action is hindere(Lin et al., 2010)

II. MATERIAL AND METHOD-

S.no	Formula code	Drug (mg)	Polymer (mg)	PVA conc. (%)	Organic solvent (ml)	Water (ml)	Drug:polymer
1	**F1**	50	50	0.5	10	90	1:1
2	**F2**	50	100	0.5	10	90	1:2
3	**F3**	50	150	0.5	10	90	1:3
4	**F4**	50	200	0.5	10	90	1:4

Formulation Table

Preparation of Metformin hydrochloride Nanoparticles:

The concept behind this novel drug delivery system was to design and fabricate a dosage form which will have the better bioavailability and a better patient compliance. As a result, numerous methods for preparing such a dosage were investigated and assessed. The "Solvent evaporation method" was selected as the most appropriate approach for this project task out of all the ways available for the manufacture of nanoparticles.

-The polymer (chitosan) is dissolved in volatile organic solvent (Ethyl acetate) into which the drug (metformin hydrochloride) is dissolved.

-Afterwards, the organic solution is dissolved in aqueous phase, which contains a surfactant (Polyvinyl alcohol), and the mixture is homogenised at a high rate (18000 rpm by magnetic stirring for 3 hours) to form an emulsion.

-After forming a stable emulsion, the organic solvent is removed from the polymer solution by increasing the temperature under reduced pressure or stirring continuously at 300rpm for 2 hours.

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-The nanoparticle are collected by ultracentrifugation at 25000rpm for 15 min.
-After that, the sample was washed multiple times with distilled water to eliminate any stabiliser residue or free drugs, and it was lyophilized for storage.

Evaluation of formed dosage form

A) Physical appearance and morphological characteristics-For the physical appearance, shape, and size of the nanoparticles that were synthesised, they were examined carefully. The nanoparticles were chosen at random, and from each batch of preparations, approximately 100 pieces of each type of nanoparticle were selected at random. All of the selected particulate matter was examined under an ocular microscope equipped with a calibrated stage nano ruler to ensure that it was free of contaminants(Bahaman et al., 2019, Kumar santosh et al.,2017)

B) Scanning Electron Microscopy-Scanning electron microscopy (SEM) is a technique that allows for direct observation and morphological evaluation. Using electron microscopy for morphological and sizing study has various advantages.SEM characterization necessitates the first step of drying out the nanoparticle solution, which is then mounted on a sample holder and sputter-coated with an electrically conducting metal, such as gold, before proceeding with the SEM analysis itself. The sample is next scanned with a finely focused electron beam to determine its composition. The secondary electrons that emitted from the sample surface are used to determine the surface properties of the sample under investigation. In order to survive in a vacuum, the nanoparticles must be resistant to vacuum, and the electron beam may cause harm to the polymer(Durah et al.,2015, Pal lal et al.,2011)

C) Percentage yield- The percentage yield can be used to gain an understanding of the proficiency with which nanoparticles are produced. A rough estimate of how to calculate the number of ingredients to be used in relation to the amount of dosage form that is being manufactured is provided. It is calculated with the use of a formula(Thomas et al.,2015, Pal lal et al., 2011)

$$\% \text{ Yield} = \frac{\text{Amount of nanoparticle}}{\text{Amount of drug + polymer}} \times 100\ \%$$

D) Drug Entrapment efficiency- An entrapment efficiency test is performed on 10 mL of the sample to determine the proportion of the medication that has been integrated. UV–visible Spectrophotometer is used to estimate the % entrapment effectiveness by spectrophotometrically measuring it at a fixed nm. Previously, 10 mL of nanoparticles were made and centrifuged at 5000 rpm for 45 minutes to obtain the desired result. The sum of free metformin in the supernatant was calculated by UV spectrophotometer at 232nm(Tatusumi et al.,2007, Kamboj et al.,2018)

The percentage drug entrapment efficiency can be estimated with the help of the following equation:

$$\% \text{ Drug Entrapment} = \frac{\text{Mass of drug in nanoparticle}}{\text{Mass of drug in formulation}} \times 100$$

E) FTIR Spectrophotometer- When it comes to pharmaceuticals, FTIR spectroscopy is a straightforward, sensitive, and versatile analytical method that may be used to detect changes in the functional groups of medications. The FTIR spectra Chitosan, pure metformin, and MN were all taken independently by KBr Pellet and analysed for differences(Kumar Sandeep et al.,2017, Durah et al.,2015)

Aspect ratios between 4000 and 400 cm^{-1} were seen in the spectra of the samples studied.

F) **In-Vitro Release Study**- It was done in a USP Type II (paddle type) dissolving equipment with a 50rpm-100rpm rotating speed. The produced nanoparticle suspension was immersed in 900ml of phosphate buffer solution in a vessel at 37 ± 0.20℃. A certain amount of medium (5ml) was removed at a specific time interval, and the same volume of dissolution medium was replenished in the flask to maintain a constant volume. A UV spectrophotometer at wavelength of 233nm

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was used to examine the withdrawn sample(Murthy et al.,2007, Kaul et al.,2018)

III. OBSERVATION-

1) Physical appearance and Morphological characteristics-

Table no.1 Particle size determined by Optical microscope

Sr. No.	Formula code	Average Particle size (nm)
1)	F1	558
2)	F2	660
3)	F3	740
4)	F4	790

The particle size of the nanoparticles was determined after they had been dried fully at room temperature. After an evaluation of the particle size of each formulation, it was determined that an increase in the concentration of polymers results in an increase in the diameter of the nanoparticles. It was discovered that formula F4 has the largest particle size in compare to the other formulations tested.Nanoparticles having a relatively large size are expected to enhance fast drug release as well as more effective polymer breakdown than smaller particles.

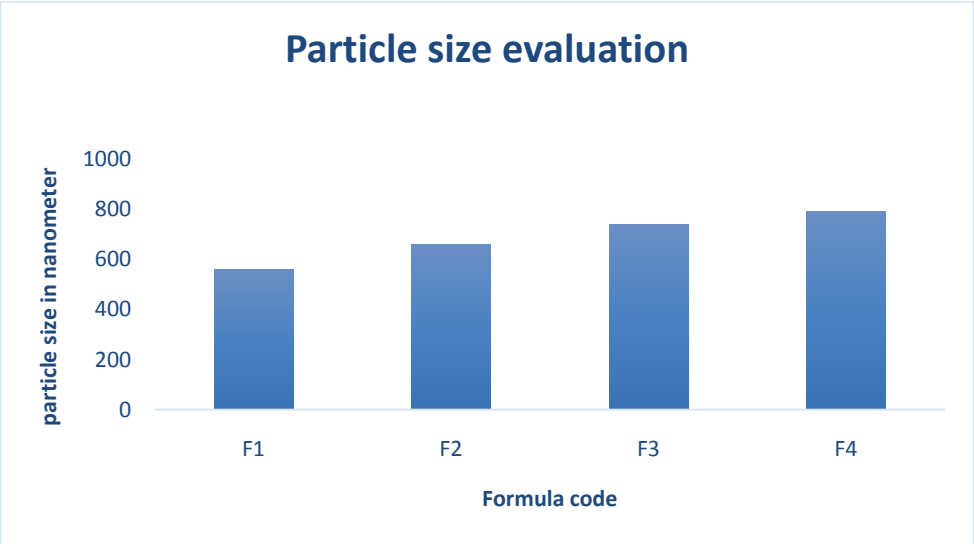

Fig 1 Graphical Representation of Particle size evaluation

2) Scanning Electron Microscopy-

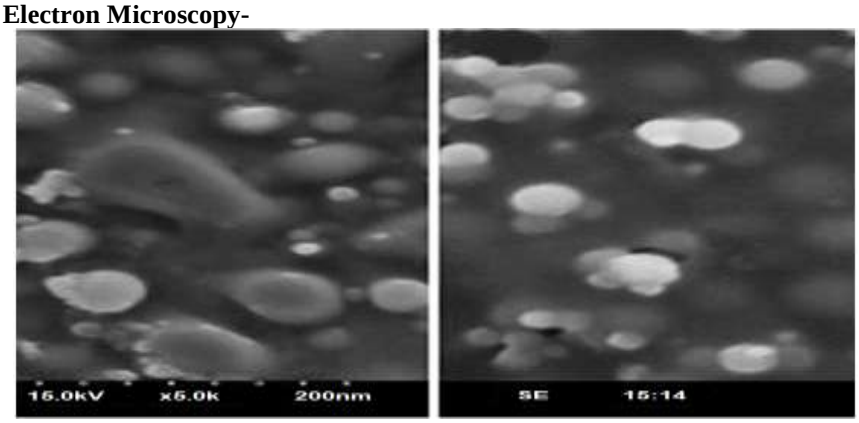

Fig 2 Scanning electron microscopy of F4 nanoparticles a) X10,000 magnification, b) X20,000 magnification.

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With the help of scanning electron microscopy, the exterior morphological properties of Metformin hydrochloride nanoparticles were examined (SEM). The SEM images and data of dried microspheres revealed that the upper surface of the microsphere was smooth, but a close inspection of the microsphere during SEM revealed a textured surface. It was discovered that the existence of rough surfaces indicated the presence of pores, which allowed for the easy sliding of active medicinal ingredient from the core of nanoparticles, leading to the elicitation of a biological response at the site of action.

3) Drug Entrapment Efficiency-

Table no. 2 Percentage Drug Entrapment Efficiency

Sr. No.	Formula Code	Drug entrapment efficiency (%)
1)	F1	72.84
2)	F2	78.65
3)	F3	8O.48
4)	F4	83.67

It is obvious from the tabular results of nanoparticles when examined by assessment tests such as drug entrapment efficiency that the formula code F4 has more ability to encapsulate the API when compared to other formula codes. What we can conclude from this result is that the greater the amount of coating on the core material, the greater the amount of absorption, that occurs due to the bioadhesive nature of the Polymer used in the manufacture of these nanoparticles, and the greater the amount of drug entrapment efficiency.

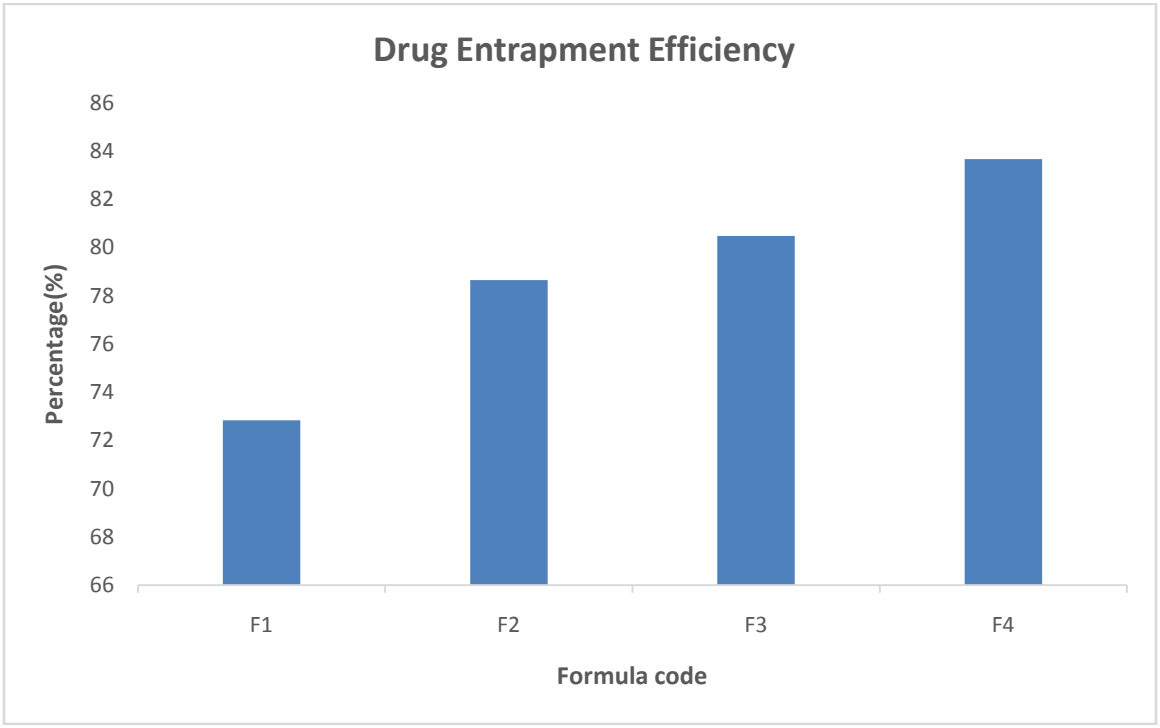

Fig 3 Graphical Representation of Drug Entrapment efficiency

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4) Percentage Yield-

Table No. 3 Percentage yield of various formulations

Sr. No.	Formula Code	Percentage Yield (%)
1)	F1	67.54
2)	F2	70.65
3)	F3	74.87
4)	F4	77.42

The amount of drug and polymer obtained before and after drying of nanoparticles was represented by the percentage yield. Using excipients such as polymer Chitosan, the findings obtained clearly demonstrated that formula F4 had more drug retention capability than the other formulations. It appears that formulation F4 has a higher compatibility with polymer when compared to the other formulation and that is the reason for higher percentage yield.

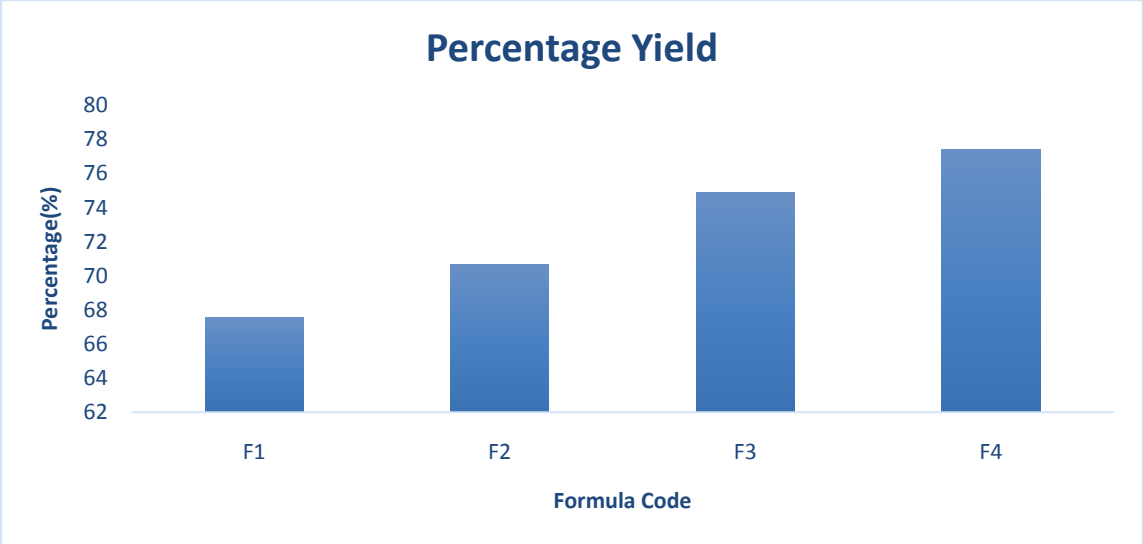

Fig 4 Graphical Representation of Percentage Yield

5) FTIR Spectroscopy-

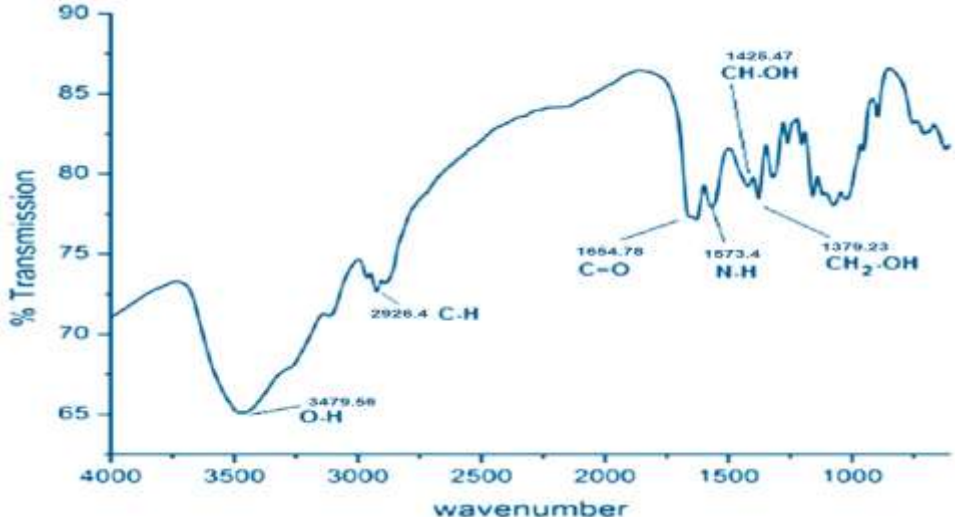

Fig 5.1 FTIR Spectrum of Chitosan

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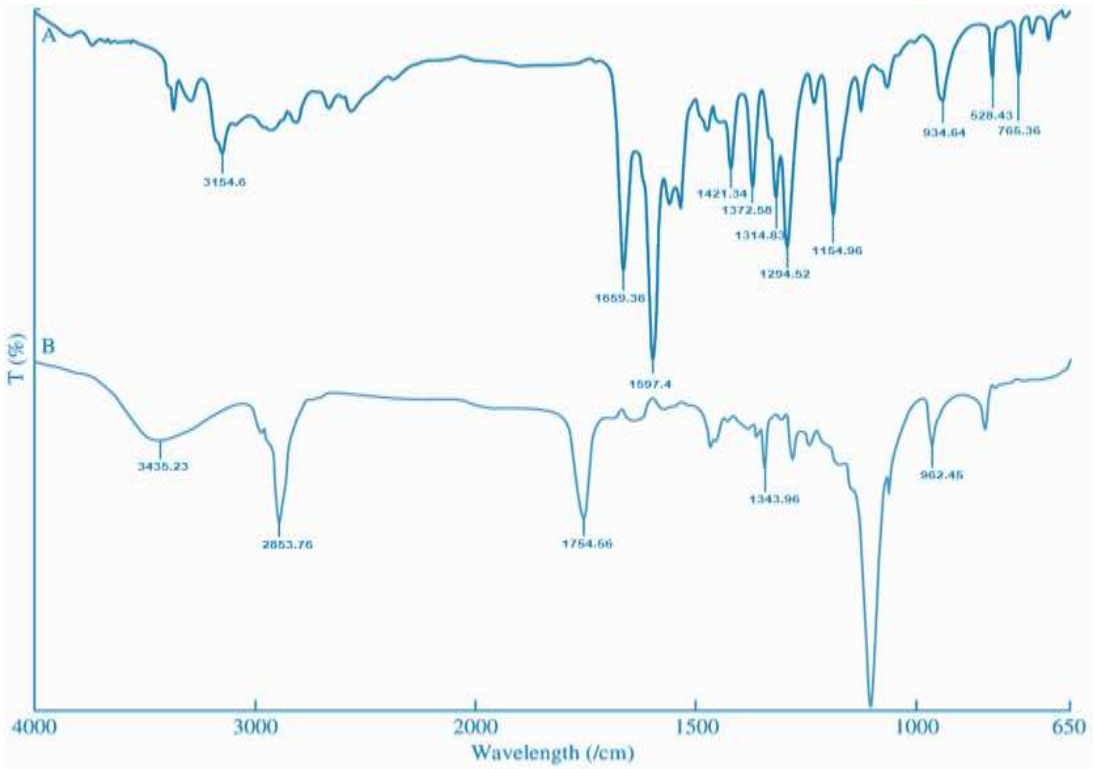

Fig 5.2 FTIR Spectrum of Metformin HCl and Metformin nanoparticles

The Infrared spectra for chitosan are shown in Fig 6.5.1. The stretching vibrations of -OH bond of the prepared chitosan was found at 3479.56cm^{-1} and that for C–H were observed at 2926.14 cm^{-1}.The existence of C=O stretching of the amide I band, bending vibrations of the N–H (N-acetylated residues, amide II band), C–H bending, and OH bending were related with the absorption peaks at 1654.78 cm^{-1}, 1573.04 cm^{-1}, 1425.47 cm^{-1}, and 1379.23 cm^{-1}, respectively.

Pure metformin and prepared nanoparticles were analysed using FTIR spectroscopy, which revealed typical characteristic peaks, such as N-H asymmetric stretching (3367.13cm^{-1}), N-H symmetric stretching (3154.6cm^{-1}), C=N stretching (1659.36cm^{-1}), N-H bending (1597.14cm^{-1}), and N-H bending (1421.34cm^{-1}).The distinctive spectrum peaks corresponding to metformin functional groups were observed in both pure metformin and metformin nanoparticles, demonstrating that metformin and polymers such as Chitosan were compatible in the design and manufacture of chitosan loaded metformin nanoparticles.

6) In-Vitro Studies-

Table no.4 Data of In-vitro test

Time(hrs)	F1(%)	F2(%)	F3(%)	F4(%)
0.5	0.81	1.02	1.31	2.64
1	6.03	8.76	12.05	16.7
2	15.06	17.58	27.9	33.02
3	25.36	28.96	32.34	38.64
4	32.70	36.36	40.76	48.33
6	39.09	44.64	49.43	54.89
8	41.83	46.65	52.56	58.09
10	45.78	49.54	57.66	66.02
12	55.56	57.89	69.70	71.11
14	67.82	71.13	78.48	79.64

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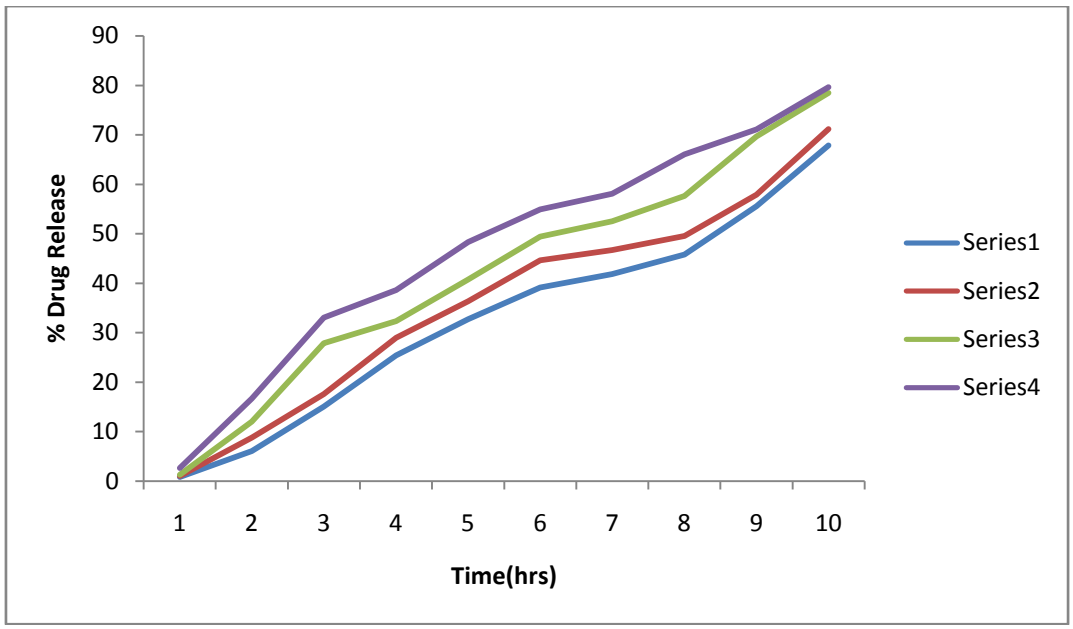
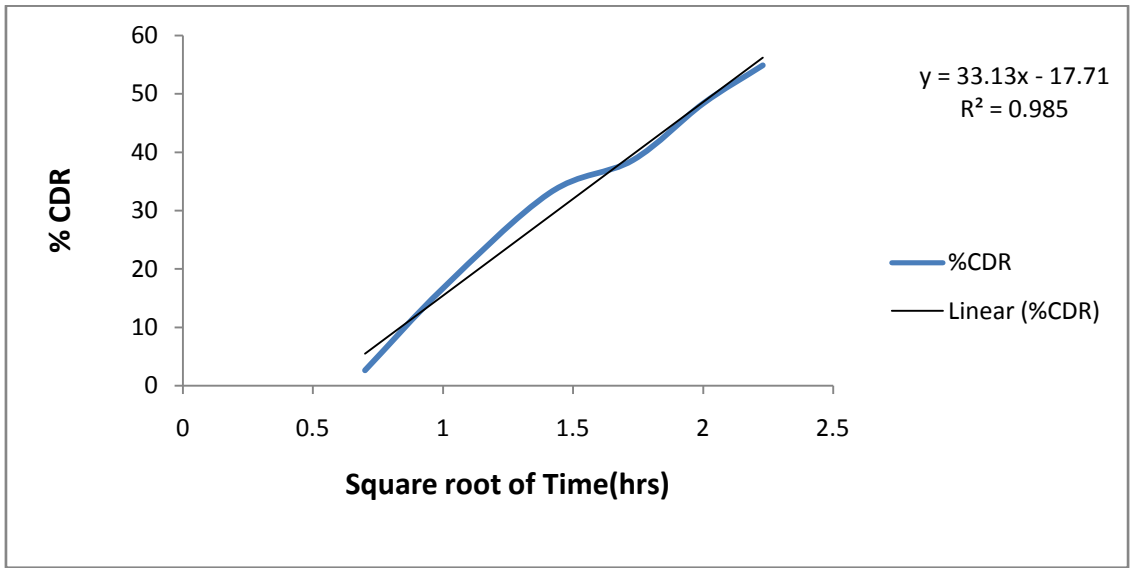


Fig 6 Graphical representation of In-vitro test

The in vitro drug release parameter was used to investigate the percent drug release pattern of the drug Metformin hydrochloride. According to the findings, as the experiment progresses, the formulation slowly and gradually releases its content into the media, resulting in an evocative action and the generation of controlled release action.

Release kinetics model-

Release kinetic models	Regression (r2) coefficient
Zero Order	0.978
First Order	0.978
Higuchi	0.985
Korsmeyer – peppas	0.972



Higuchi Release Model

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The release profile of F4 nanoparticles best fit into the Higuchi model that describes the diffusion of drug from homogeneous and granular matrix systems. The drug release from a matrix system is said to follow Higuchi's release kinetics and allows follows highest linearity (r^2) 0.985.

IV. CONCLUSION-

When it comes to treating type 2 diabetes, metformin is the widely prescribed oral antihyperglycemic agent on the market. On the other hand, it has a sluggish and incomplete absorption after oral administration of drug, and because of its short biological half-life, frequent applications of high dosages of metformin (as rapid release formulations) are required for an effective treatment.To address the challenges connected with conventional dosage forms, drug delivery systems are extremely useful technologies to have.

As a result, the development of drug delivery systems (e.g., microparticles, nanoparticles) strategies for metformin may be beneficial for improving its bioavailability, reducing the frequency of dosing, decreasing gastrointestinal side effects and its toxicity etc.

We prepared the metformin hydrochloride nanoparticles by solvent evaporation method and used the Chitosan as a polymer in different concentration. And observed that the formulation F4 which have the high concentration of polymer having the high drug entrapment efficiency and percentage yield and shows the max. In-vitro drug release.

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