

**PREPARATION AND PHARMACOLOGICAL EVALUATION OF
METFORMIN LOADED MICROSPHERES**

**Thesis Submitted By
Biplab Kumar Chakra, M. Pharm**

Doctor of Philosophy (Pharmacy)

Department of Pharmaceutical Technology
Faculty Council of Engineering & Technology Jadavpur University
Kolkata, India
Year 2022

SYNOPSIS

Microsphere is a targeted Drug Delivery tool to provide sufficient drug at the specific site of action. The therapeutic efficacy of the drug is maximum. It is an effective carrier of the drug at the target site. It is a controlled Drug Delivery System. As it is in the spherical form so surface area is larger. That helps in the adsorption of the drug in the intestine.

Metformin is a drug of choice for type 2 diabetes patient. It is biguanide class of drug. It is having a side effect on lactic acidosis for some patient.

It does not work on pancreas. Does it does not simulate the pancreas. Its main function is to act on neoglucogenesis in liver. That's production of glucose from fat protein and other dietary sources is completely blocked with the introduction of metformin. Reaction of metformin is potentiated by amp dependent protein kinase is rather it potential the ampk or amp dependent protein kinase for its action.

The microsphere type of drug delivery system comes under Novel Drug Delivery System. The word novel implies Novelty in the dosage form. Frequent dosing is a problem in traditional medicine. The dosage form should be long acting to provide longer action in its therapeutic efficacy. Microspheres provide prolonged release profile in its action to provide sustained release pattern. This is an example of modern Novel Drug Delivery System.

The Michael spheres are smaller particles with a size range of (1-1000nm)

There are different types of microspheres available. They are maybe like this -

1. **Magnetic microsphere**

Used in Pacific drug delivery at the targeted site to deliver in the liver carcinoma. The drug maybe incorporated in the microsphere and a magnetic field is created. Generally chitosan son and dextrin can be used.

2. **Floating microspheres**

The drug is embedded in the matrix. The release of the drug is a slow and continuous. Generally long and constant release of the drug is found. The therapeutic effect is prolonged.

3. **Radioactive microsphere**

These are used as injectables. Very small size of microsphere are prepared. These are injected to the targeted area. And their microspheres are injected to the targeted area mainly arteries.

4. **Polymeric microspheres**

Various Polymers are used in this kind of microsphere. Polymers are used to prepare the microsphere. They are maybe of two types a) biodegradable polymeric microsphere b) synthetic polymeric microsphere.

- a) Biodegradable polymeric microsphere - there are used very widely for the preparation of microsphere. They can provide longer release of the drug as they are having good swelling properties. The amount of polymer is important to control the release of the drug. But the problem with this kind of

microsphere is with its low drug entrapment efficiency. Birthday are very useful in microsphere related development and its use.

- b) Synthetic polymeric microsphere - they are very useful in the preparation of the microsphere. They can provide good drug Entrapment efficiency and release profile. But it problem lies with its application. They can shift from the site of application. All though it is having a wide application.

Method -

There are various methods for the preparation of the microspheres. There are so many method for the preparation of the microsphere. It depends on the particle size, route of Administration, duration of drug release etc. There are so many techniques for the preparation of the microsphere.

Emulsion solvent evaporation technique

Fast of all drug is embedded in the matrix and one primary emulsion is prepared. At the same time the microspheres are prepared by the Rapid evaporation of the solvent.

Emulsion crossing method

That is medicament was soluble in aqueous solution of gelatin and the solution was heated 40 degree c. The solution was then added drop wise to liquid paraffin while steering the mixture at 1500 rpm for 10 minute at 35°c and it produced W/O then again it is seed for 10 minutes at 15 degree c.

The produced microspheres where was at least 3 times with Acetone and isopropyl alcohol and then air dried at least three times with Acetone and isopropyl alcohol and and then it is Airdried and dispersed in 5 ml of glutaraldehyde saturated toluene solution at room temperature for 3 hours. For crossing game and then treated with 100 ml of 10 mm glycerine solution containing 0.1% W/V of tween 80 at 37° C for 10 minutes two block unreacted glutaraldehyde.

There are so many other methods are there for the preparation of the microsphere. Those are co acceleration method, spray drying technique, multiple emulsion technique, ionic gelation method etc.

Characterization -

There are so many techniques for the characterization of the prepared microsphere. As it is microsphere so they are must be analysis of the proper size and shape by the suitable technique.

Particle size analyser

The microsphere maybe 50 mg would be dissolved in distilled water to make the suspension. The distilled water maybe of 5 ml in volume. Tween 80 (2%) maybe added into the suspension. After the microsphere aggregation can be prevented by sonicating into the water bath. The particle size may be found in micrometre.

Optical microscopy

For the determination of the particle size this method was used. The measurement was made by 450 x (10 x eye piece and 45x objective) and particle size was calculated.

Scanning electron microscopy (SEM)

In this method particle size was measured. Measurements was recorded in micrometre. Particle surface morphology is found by this method. This is also helpful in comparison of different particular dimension.

Swelling Index

It is done for the measurement of swelling. As the gum is used so there will be a swelling in the microsphere volume. This maybe done by distilled water and buffer solution of PH 1.2, 4.5, 7.4 was used for that. The swelling was measured at 37 degree c.

Entrapment efficiency

Drug entrapment is a very important factor for the preparation of the microsphere. This is done for the final efficiency of the microsphere particle and its drug Entrapment denotes the final Entrapment of the drug in the microsphere. Spectrophotometric Analysis was done for the measurement.

X-ray diffraction (XRD) studies

This is done to find the polymorphic form in the microchip picture. This is due to the fact that gum is present in the microsphere preparation. Xrd pictures are analysed after prepared microsphere and the blank and the gum for the determination of the swelling.

Thermal analysis

Generally DSC is done to study the thermal analysis of the microsphere. There are other methods also available. Such as thermogravimetric analysis (TGA) and differentiated thermometric analysis (DTA)

UV FTIR (Fourier Transform Infrared Spectroscopy)

The drug polymer interaction study is generally found out

Stability studies

Stabilities studies of the microsphere is performed. It is found for different conditions like

1. Ambient humid condition
2. Room temperature
3. Overall temperature

In this present work metformin microspheres is prepared. The half life of the metformin is 3.4 + - 0.7 hours. So for a constant flow of medicine in the blood some novel approaches should be followed. Microsphere scan stimulate the regularity in supplying the drug for a longer period of time.

This may help in controlling blood glucose level so that diabetes is controlled. This particular function of made for me will be more prompt when it is delivered in the form of microsphere.

Now a days we have seen anti- tumor effects of metformin also. This is father potential with the use of microsphere of metformin. By this work we have seen the anti cancer activities of metformin in Swiss Albino mice model.

The Anti Diabetic activity of the prepared microsphere is done by animal model. Sweets Albino rates are chosen for Anti Diabetic activity. They were made diabetic with streptozotocin. After that the Swiss albino rat were divided in 5 group.

Group 1 normal rat

Group 2 diabetic control

Group 3 standard

Group 4 formulation (metformin microsphere)

Group 5 metformin microsphere class thiamine

Then all these groups were treated for fasting blood sugar level. It was observed that fasting blood glucose level was best controlled by metformin microsphere and made form in microsphere with thiamine treated group.

After that the Lipid profile of the Swiss Albino rats were also checked. It was done by various method. It was observed the best lipid profile experiments was seen with made form in metformin microsphere treated group and metformin microsphere with the thiamin treated group.

It was also found that urea and creatine in levels are also controlled by medicine. The best result where observed with the microsphere formulation and formulation with thiamine treatedgroup to provide best result to control the levels of Urea and creatine.

The gluconeogenic enzyme are very important for formation of glucose and their control. It was observe that those enzymes I control with the help of the metformin microsphere and metformin microspheres with thiamine.

Dadar carbohydrate metabolic enzymes level also controlled with the help of made form in microsphere and made form in microsphere with thiamine treated group.

Anti cancer studies

Various anti cancer studies was perform to established the antique answer effect of the formulated microsphere of metformin.

The tumor volume and tumor weight measurement is very important for anti cancer work. This is due to the fact that cancer cells apoptotic signs are reduction in tumor volume and tumor cell size and tumor weight too.

Intracellular ROS production was detected. At the same time in Vivo flow cytometric analysis of mitochondrial membrane potential was done.

In Vivo cleaved Caspase 3 flow cytometric apoptotic analysis was measured.

In vivo cleaved PARP flow cytometric apoptotic analysis was done.

In vivo P53 flow cytometric apoptotic analysis was measured.

Various in tro studies were performed with the metformin loaded microsphere.

In vitro cell cytotoxicity assay was performed with metformin loaded microsphere. On various cancer cell lines in vitro cell cytotoxicity assay was performed. It was found that proliferation of cancer cell lines i.e MDA-MB- 231 breast cancer cell lines and EAC cell controlled by metformin loaded microsphere. The reports of the MTT assay supported the fact that there was no cytotoxicity.

At the same time determination of intracellular ROS in the in vitro MDA-MB-231 breast cancer cell lines was performed.

Flow cytometric analysis of mitochondrial membrane potential was performed . it was done in MDA-MB-231 breast cancer cell lines.

The mean body weight changes with various doses of metformin microsphere 250mg, 500mg, 850mg, body wt. was performed. No significant changes in body wt. was found with the experimental mice after using the formulation.

The toxicity study of the metformin loaded microsphere was performed. According to the guidelines of the animal ethics committee and the OECD. We have to perform the toxicity study. Here we took various doses of metformin loaded microspheres. The doses may be of 250, 500mg/kg body wt. It was found that haematological and biochemical parameters was not changed.

So it can be said that the prepared formulation was safe and non-toxic.