Design and development of polymeric microspheres loaded with model drug to provide sustained-release

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Abstract-

Introduction: Diabetes is defined as a state of hyperglycemia in either fasting or postprandial states. The chronic hyperglycemia of diabetes mellitus (DM) is associated with end-organ damage, dysfunction, and failure in organs and tissues including the retina, kidney, nerves, heart, and blood vessels. Microspheres constitute an important part of particulate drug delivery systems by their small size and efficient drug entrapment capacity, deliver the therapeutic amount of the drug to the proper site in the body and then maintain, the desired drug concentration. In the present studies, it was planned to develop gastroretentive mucoadhesive microspheres of metformin by spray drying technique.

Material and methods: The selection of the excipients and process was done by pre-optimization studies and for the final batch formulation optimization studies were performed. From the pre-optimization and optimization studies, it was concluded that the spray drying process is the most suitable process for the proposed formulation, and the more suitable excipients are Eudragit RSPO and HPMC K4M. The process parameters were also selected for drying rate and temperature. The selection of response variables was also done from the results of pre-optimization studies such as particle size, entrapment efficiency, and in-vitro drug release.

Result and Discussion: The optimized batch's in-vitro drug release research revealed consistent drug release for up to 3 hours and mucoadhesion for up to 5 hours. The optimized mucoadhesive microspheres' micrometric characteristics, including bulk density, tapped density, compressibility index, Hausner ratio, and angle of repose, were found to be 0.16 g/cm^3, 0.14 g/cm^3, 10.1%, and 35.1°, respectively. The entrapment efficiency was found to be 69.4%. The image of SEM confirmed that microspheres were developed in regular size with the loading of the drug.

Conclusion: The formulated polymeric microspheres containing metformin helped to improve the oral bioavailability of the drug and controlled its release rate, which lowers the frequency of dose and is beneficial for long-term treatment in the case of diabetes.

Keywords: **Polymeric microspheres, mucoadhesive microspheres, spray drying method, metformin, controlled release.**

1. INTRODUCTION

Diabetes is a chronic condition brought on by either insufficient insulin production by the pancreas or inefficient insulin use by the body. A hormone called insulin controls blood sugar levels[5]. Uncontrolled diabetes frequently causes hyperglycemia, also known as high blood glucose or raised blood sugar, which over time can seriously harm many different bodily systems, including the neurons and blood vessels. It affects men, women, and children of all ages in every country, and more than half a billion people globally have diabetes. In the next 30 years, that figure is expected to more than double to 1.3 billion people, with a rise in every country[1].

Metformin Hydrochloride (MF) is a BCS class-2 drug most commonly and widely used in the treatment of diabetes mellitus. Metformin reduces hepatic production of glucose, decreases the intestinal absorption of glucose, and enhances insulin sensitivity by increasing both peripheral glucose uptake and utilization. Metformin Hydrochloride (MF) is a glucose-lowering agent that is widely used for the management of type II diabetes. MF is reported to be absorbed mainly in the upper part of GIT. It has having narrow absorption window and high water solubility, and it would be more beneficial to retain the drug in the stomach for a prolonged duration to achieve maximum absorption and better bioavailability[2].

A conventional oral CR formulation releases most of the drug content in the colon, which requires that the drug be absorbed from the colon. The present investigation aims to develop a novel gastroretentive (GR) drug delivery system, which not only releases the drug in the absorption window but also provides a controlled release drug profile that may result in patient compliance and therapeutic success. A gastroretentive drug delivery system is a type of controlled drug delivery system that can retain and prolong the release of the drug in a particular region of the gastrointestinal tract (GIT) and thereby increase the gastric residence time of drugs. The gastro retentive drug delivery systems are designed

based on delayed gastric emptying and controlled release principles. The present study aimed to prolong the residence time of dosage form in the stomach to improve the absorption of the drug throughout the upper gastrointestinal tract and subsequently bioavailability by formulating gastro retentive bioadhesive microspheres[3]."
Microspheres constitute an important part of these particulate drug delivery systems by their small size and efficient drug entrapment capacity, deliver the therapeutic amount of the drug to the proper site in the body and then maintain, the desired drug concentration. However, the success of these microspheres is limited due to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing longer retention of the drug delivery system within the absorbing site. This can be achieved by coupling adhesive characteristics to microspheres and developing bioadhesive microspheres[4,5].

2. EXPERIMENT

2.1. Material and methods

2.1.1. Materials

Selection of the excipients and process was done by pre-optimization studies and for the final batch formulation optimization studies were performed. From the pre-optimization and optimization studies, it was concluded that the spray drying process is the most suitable process for the proposed formulation, and the more suitable excipients are Eudragit RSPO and HPMC K4M. Metformin, Eudragit RSPO, and HPMC K4M were selected for the formulation.

2.1.2. Methods

2.1.2.1. Preparation of polymeric microspheres

The optimized batch of polymeric microspheres was prepared using a spray drying method with slight modification. Mucoadhesive microspheres of metformin were prepared by spray dryer (make- JISL, Jay Instruments, and systems) Model- Spraymate/software-spray-in). Hot water was used to dissolve HPMC K4M while being constantly stirred. In ethanol, metformin Eudragit RSPO was dissolved. HPMC solution was stirred while an ethanol solution containing medication and Eudragit RSPO was added. Using a magnetic stirrer, the finished solution was subsequently agitated for an additional 10 minutes. The product was then collected after this solution was sprayed using a spray drier. Below are descriptions of the spray drying parameters[6,7].

Table 1: Factors selected for optimization batches

Variable	Unit	Type	Low Actual (-1)	Middle Actual	High Actual
Metformin	mg	Numeric	50	125	200
Eudragit RSPO	mg	Numeric	100	300	500
HPMC K4M	mg	Numeric	500	750	1000

2.1.2.2. Selection of response variables

The responsible variables were selected on the basis desired characteristics of microspheres. The particle size, entrapment efficiency, and % drug release were selected as response variables. Response variables are shown in Table 2.

Table 2: Response variables selected for optimization in Box-Behnken design

Response variables	Unit
Particle size	Micron
Entrapment efficiency	%
Drug release in 15 min	%
Drug release in 360 min	%

2.2. Optimization of Spray Drying Parameters (Process Variables)

Metformin-loaded loaded spray dried microspheres were prepared using a spray dryer (Model-spraymate, Manufacturer- JISL, Mumbai).

- **Optimization of Inlet and Outlet temperature:** Inlet and outlet temperatures are directly related to the residual moisture of solids. The temperature difference between the inlet and outlet temperature should not be too high.
- **Optimization of the feed rate of liquid:** The feed rate of liquid is controlled by a peristaltic pump. The rotational speed of the peristaltic pump is expressed in RPM (rotations per minute). Feed rate has a direct effect on particle size.

- **Optimization of atomization pressure:** Atomization pressure is the pressure required to atomize the fluid stream into droplets.

The microspheres of trial batches were prepared by a similar procedure. The volume of solvents (water and ethanol) was kept constant throughout the optimization of formulation.

Table 3: Experimental plan for optimization of Metformin HCl mucoadhesive microspheres

Formulation Code	Eudragit RSPO	Hydroxypropyl methylcellulose K4M
MM 1	100	700
MM 2	200	500
MM 3	300	850
MM 4	500	1000
MM 5	300	700
MM 6	300	1000
MM 7	100	500
MM 8	500	1000
MM 9	100	850
MM 10	100	1000
MM 11	500	700

2.3. Evaluation of Metformin Loaded Microspheres

The prepared microspheres optimization batches were evaluated for the following parameters: percentage yield, entrapment efficiency, particle size distribution, and in-vitro drug release profile[8,9]. The results are shown in Table 4.

Table 4: Evaluation of Percentage Yield and Entrapment Efficiency of Microspheres (Optimization Batches)

S.No.	Formulation Code	Theoretical Yield (mg)	Practical Yield (mg)	Percentage Yield (%)	Entrapment Efficiency (%)
1	MM 1	1020	300	35.4	82.3
2	MM 2	1300	720	40.3	70.5
3	MM 3	1450	330	38.7	80.5
4	MM 4	1170	250	25.6	75.5
5	MM 5	1120	800	50.3	90.5

6	MM 6	850	550	51.2	95.3
7	MM 7	1300	250	39.0	79.5
8	MM 8	1175	330	47.2	81.5
9	MM 9	1000	700	52.8	83.7
10	MM 10	1200	213	37.4	88.8
11	MM 11	900	230	48.9	85.7

MM: Metformin Microspheres

2.3.1. In-vitro drug release study

To ascertain the percentage of drug release from the produced formulation, an in vitro dissolving test of microspheres was conducted. The study was carried out using 0.01 M sodium phosphate containing 0.5% SLS (pH 7.0) as a dissolution medium. The dissolving media was filled with microspheres. After each withdrawal at a specific period, samples were taken out, filtered through 0.45µ membrane filters and the medium refilled into the same volume of fresh dissolving media while maintaining the same temperature. The withdrawn samples are diluted and examined at 234 nm using a UV spectrophotometer. The table below lists the requirements for dissolution[10].

Table 5: Parameters and their description for dissolution study

S. No	Parameters	Description
1.	Apparatus	Electro lab TDT06P
2.	Dissolution medium	0.01 M Sodium phosphate containing 0.5% SLS (pH 7.0)
3.	Temperature of medium	37°C ± *0.5*°C
4.	Volume of medium	900 ml
5.	Speed	50 RPM
6.	Sampling interval	15, 30, 60, 120, 180, 240 and 360 minutes

Table 6: In-vitro drug release data for optimized batches of metformin microspheres

S.No.	Formulation code	Cumulative drug release in 15 mins.	Cumulative drug release in 360 mins.
1	MM 1	13.3	96.5
2	MM 2	12.5	84.2
3	MM 3	12.6	79.3
4	MM 4	14.2	82.5
5	MM 5	8.5	60.7

6	MM 6	9.7	75.8
7	MM 7	12.1	85.1
8	MM 8	16.4	95.6
9	MM 9	11.5	73.1
10	MM 10	13.3	98.7
11	MM 11	10.7	82.9

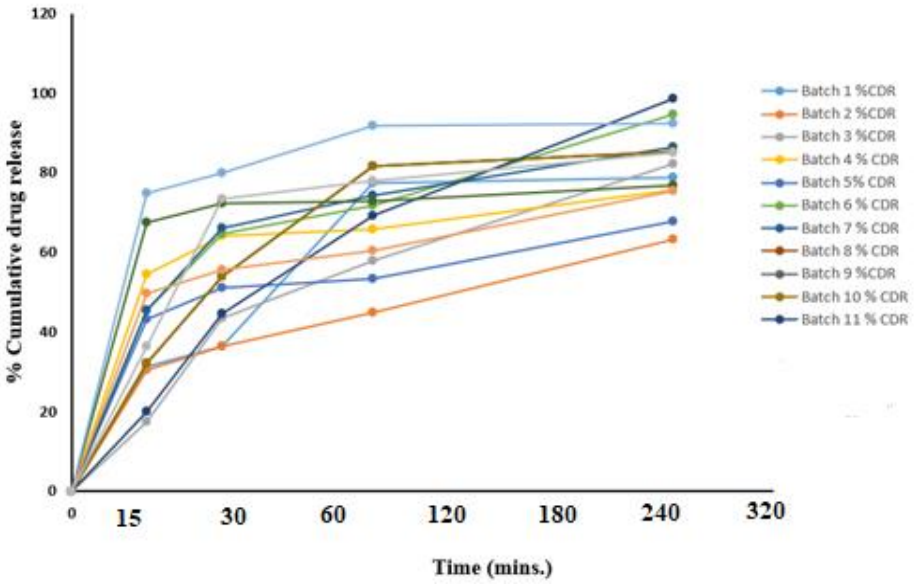

Fig. 1: In-vitro drug release profile drug-loaded Microspheres

2.4. Prediction of Optimized Mucoadhesive Microspheres of Metformin HCl

To obtain the optimized formulation, the desired criteria were set, and based on the results of the optimization batches the Final formula for the final batch was selected as shown in the below table 7.

Table 7: Factors for the optimization batches of microspheres

S. No.	Ingredients	Quantity
1.	Eudragit RSPO	160
2.	HPMC K4M	800

The final batch was prepared by using the above formula through the process which was used for optimization batches as described in section 2.1.2.1 via spray dryer.

3. EVALUATION OF THE METFORMIN LOADED MICROSPHERES FINAL BATCH

3.1. Bulk density

A 10 g accurately weighed sample was filled in a 100 ml graduated cylinder and settled volume was noted. The bulk density was calculated in g/cm^3 by the following formula and recorded in the table 8. [11]

Bulk density = M/Vo

Where,

M = Mass of powder taken
Vo = Apparent volume

3.2. Tapped density

A 10 g accurately weighed sample was filled in a 100 ml graduated cylinder the tapping was done 500 times and the tapped volume was noted. Tapping was continued further for an additional 750 times and the tapped volume was less than 2%, so Vf was considered as the tapped volume. The tapped density was calculated in g/cm^3 by the following formula and recorded in the table 8.

Tapped density = M /Vf

Where,
M = Weight of the sample powder taken
Vf = Final tapped volume

3.3. Compressibility index

The compressibility index (CI) was calculated using the following formula and recorded in the table 8.

CI = { (Vo – Vf) × 100 }/Vo

3.4. Hausner ratio

Tapped density and bulk density were measured and the Hausner ratio was calculated using the following formula, recorded in table 8.

Hausner ratio = Vo / Vf

Table 8: Calculated values for micrometric properties

Parameters	Calculated values
Bulk density	0.16 g/cm^3
Tapped density	0.14 g/cm^3
Compressibility index	10.1%
Hausner's ratio	1.2
Angle of repose	35.1 degree

Inference

The flow property of microspheres was fair and % compressibility was found to be excellent as per Table 8.

3.5. Differential scanning calorimetry (DSC)

The results of DSC tests were obtained using differential scanning calorimetry (Perkin Elmer DSC 6000) that was calibrated by Indium. The analysis of samples of pure drug, drug-loaded microspheres that have been optimized, and the sample taken 3.5 mg, was placed in a standard-grade aluminum pan and sealed. The analysis of the sample was carried out in the temperature range of 50 to 300°C at a 20 ml/min scanning rate.

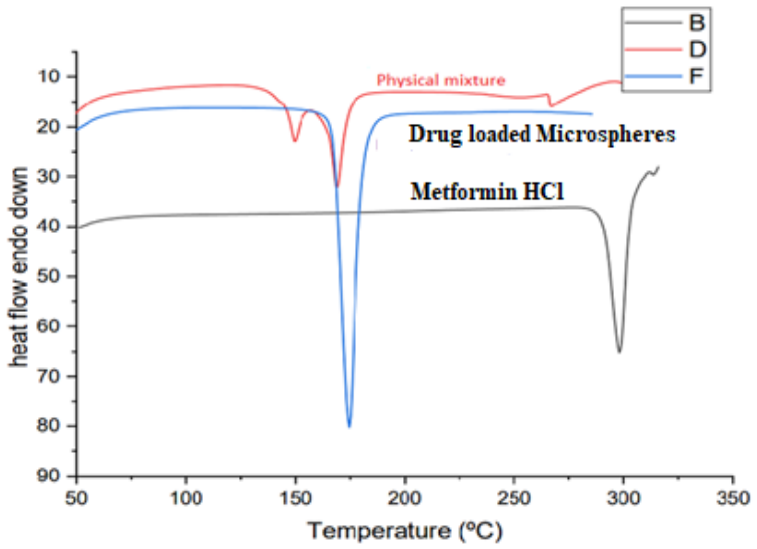

Fig. 2: DSC of the physical mixture, drug-loaded microspheres, metformin HCl

3.6. Entrapment efficiency % and loading of drug

The entrapment efficiency and loading of drug molecules in microspheres are the desirable characteristics of microspheres. The entrapment efficiency and drug loading of the final optimized metformin-loaded microspheres were found to be 76.05% and 48.56% respectively[12].

Entrapment efficiency was calculated by the following formula:

$$\%\text{Entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

3.7. In-vitro drug release studies

To ascertain the percentage of drug release from the produced formulation, an in vitro dissolving test of microspheres was conducted. The study was carried out using 0.01 M sodium phosphate containing 0.5% SLS (pH 7.0) as a dissolution medium. The dissolving media was filled with microspheres. After each withdrawal at a specific period, samples were taken out, filtered through 0.45µ membrane filters and the medium refilled into the same volume of fresh dissolving media while maintaining the same temperature. The withdrawn samples are diluted and examined at 232 nm using a UV spectrophotometer. The table below lists the requirements for dissolution[13-15].

Table 9: In-vitro drug release of drug-loaded microspheres

S. No.	Time Interval (min.)	% Cumulative drug release
1	15	13.5
2	30	25.6
3	60	38.9
4	120	53.6
5	180	65.7
6	240	80.9
7	320	93.4

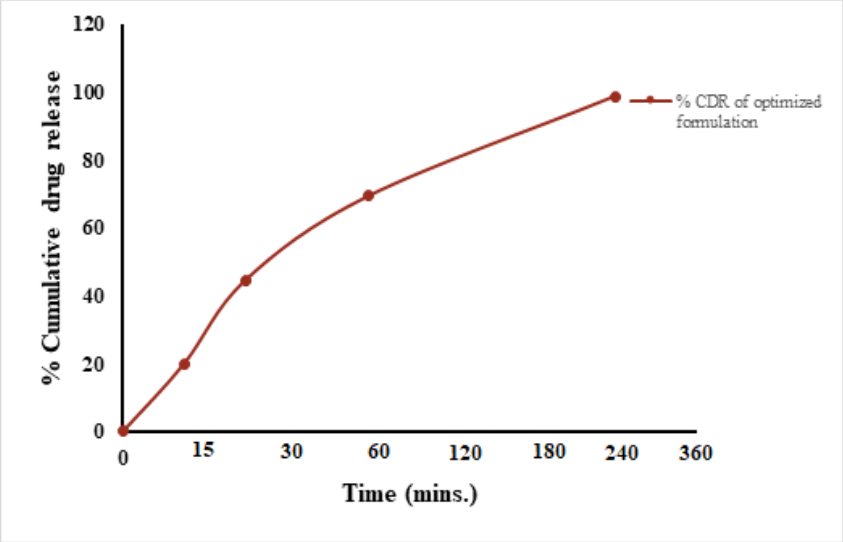

Fig. 3: In-vitro drug release of the final batch of drug-loaded microspheres

4. SUMMARY AND CONCLUSION

Due to the high prevalence of diabetes mellitus (DM), poor health outcomes, and rising costs of care, attention must be paid to disease prevention, early detection, and optimal management. Diabetes mellitus (DM) is a metabolic condition marked by unnecessarily high blood glucose levels. Type 1, type 2, maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and secondary causes owing to endocrinopathies, steroid usage, etc. are among the several kinds of DM.

Serious difficulties are brought on by the ensuing hyperglycemia. The medicine metformin has been found to reduce the majority of diabetic complications and prevent diabetes in persons who are at high risk. Recent findings on metformin not only offer positive implications, such as renoprotective characteristics, but some reports also highlight its detrimental effects, which are minor when its benefits are taken into consideration. Metformin is a highly soluble, archetypal transporter-mediated medication, yet only 50% of an oral dose is absorbed from the stomach. Metformin is a BCS Class III drug as a result. Hence metformin suffers low bioavailability and short half-life problems. To overcome these drawbacks, controlled release gastroretentive microspheres were developed.

A gastroretentive drug delivery system is a type of controlled drug delivery system that can retain and prolong the release of the drug in a particular region of the gastrointestinal tract (GIT) and thereby increase the gastric residence time of drugs.

In the present studies, it was planned to develop gastroretentive mucoadhesive microspheres of metformin by spray drying technique.

The procured sample of Metformin HCl was characterized by melting point determination, UV spectroscopy, infrared spectroscopy, and differential scanning calorimetry. The melting point of the drug sample was found to be 223-236°C. The UV, FTIR spectrum, and DSC curves were compared with the reported literature and it was concluded that the procured drug sample was pure and used for further studies.

In pre-formulation studies, solubility analysis and calibration curves were prepared in different solvents. The solubility of metformin in water, 0.1 M phosphate buffer (pH-7.4), in ethanol, in pH 6.8 buffer by using double beam UV visible spectrophotometer (Shimadzu 1700). The correlation coefficient of the calibration curve of Metformin in this solvent medium was found to be very close to one. The linearity of calibration curves showed that the Beer-Lambert law was observed in the concentration range of 5-25 µg/ml of the drug in all solvent mediums. Gastro retentive microspheres of metformin were prepared using metformin, Eudragit RSPO, and HPMC K4M by spray drying technique.

Metformin, Eudragit RSPO and HPMC K4M were selected as factors for optimization batches for the preparation of polymeric microspheres and was prepared using a spray drying method. The prepared microspheres optimization batches were evaluated for the parameters such as percentage yield, entrapment efficiency, particle size distribution, and in-vitro drug release profile. Optimization of the developed formulation was done by taking different quantities of the components and the quantity of the drug was kept constant. The microspheres of trial batches were prepared by a similar procedure. The volume of solvents (water and ethanol) was kept constant throughout the optimization of formulation. Finally, mucoadhesive metformin microspheres were created and experimentally validated using the

expected optimized formula. The optimized batch's in-vitro drug release research revealed consistent drug release for up to 3 hours and mucoadhesion for up to 5 hours. The optimized mucoadhesive microspheres' micrometric characteristics, including bulk density, tapped density, compressibility index, Hausner ratio, and angle of repose, were found to be 0.16 g/cm^3, 0.14 g/cm^3, 10.1%, and 35.1°, respectively. The entrapment efficiency and drug loading of the final optimized metformin-loaded microspheres were found to be 76.05% and 48.56% respectively.

In summation, it can be concluded that the development of metformin-loaded long-acting gastro-retentive bioadhesive microspheres was successfully done. The developed microspheres for gastro retention in the present study showed consistent drug release for 5 hours that can provide better gastric retention and long action with lesser side effects and produce more advantages over conventional drug delivery systems.

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