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Original Article

Development and Evaluation of Levosulpiride Loaded Floating Microspheres for Gastrointestinal Disorder

Anshul Tiwari*, Ankur Singai, Anurag Dubey, Sunil Kumar Jain, Mahak Jain
Adina Institute of Pharmaceutical Sciences, NH-86, Bhopal Road, Sagar (M.P.), India-470001

ABSTRACT
In the present study the floating microspheres of the levosulpiride were prepared. The sodium alginate, sodium carbonate and HPMC K15m were selected as formulation variables. The characterization of prepared formulations was carried out. The drug release study shows that the four different formulations prepared gives cumulative drug release 73.49 to 88.13% at the end of drug release study. The highest drug release was obtained in LS3 batch. *In vitro* drug release profile indicated that drug release was retarded due to the presence of higher concentration of polymer. The sodium alginate, sodium carbonate and HPMC K15m in the ratio 1:2:1 respectively shows desired drug release properties. The formulations were quite stable at the ambient temperature conditions but accelerated temperature could affect the release behavior of the floating microspheres.
Keywords: Levosulpiride, Floating microspheres, sodium alginate, HPMC, sodium carbonate

Address for Correspondence:
Anshul Tiwari
Department of Pharmaceutics,
Adina Institute of Pharmaceutical Sciences, Sagar (M.P.)-India 470001
Email: ansultiwari95@gmail.com

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INTRODUCTION

Diabetes is one of the major causes of death and disability in the world. The latest, WHO estimate for the number of people with diabetes worldwide, in 2000, is 171 million, which is likely to be at least 366 million by 2030. The focus of medical community is on the prevention and treatment of the disease, as is evident from the rising number of research papers every year on the subject (Khan et al., 2020). The primary aim of oral controlled drug delivery is the most preferable route of drug delivery system is to achieve better bioavailability and release of drug from the system which should be predictable and reproducible, easy for administration, patient compliances and flexibility in formulation for effective therapy or to improve therapeutic efficiency of the drug through improved bioavailability (Homayun, et al., 2020). Floating drug delivery systems or hydro dynamically balance systems have a bulk density lower than gastric fluids and thus remain buoyant in the stomach for a prolonged period of time without affecting the gastric emptying rate. The drug is released slowly at a desired rate from the system and drug residual systems are emptied from the stomach. This results in increase in the gastric residence time and a better control of qualification in plasma drug concentration (Kandwa et al., 2014; Parmar et al., 2014).

Levosulpiride is a substituted benzamide antipsychotic used in the treatment of psychoses such as schizophrenia, anxiety disorders, vertigo and also in peptic ulceration. It is a selective antagonist of the dopamine D2 receptors on both central and peripheral nervous systems. Levosulpiride is slowly and weakly absorbed from the gastrointestinal tract with an oral bioavailability of less than 25% and short

half-life of about 6 h. Levosulpiride is a widely used gastroprokinetic agent in the treatment of various gastric disorders; however, its short half-life and increased dosage frequency leads to non-compliance and possible adverse effects (Gupta et al., 2007).

The main objective of this study was to develop and evaluate the floating microspheres of Levosulpiride used as anti-hyperglycemic agent, in order to increase its residence time at the site of absorption and thus improve its bioavailability; and to extend the duration of action along with possibilities of dose reduction.

2. MATERIALS AND METHODS

Chemicals

Levosulpiride was provided by Sun Pharmaceuticals Ltd. Ahmedabad, Sodium Alginate was provided by S.D. Fine Chemicals, Mumbai and HPMC-K15 obtained from Emcure Pharmaceuticals, Pune. Other chemical were of analytical grade obtained from market and used without further purification or investigation.

Preformulation study

The obtained drug was evaluated for the organoleptic characteristics, solubility, melting point and partition coefficient.

Partition coefficient study

Partition coefficient is a measure of drugs lipophilicity and an indicator of its ability to cross bio-membranes. It can be defined as the ratio of unionised drug in organic phase and aqueous phase at equilibrium.

Po/w= Coil/Cwater

The partition coefficient of levosulpiride was determined in n-octanol/water (pH 7.0), and n-octanol/PBS (pH 7.4) system employing the method reported by (Finizo, 1997).

10 mg of levosulpiride was accurately weighed and taken in a stopper tube containing 10 ml each of n-octanol and aqueous phase. The tubes were placed on a wrist shaker for 6 hours until equilibrium was reached. Phases were separated using the separating funnel and the aqueous phase was analysed for amount of drug after appropriate dilution.

Solubility study

The solubility of Levosulpiride was determined in solvents of different polarities. The solubility of Levosulpiride is usually determined by the equilibrium solubility method, which employs a saturated solution of Levosulpiride, obtained by adding an excess amount of Levosulpiride in the solvent to promote drug precipitation, and then stirring for 6 hrs until equilibrium was reached. The mixture was filtered and amount of Levosulpiride was determined by using UV Spectrophotomer at 290 nm .

Fourier Transform Infrared Spectroscopy

The FT-IR analysis of the Levosulpiride was carried out for qualitative compound identification. The FT-IR spectra for pure drug and with other excipients was obtained by placing the drug directly into the cavity and was determined by FT-IR spectrophotometer in the wave number region of 4000-400 cm^{-1}. The drug excipient compatibility was also checked using FTIR studies. The drug excipients compatibility study performed by mixing the drug with excipients in 1:1 ratio. The mixture was kept in hot air oven at 60^{o}C for a week. The DSC study of the mixture was performed.

Differential scanning calorimetry

DSC curves were obtained in a Perkin-Elmer (Pyris 1 DSC) cell using aluminium crucibles with about 2 mg of samples, under a dynamic N_2 atmosphere (flow rate: 50 mL/min) and at a heating rate of 10°C/min in the temperature range 25 – 400°C.

Preparation of floating microsphere

The formulation and process variables such as stirring speed and concentration of calcium chloride which could affect the preparation and properties of floating microspheres were identified and studied. The optimization was done on the basis of particle size and the shape of the microspheres.

The drug and polymer solution were added dropwise through a syringe with a 25-gauge needle into 100 mL of calcium chloride solution and stirred at 200 rpm. The microspheres formed were kept suspended in

the solution for 1 h to improve their mechanical strength and then collected by filtration. After that, the floated microspheres were washed with 100 mL of distilled water 3 times and then dried in a hot air oven for 12 h at 50°C and then stored in a desiccator (Abbas AK, et al. 2020). The composition of the floating microspheres is given in following Table 1.

Table 1 Formulations of the Floating Microspheres

Sr. No	Formulation Code	Drug (mg)	Sodium alginate (mg)	Sodium bicarbonate (mg)	HPMC-K15m	Calcium Chloride (%)
1.	LS_1	75	100	200	100	2
2.	LS_2	75	200	100	100	2
3.	LS_3	75	100	200	100	2
4.	LS_4	75	200	100	100	2

Optimization of the stirring speed

Stirring speed from mechanical shaker was varied from 100 to 500 rpm for floating microspheres preparation of selected formulation LS3 based on the drug release characteristics while keeping the other variables constant. The particle size and shape were determined which are recorded

Optimization of concentration of calcium chloride

Concentration of calcium chloride was varied from 1% to 4%, to study its effect on the particle size and shape of the microspheres that are formed. 2% solution of the calcium chloride was suitable for the formulation of microspheres.

Determination of Percentage Yield

The percentage yield was calculated using the weight of a dried final product regarding the total weight of the drug and polymer measured initially and used for the preparation of microspheres. The percentage

yields were calculated as per the formula **(Veseli *et al*. 2019)**

Percentage yield = [Weight of microspheres obtained/(Weight of drug+polymer)]×100

Determination of entrapment efficiency

Floating microspheres weighed and crushed in 100 mL of 0.1 N HCl. An aliquot of 1 mL was taken and diluted to 10 mL; after that the mixture was shaken and filtered through a 0.45 µm filter and then analyzed using ultraviolet (UV) spectrophotometer (Shimadzu 8400S, Japan) using the prepared calibration curve. Each batch should be examined for drug entrapment in triplicate (Prajapati et. al. 2012).

% Entrapment efficiency

= (Actual drug content) ×100/ (Theoretical drug content)

Determination of *in vitro* Buoyancy

In vitro buoyancy was determined to study the floating behavior of microspheres in the prepared formulations. First 100 mg of microspheres was spread in 0.1 N HCl (pH 1.2; 250 mL). The mixture was then stirred at 100 rpm in a magnetic stirrer. After 16 h, the buoyant microparticles layer was collected by pipette and separated by filtration. The particulate sinking layer particles were separated by filtration. Particles of both types were dried in a desiccator until a constant weight was obtained (Kolagani et al 2020). The buoyancy percentage was calculated by the following equation:

% Buoyancy= (Weight of floating microspheres)/(Total weight of floating and settled microspheres)×100

***In vitro* drug release study**

The in vitro drug release rate from the floating microspheres was performed using a paddle type six-station dissolution test apparatus. An accurate amount of floating microspheres equivalent to 75 mg of drug was kept in 0.1 N HCl (1.2 pH) and the dissolution fluid was maintained at 37±0.5°C at a speed of rotation of 50 rpm. Sink

conditions prevailed during the in vitro drug release study. The sampling was done at various intervals and concentration was determined by U. V. Spectrophotometer.

Scanning Electron Microscopy

Morphology details of the specimens were determined by using a Scanning Electron Microscope (SEM), Model JSM 35CF, JEOL, Japan.

The samples were dried thoroughly in vaccum dessicator before mounting on brass specimen studies. The samples were mounted on specimen studies using Double sided adhesive tape. The sputtering was done for nearly 3 minutes to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage. The condenser lens position was maintained between 4.4-5.1. The objective lens aperture has a diameter of 240 microns and the Working Distance WD is 39 mm.

Drug Release Kinetics

To determine the mechanism and kinetics of drug release, the results of the in vitro dissolution study of enalapril microspheres were obtained for various kinetic equations. The kinetics models used were zero order, first order, Higuchi's, and Korsmeyer Peppas. Correlation coefficient (R2) values were calculated for the linear curves obtained by regression analysis (Paarakh et. al. 2018).

Zero order Drug release

$Qt = Qo + Kot$ (1)

First order drug release

$\log Qt = \log Qo + K_1 t /2.303$ (2)

Higuchi model

$Qt = KH \sqrt{t}$ (3)

Korsemeyer-Peppas model:

$Mt /M\infty = Kt\ n$ (4)

Hixon Crowell cube root law

$\mathbf{Mo^{1/3}-Mt^{1/3}=kt}$

Where Qt is the amount of drug dissolved at time, t; Q0 is the initial amount of drug in the solution at time t=0, Q is the amount of drug remaining at time, t; Mt/M∞ is the fraction of drug released at time, t and n is diffusion exponent. K0, K1, KH and K refer to the rate constants of respective kinetic models.

Stability Study

Stability studies of the optimized formulation LS3, packed in HDPE containers up to 6 months were carried according to International Confere1nce on Harmonization (ICH) guidelines; in a humidity chamber maintained at 45°C ± 2°C and 75% ± 5% RH . At the end of every month up to 6 months, the samples were withdrawn and evaluated for post compression studies. The consolidated results of accelerated stability studies were tabulated. Comparative in vitro dissolution profiles of initial and accelerated stability samples were shown in figure. The chemical stability of drug in the 6M-accelerated stability sample of optimized (LS3); which will influence it's in vitro dissolution characteristics, was investigated.

3. RESULTS AND DISCUSSION

The drug solubility study was performed in various solvents shows that drug is more soluble in the alkaline conditions Phosphate 7.4 while it has lower solubility in acidic conditions (pH 1.2). It was soluble in the organic solvents.

The drugs were identified by the melting point of the drug. The partition coefficient of the levosulpiride was 3.0 in n-octanol/water and 1.2 in n-octanol/PBS system showing the drug is hydrophobic in nature

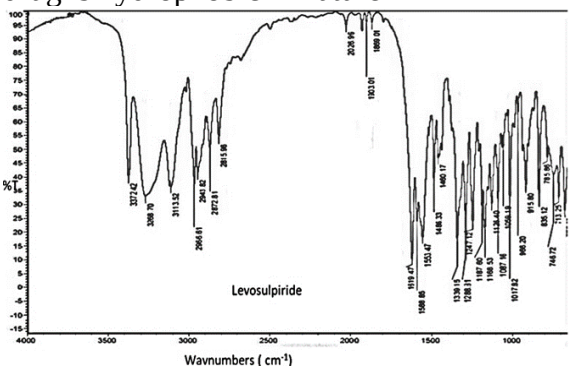

Fig.1 F.T.I.R. Spectra of Levosulpiride

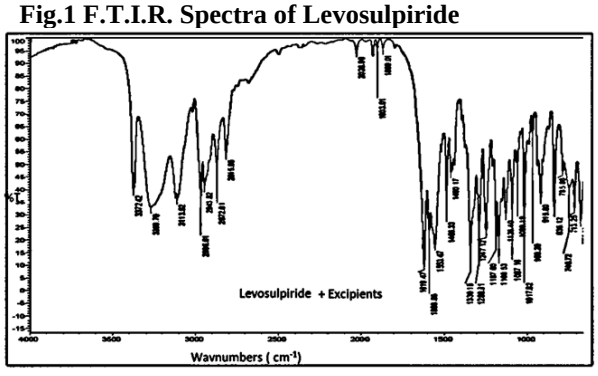

Fig.2 F.T.I.R. Spectra of Levosulpiride in Combination with excipients

The major peak assignment is shown in the table 2. The assignment of peaks in FTIR shows obtained drug was levosulpiride and it was compatible with the excipients in the physical mixture.

Table 2 F.T.I.R. Spectra of Levosulpiride and in Combination with excipients

Sr No	Functional group	Levosulpiride	Levosulpiride+Excipients
1	O-H stretching	3268.70	3268.70
2	C-H stretching	2872.81	22872.61
3	C=O stretching	1869.01	1869.01
4	S=O stretching	1059.19	1017.82

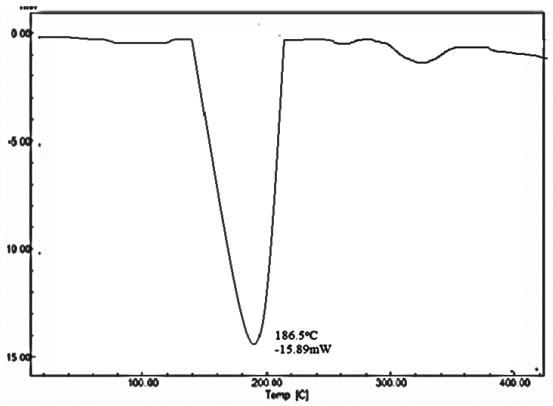

Fig. 3 DSC Thermogram of Levosulipiride

The levosulride melts at 186.5^{o}C while its mixture with excipients melts at the187.33^{o}C revealing that the drug is compatible with the formula ingredients as shown in fig. 2 and 3. The drug was found to be compatible with the excipients. Hence, the selected excipients are suitable for the formulation of the microspheres.

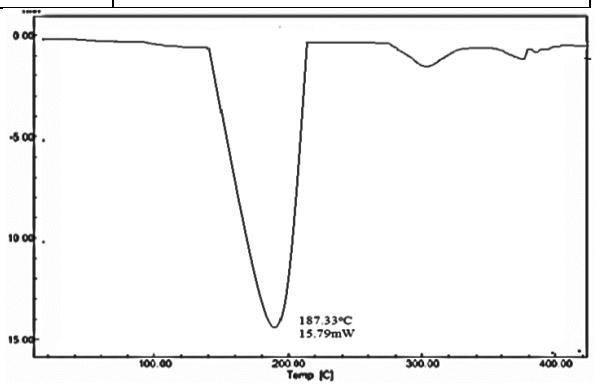

Fig. 4 DSC Thermogram of Levosulipiride +Excipients

The experiments were carried out for optimization of the stirring speed and concentration of calcium chloride. The selected stirring speed was 200 rpm and selected concentration of calcium chloride was 2% as microspheres of lower particle sizes were obtained compared with other batches. Further formulations were carried out at 200rpm and using 2% solution of calcium chloride. The results of the various optimization parameters are shown in table

Table 3 Effect of stirring speed on particle size and shape of floating microspheres

Speed (rpm)	Particle size (µm)	Shape
100	785.85±4.58	Oval

200	438.96±3.80	Spherical
300	589.45±5.46	Spherical
400	709.30±8.75	Elongated

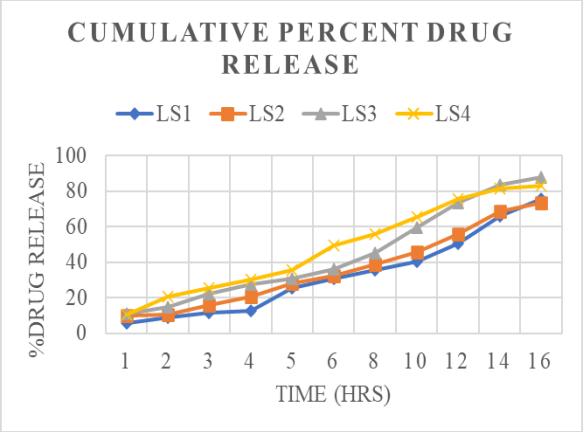

Table 4 Optimization of concentration of calcium chloride

Calcium Chloride (%)	Particle size (µm)	Shape	Percentage yield	In Vitro Buoyancy
1	900.56±8.54	Elongated	65.29±2.51	50.56±2.29
2	620.78±3.35	Spherical	75.33±1.51	65.79±0.75
3	690.56±5.16	Spherical	78.19±1.26	70.56±1.33
4	750.89±6.27	Oval	60.76±2.89	55.95±2.76

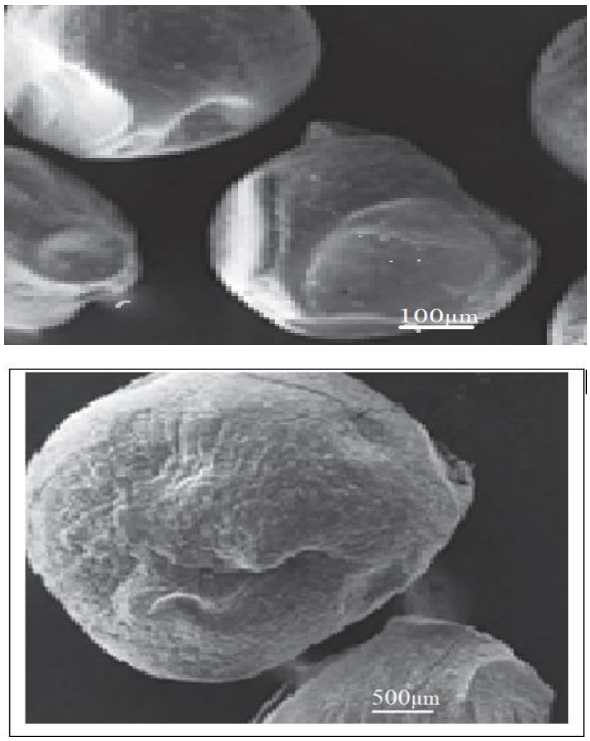

Fig. 6 Scanning Electron Microscopy

The percentage yield was determined and shown in the table given below. It reveals that the batch LS3 shows highest 78.19±1.26% yield. The results of drug entrapment studies are shown the table reflects that batch LS3 exhibits the 78.00±0.89 which is more as compared to other formulation batches. The results of In Vitro Buoyancy of microspheres shows that about 70.56±1.33% microsphere were floating at the end of the test period indicating that Batch LS3 can be considered for the further optimization studies. The results of cumulative drug release are mentioned in the following table and figure batch LS1, LS2, LS3 and LS4 shows 75.66%, 73.49%, 88.13% and 83.15% drug release at the end of 16 hrs thererefore Batch LS3 was selected as optimized batch.

The scanning electron microscopy shows the almost spherical nature of the floating microspheres. The particle size of the microspheres was 435±97µm. Additionally,

the porous structures were observed on the microspheres which may help in controlled drug release from the floating microspheres.

The optimized formulation LS3 found to follow zero order drug release with r2 value 0.9908. The r2 value for the Korsmeyer Peppas model was 0.9402, for first order model it was 0.2646, for Hixon Crowell model it was 0.9603 and for Higuchi Model the value was 0.9832.µ

Stability studies of the optimized formulation LS3, packed in HDPE containers up to 6 months were carried according to International Confere1nce on Harmonization (ICH) guidelines; in a humidity chamber maintained at 45°C ± 2°C and 75% ± 5% RH . At the end of every month up to 6 months, the samples were withdrawn and evaluated for post compression studies. The consolidated results of accelerated stability studies were tabulated. Comparative in vitro dissolution profiles of initial and accelerated stability samples were shown in figure. The chemical stability of drug in the 6M-accelerated stability sample of optimized (LS3); which will influence it's in vitro dissolution characteristics, was investigated.

Table 10.1. Accelerated Stability Testing of Optimized Batch

Sr No.	Time interval	Drug Content
1	Initial	99.69±2.56
2	1 months	99.56±1.33
3	3 Months	98.71±1.09
4	6 Months	98.37±0.93

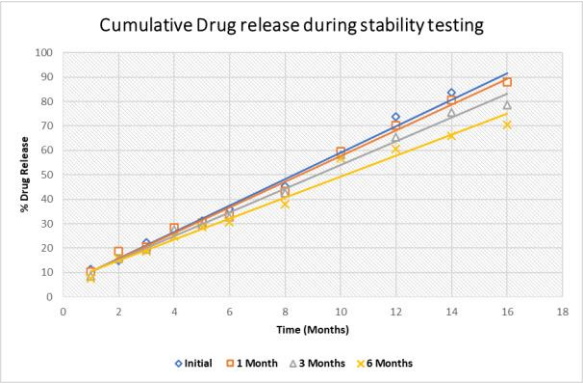

Fig. 7 Cumulative drug release during accelerated stability test

The results of stability testing shows that the drug content decreases with the increase in time interval during the accelerated stability test. The cumulative drug release was also diminished with time. The reason may be assigned to the crosslinking of the polymers during the storage at accelerated temperature.

4. CONCLUSION

The result obtained from all the experimental data collected in the dissertation revels that it is possible to prepare an intragastric floating and extended release floating microspheres preparation by using sodium alginate, HPMC-K15m and sodium bicarbonate (1:2:1 ratio) floating microspheres drug delivery system provides the possibility of enhancing the bioavailability and control the release of levosulpiride exhibiting absorption window by prolonging the gastric emptying time of the dosage form ensuring availability of drug at the absorption site for the desired period of time. The floating microspheres showed a good buoyancy and drug release properties. The optimized formulation shows the zero-order release pattern exhibiting the controlled release behaviour as desired for the floating microspheres. The developed formulation has great potential to use it for the industrial applications provided that further scale up studies are performed.

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