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Formulation and Evaluation of Microspheres using Metoprolol Succinate

Gajanan J Deshmukh[1,2*], Mohan Varma. M[3]

1.Department of Pharmacy, Govt. Polytechnic, Amravati-444603, Maharashtra, India.

2.Jawaharlal Nehru Technological University, Kukatpally, Hyderabad-500072, Telangana, India.

3.Shri Vishnu College of Pharmacy, Bhimavaram-534202, Andhra Pradesh, India

ABSTRACT

Present study aims to prepare and evaluate Metoprolol succinate microspheres by ionotropic gelation method. Among all the formulations S7 was selected as optimized formulations for based on the physico chemical parameters and drug release studies. In the *in vitro* release study of formulation S7 showed 96.29% after 12 h in a controlled manner, which is essential for disease like peptic ulcer. The *in vitro* release profiles from optimized formulations were applied on various kinetic models. The best fit with the highest correlation coefficient was observed in Higuchi model, indicating diffusion controlled principle. FT-IR and DSC analyses confirmed the absence of drug-polymer interaction. The results obtained from evaluation and performance study of different types of Metoprolol succinate microspheres that system may be useful to achieve a controlled drug release profile suitable for peroral administration and may help to reduce the dose of drug, dosing frequency and improve patient compliance when compared with marketed product.

Keywords: Metoprolol succinate, ionotropic gelation, microspheres, hypertension, Higuchi.

*Corresponding Author Email: gjdeshmukh@gmail.com

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INTRODUCTION

Controlled drug delivery by encapsulating the drug inside polymeric carriers has made great progress in last two decades as it can enhance the drug release and decrease adverse effects[1, 2, 3, 4] by drug localization at the site of action and by controlling the drug release[5]. The term microsphere is defined as a spherical particle with size varying with diameters in the micrometer range (typically 1µm to 1000µm), containing a core substance. The microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature and ideally having a particle size less than 200 micrometer[6] Moreover, entrapment inside the polymers can also shield the sensitive drugs (e.g., peptides/proteins) from chemical and enzymatic decomposition. Microspheres developed using biodegradable polymers are widely used to achieve controlled release of drugs[7, 9]. The chief advantage of using biodegradable polymers is that after performing their tasks they break down in a biologically friendly manner.

For the treatment of chronic diseases it is important to take medication several times, this may lead to fluctuating drug level in body. In order to avoid frequent drug administration and maintenance of therapeutic drug level in body it is essential to administer drug by a sustained release system. Drugs with short elimination half life are most suitable for sustained release formulations. Sustained delivery of drugs can be achieved by microspheres formulation[9].

Metoprolol succinate is a selective β_1 receptor blocker medication. It is used to treat high blood pressure, chest pain due to poor blood flow to the heart, and a number of conditions involving an abnormally fast heart rate. It is also used to prevent further heart problems after myocardial infarction and to prevent headaches in those with migraines. It has an absorption window and is mainly absorbed from the upper parts of GIT, and good stability in the acidic environment of the stomach makes it a suitable candidate to formulate in a GRDF. Moreover it has a half-life of 3-7 hours[10]

The aim of present work is to design and in vitro evaluation of microspheres of Metoprolol succinate to enhance its bioavailability and prolonged residence time in stomach.

MATERIALS AND METHOD

Materials:

Metoprolol Succinate pure drug was generous gift from Macleods Labs, Mumbai, India. Sodium alginate was obtained from Pruthvi Chemicals, Mumbai. Calcium chloride was received from SD Fine Ltd, Mumbai. All other chemicals used were of analytical grade.

Preparation of Metoprolol Succinate microspheres:

Metoprolol Succinate microspheres were prepared with polymers like sodium alginate and calcium chloride by Ionotropic gelation method. Different formulation trials of Metoprolol Succinate were prepared using different concentration of polymer and cross linking agent. Total 14 formulations are developed using sodium alginate and calcium chloride in different concentrations. In this method weighed quantity of Metoprolol Succinate was added to 100ml sodium alginate solution and thoroughly mixed at 500 rpm. Resultant solution was extruded drop wise with the help of syringe and needle into 100ml aqueous calcium chloride solution and stirred at 100 rpm. After stirring for 10 minutes the obtained microspheres were washed with water and dried at 60 degrees-2hours in a hot air oven and stored in dessicater[11].

Table 1: Formulation trials for Metoprolol succinate microspheres

Formulation code	Metoprolol succinate (g)	Sodium alginate	Calcium chloride
S1	1	1%	7%
S2	1	1.2 %	7%
S3	1	1.4%	7%
S4	1	1.6%	7%
S5	1	1.8%	7%
S6	1	2%	7%
S7	1	2.2%	7%
S8	1	1%	10%
S9	1	1.2%	10%
S10	1	1.4%	10%
S11	1	1.6%	10%
S12	1	1.8%	10%
S13	1	2%	10%
S14	1	2.2%	10%

Micromeretic properties of Metoprolol Succinate microspheres:

Particle size:

The 100 microspheres were evaluated with respect to their size and shape using optical microscope fitted with an ocular micrometer and a stage micrometer. The particle diameters of more than 100 microspheres were measured randomly by optical microscope[12].

Angle of repose:

Angle of repose (θ) of microspheres measures the resistance to particles flow, and is calculated according to fixed funnel standing cone method. Where (θ) is angle of repose, H/D is surface area of the free standing height of the microspheres heap that is formed on a graph paper after making the microspheres flow from glass funnel.

$$\theta = \tan^{-1} (h/r)$$

Bulk density:

Volume of the microspheres in the measuring cylinder was noted as bulk density.

$$\text{Bulk density} = \frac{\text{Wt of powder}}{\text{Bulk volume of powder}}$$

Tapped density:

Change in the microspheres volume was observed in mechanical tapping apparatus.

$$\text{Tapped density} = \frac{\text{Wt of microspheres}}{\text{Tapped volume of microspheres}}$$

Compressibility index:

Also called as Carr's index and is computed according to the following equation.

$$\text{Carr's compressibility index} = \frac{\text{Tapped density - Bulk density}}{\text{Tapped density}} \times 100$$

Hausner's ratio:

Hausner's ratio of microspheres is determined by comparing the tapped density to the fluff density using the equation[13].

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Evaluation of Metoprolol Succinate microspheres:

Swelling index:

Swelling index was determined by measuring the extent of swelling of microspheres in the given medium. Exactly weighed amount of microspheres were allowed to swell in given medium. The excess surface adhered liquid drops were removed by blotting and the swollen microspheres were weighed by using microbalance. The hydro gel microspheres then dried in an oven at 60 degrees for 5h until there was no change in the dried mass of sample. The swelling index of the microsphere was calculated by using the formula[14].

Swelling index= (Mass of swollen microspheres - Mass of dry microspheres/mass of dried microspheres) X 100.

Drug entrapment efficiency and % yield:

In order to determine the entrapment efficiency, microspheres equivalent to 100mg was thoroughly crushed by triturating and suspended in required quantity of methanol followed by agitation to dissolve the polymer and extract the drug. After filtration, suitable dilutions were made and drug content assayed spectrophotometrically at 225nm using calibration curve. Each batch should be examined for drug content in a triplicate manner[15].

% Drug entrapment = Calculated drug concentration /Theoretical drug concentration x 100

% yield = [Total weight of microspheres / Total weight of drug and polymer] x 100

In vitro drug release studies:

In vitro drug release studies for developed Metoprolol Succinate microspheres were carried out by using dissolution apparatus II paddle type (Electrolab TDL-08L). The drug release profile was studied in 900 ml of 6.8 phosphate buffer at 37± 0.5^0C temperature at 100 rpm. The amount of drug release was determined at different time intervals of 0, 1, 2, 3, 4, 6, 8, 10 & 12 hours by UV visible spectrophotometer (Shimadzu UV 1800) at 225nm.

Kinetic modeling of drug release:

In order to understand the mechanism and kinetics of drug release, the result of the *in vitro* dissolution study of microspheres were fitted with various kinetic equations, like zero order[16] (percentage release Vs. time), first order[17] (log percentage of drug remaining to be released vs. time) and Higuchi's model[18] (Percentage drug release vs. square root of time). Correlation coefficient (r^2) values were calculated for the linear curves obtained by regression analysis of the above plots.

Drug excipient compatibility studies

The drug excipient compatibility studies were carried out by Fourier transmission infrared spectroscopy (FTIR) method, Differential Scanning Calorimetry (DSC) and SEM.

Fourier transforms infrared spectroscopy (FTIR)

FTIR spectra for pure drug, physical mixture and optimized formulations were recorded using a Fourier transform Infrared spectrophotometer. The analysis was carried out in Shimadzu-IR Affinity 1 Spectrophotometer. The samples were dispersed in KBr and compressed into disc/pellet by application of pressure. The pellets were placed in the light path for recording the IR spectra. The scanning range was 400-4000 cm^{-1} and the resolution was 1 cm^{-1}.

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Samples were accurately weighed and heated in sealed aluminum pans at a rate

of 10°C/min between 25 and 350°C temperature rang under nitrogen atmosphere, empty aluminum pan was used as a reference.

SEM studies

The surface and shape characteristics of pellets were determined by scanning electron microscopy (SEM) (HITACHI, S-3700N). Photographs were taken and recorded at suitable magnification.

Stability studies

The stability study of the optimized formulation was carried out under different conditions according to ICH guidelines. The optimized microspheres were stored in a stability chamber for stability studies (REMI make). Accelerated Stability studies were carried out at 40 ^{0}C / 75 % RH for the best formulations for 6 months. The microspheres were characterized for the percentage yield, entrapment efficiency & cumulative % drug released during the stability study period[19]

RESULTS AND DISCUSSION

Preparation of Metoprolol Succinate microspheres:

Metoprolol succinate microspheres were prepared by ionic gelation method, using different polymers like sodium alginate, calcium chloride in different concentration and the formulation codes S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13 and S14 were prepared and shown in Figure. All the formulations were evaluated for their various physicochemical parameters.

Figure 1: Metoprolol succinate microspheres

Micromeretic properties of Metoprolol succinate microspheres:

Table 2: Micromeritic properties of Metoprolol succinate microspheres

Formulation code	Particle size (µm)	Bulk density (g/cc^3)	Tapped density(g/cc^3)	Angle of repose(θ)	Carr's index (%)
S1	71.12±0.08	0.68	0.68	27˚.74	11.34%
S2	75.29±0.13	0.73	0.71	28˚.67	12.34%
S3	77.43±0.04	0.75	0.73	30˚.54	12.12%
S4	79.67±0.09	0.78	0.74	30˚.15	11.23%

S5	83.45±0.04	0.81	0.76	28˚.93	14.56%
S6	92.45±0.09	0.90	0.82	25˚.24	13.95%
S7	73.45±0.09	0.92	0.85	21˚.54	9.32%
S8	66.45±0.04	0.65	0.63	27˚.93	14.56%
S9	77.45±0.09	0.66	0.65	25˚.54	13.95%
S10	80.23±0.14	0.68	0.66	23˚.91	13.32%
S11	84.12±0.08	0.70	0.69	23˚.74	13.34%
S12	86.29±0.13	0.73	0.72	25˚.67	12.34%
S13	90.43±0.04	0.75	0.73	25˚.54	13.12%
S14	93.45±0.09	0.86	0.78	25˚.15	12.23%

Metoprolol succinate microspheres of 14 formulations were prepared by ionic gelation method and evaluated for their various physic chemical parameters. All the formulations were evaluated for particle size, bulk density, tapped density, angle of repose and carr's index and found to be within the limits, the results were depicted in Table 2.

The results of % yield, entrapment efficiency and swelling index was found to be satisfactory which shown in Table 3. The formulation S7 showed the best percentage yield, entrapment efficiency and swelling index values of 96.50%, 95.30% and 95% respectively.

Table 3: Percentage drug yield and entrapment efficiency Metoprolol succinate microspheres:

Formulation code	Percentage yield	Entrapment efficiency	Swelling index(%)
S1	80.00%	81.00%	74%
S2	82.00%	82.00%	79%
S3	84.00%	84.00%	81%
S4	86.87%	87.30%	84%
S5	90.30%	90.20%	89%
S6	92.30%	93.10%	91%
S7	96.50%	95.30%	95%
S8	85.00%	83.03%	69%
S9	87.00%	87.00%	71%
S10	88.00%	88.00%	74%
S11	89.09%	90.00%	85%
S12	91.50%	91.66%	93%
S13	94.30%	95.03%	92%
S14	85.30%	84.88%	89.2%

***In vitro* drug release studies**:

The drug release from different formulations of Metoprolol succinate microspheres were carried out in pH 6.8 phosphate buffer using USP Type II (paddle) apparatus. The samples were withdrawn at the specified time intervals upto 12 h and the absorbance was measured using UV-visible spectrophotometer at 225 nm. The cumulative percentage drug release was calculated for all the 14 formulations, the results are depicted for S1-S7 in **Table 4& Figure 2** and the results of

S8-S14 formulations are summarized in **Table 5 & Figure 3**. The formulation S7 was shown highest percentage of drug release i.e, 96.29% in 12 hrs when compared with other formulations.

Table 4: *in vitro* cumulative % drug release of Metoprolol succinate sodium alginate microspheres formulations S1 to S7

Time in hours	S1	S2	S3	S4	S5	S6	S7
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	17.09±0.33	15.65±0.14	15.45±0.21	18.21±0.11	16.23±0.21	13.31±0.11	14.10±0.12
2	25.08±0.21	24.76±0.21	22.34±0.21	26.34±0.15	25.34±0.21	20.15±0.18	22.30±0.21
3	27.05±0.22	30.88±0.16	31.04±0.11	34.45±0.18	33.22±0.31	27.19±0.15	30.20±0.14
4	38.77±0.16	39.20±0.18	37.90±0.15	44.40±0.18	38.20±0.21	36.23±0.16	38.40±0.45
6	51.39±0.21	53.30±0.11	49.90±0.17	51.70±0.19	51.30±0.15	50.73±0.11	54.80±0.18
8	64.23±0.44	63.30±0.13	61.20±0.18	60.30±0.12	63.30±0.22	65.46±0.31	70.60±0.16
10	70.25±0.18	72.90±0.16	71.20±0.18	70.30±0.22	73.30±0.19	80.45±0.14	84.90±0.15
12	81.34±0.32	83.30±0.12	85.20±0.17	87.50±0.23	90.30±0.15	92.17±0.16	96.29±0.11

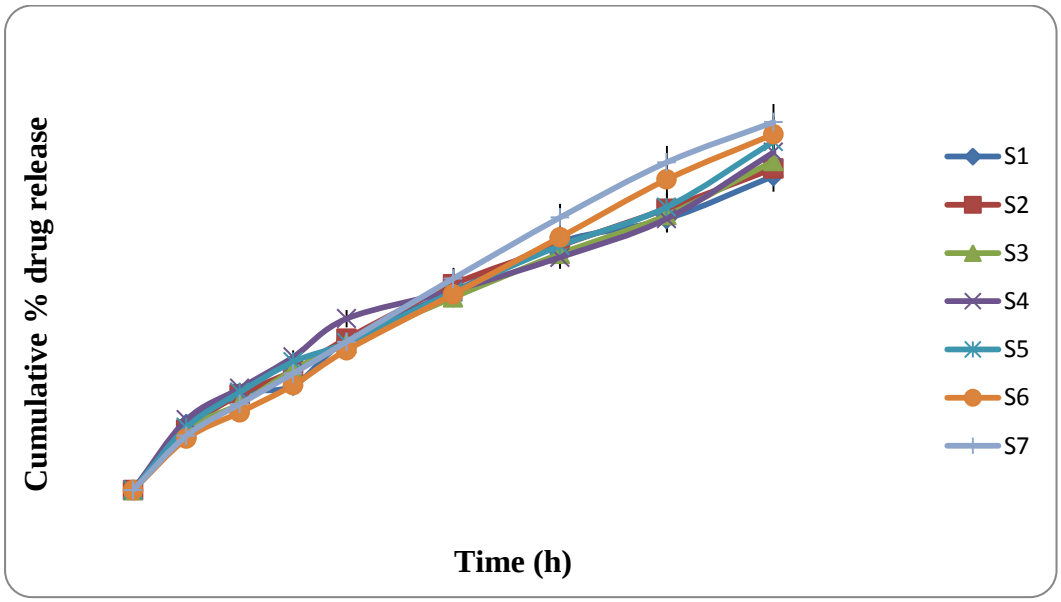

Figure 2:*In vitro* cumulative % drug release of Metoprolol succinate sodium alginate microspheres formulations S1 to S7

Table 5: *In vitro* cumulative % drug Metoprolol succinate sodium alginate release of microspheres formulations S8 to S14:

Time in hours	S8	S9	S10	S11	S12	S13	S14
0	0±0	0±0	0±	0±0	0±0	0±0	0±0
1	16.06±0.11	14.23±0.16	15.21±	17.54±0.32	14.21±0.18	14.62±0.15	12.63±0.18
2	26.40±0.21	24.23±0.18	25.80±	27.40±0.21	24.23±0.16	24.01±0.25	32.01±0.15
3	31.01±0.18	30.56±0.55	32.30±	34.33±0.22	31.94±0.25	30.11±0.23	37.11±0.22
4	38.20±0.16	39.90±0.32	45.40±	39.20±0.25	40.10±0.11	39.24±0.22	44.83±0.19

6	54.30±0.11	49.92±0.26	53.60±	54.30±0.16	54.20±0.19	53.83±0.16	57.79±0.15
8	63.35±0.21	65.27±0.18	62.30±	63.30±0.21	68.20±0.18	68.03±0.22	64.60±0.16
10	69.90±0.25	71.10±0.15	72.60±	69.90±0.52	72.32±0.22	83.22±0.15	75.56±0.22
12	83.30±0.15	86.20±0.11	88.50±	89.42±0.18	94.41±0.31	91.36±0.11	85.70±0.15

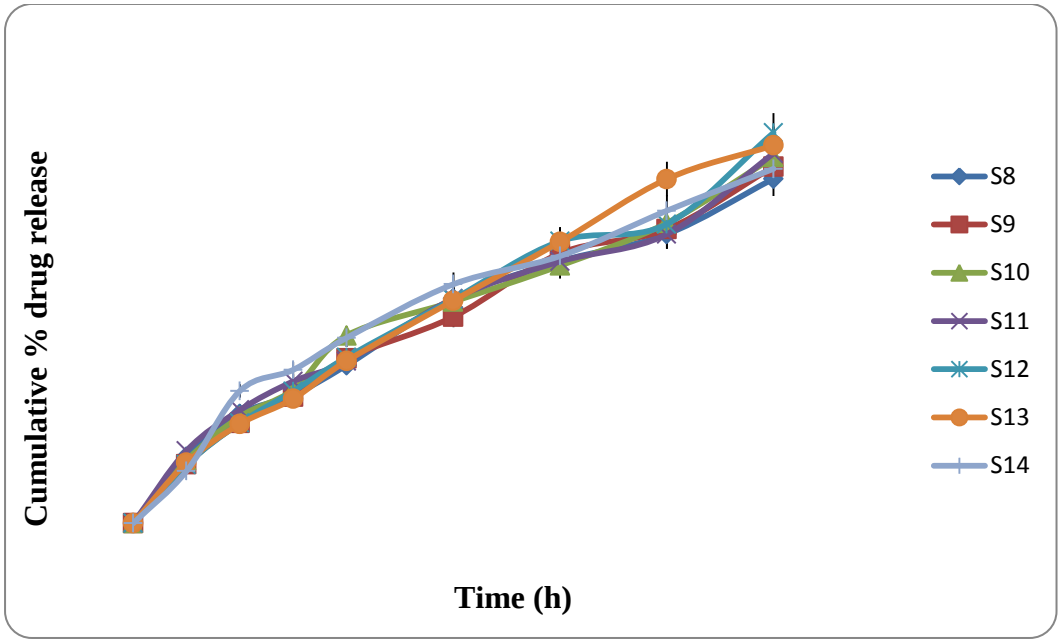

Figure 3: *In vitro* cumulative % drug release of Metoprolol succinate sodium alginate microspheres:

Among all the 14 Metoprolol succinate microspheres, formulation S7 was found to be optimized formulation based on micromeretic properties, particle size, swelling index, %yield, entrapment efficiency and drug dissolution studies.

Mathematical modeling of optimized Metoprolol succinate microspheres:

Mathematical modeling of the relese kinetics of specific classes of controlled-relese systems may be used to predict solute release rates from and solute diffusion behavior through polymers and elucidate the physical mechanisms of solute transport by simply comparing the relese data to mathematical models.

In the view of establishment of release mechanism and quatitatively interpreting and translate mathematically the dissolution date being plotted.

***In-vitro* drug release order kinetics of optimized formulation (S7):**

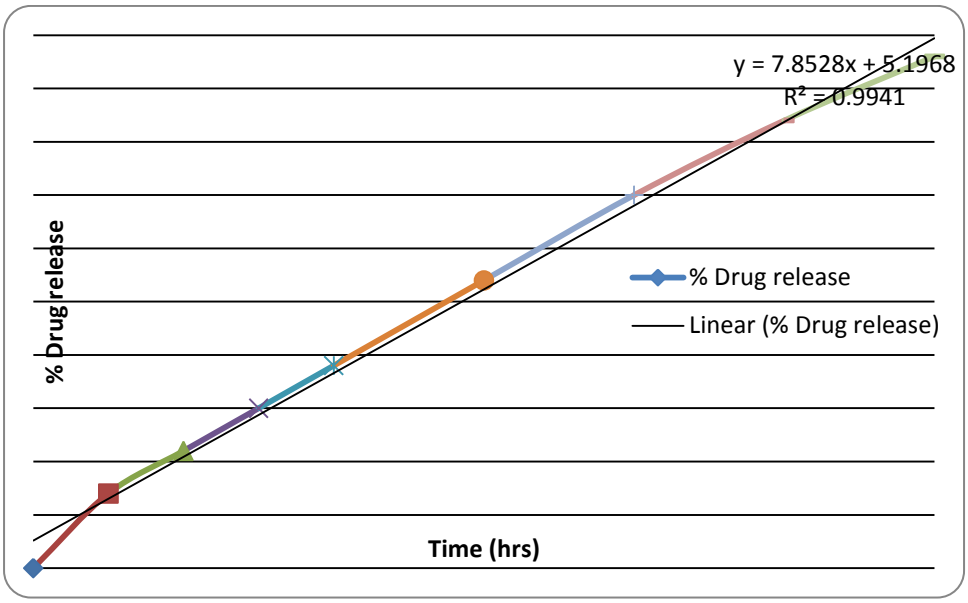

Figure 4: Zero order plot for the Metoprolol optimized formulation S7

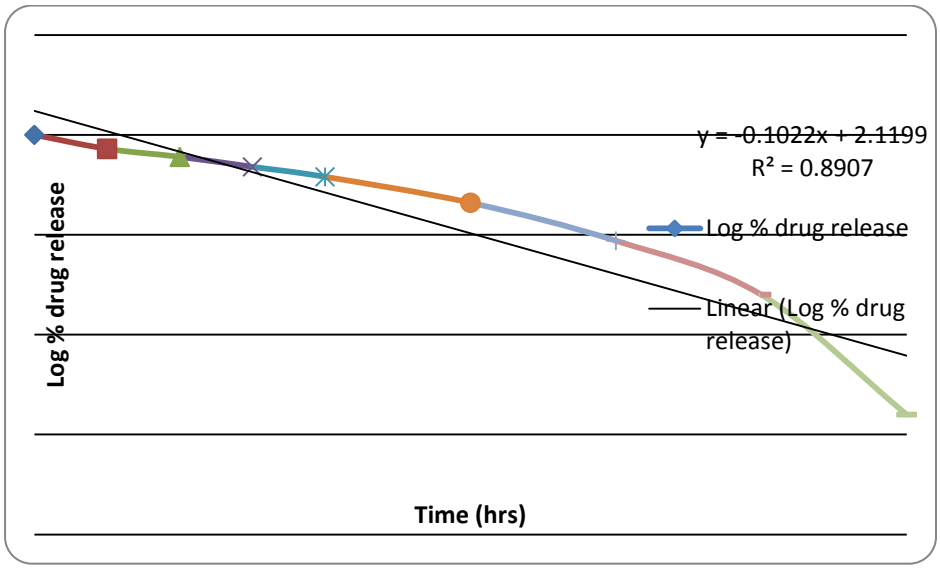

Figure 5:First order plot for the Metoprolol optimized formulation S7

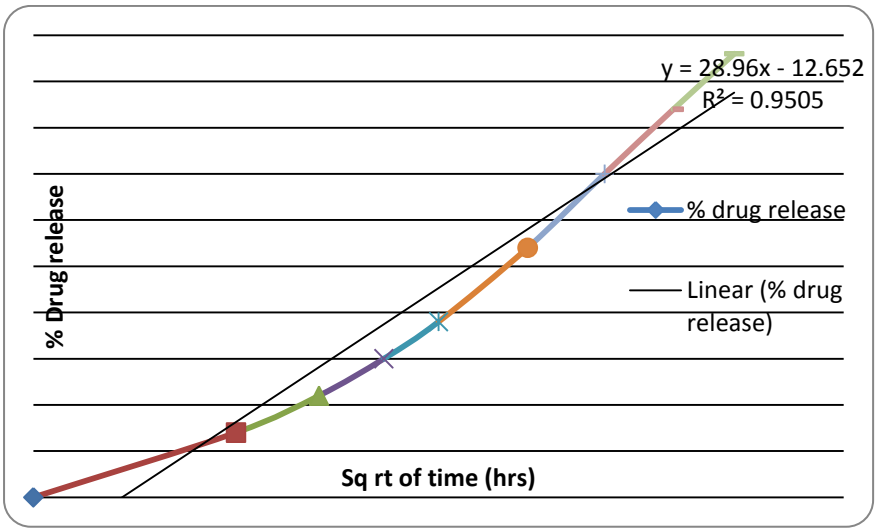

Figure 6: Higuchi plot for the Metoprolol optimized formulation S7

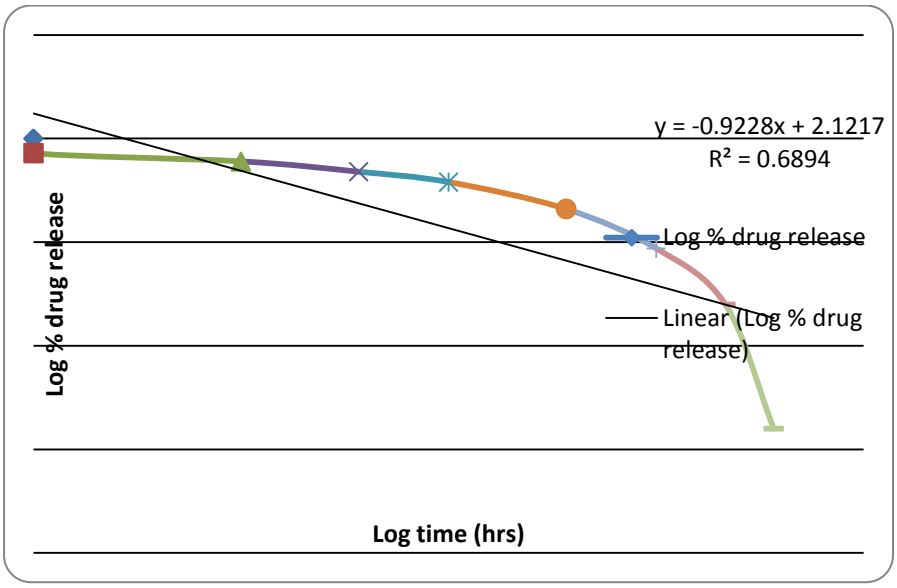

Figure 7:Korsmeyer-Peppas plot for the optimimized formulation of microspheres S7

Table: Release order kinetics of optimized normal microspheres (S7):

Formulation	Zero Order		First Order		Higuchi		Korsmeyer Peppas	
Code	R^2	K	R^2	K	R^2	K	R^2	N
S7	0.994	7.852	0.890	0.102	0.950	28.96	0.689	2.121

From the above results it is apparent that the regression coefficient value closer to unity in case of zero order plot i.e.0.994 indicates that the drug release follows a zero order mechanism. This data indicates a lesser amount of linearity when plotted by the first order equation. Hence it can be concluded that the major mechanism of drug release follows zero order kinetics.

Further, the translation of the data from the dissolution studies suggested possibility of understanding the mechanism of drug release by configuring the data in to various mathematical modeling such as Higuchi and Korsmeyer - Peppas plots.

The mass transfer with respect to square root of the time has been plotted, revealed a linear graph with regression value close to one i.e. 0.950 starting that the release from the matrix was through diffusion. Further the n value obtained from the Korsmeyer - Peppas plots i.e. 0.689 suggest that the drug release from microspheres was anomalous Non-fickian diffusion.

CHARACTERIZATION:

Fourier Transform Infrared Spectroscopy (FTIR):

Drug polymer interaction was checked by comparing the IR spectra of the physical mixture (Figure 9) of drug with the excipients used with the IR spectrum of Metoprolol succinate pure drug (Figure 8) and optimized formulation (Figure 10). As shown in Figure, Metoprolol succinate pure drug gives the peaks in IR spectrum nearby at 1050.25 cm^{-1} due to the presence of O-H bending, 1316.54 cm^{-1} due to the C-N stretching, 1448.68 cm^{-1} is due to CH_3-O Bending, 1614.54 cm^{-1} is due to N-H Bending, 2735.30 cm^{-1} is responsible for CH_3-O Stretching, 2945.10 cm^{-1} is due to C-H Streching (Aliphatic) and 3149.80 cm^{-1} is due to C-H Streching (Aromatic).

Frequencies of functional groups of pure drug remained intact in physical mixture and optimized formulation containing different polymers; hence, there was no major interaction between the drug and excipients used in the study.

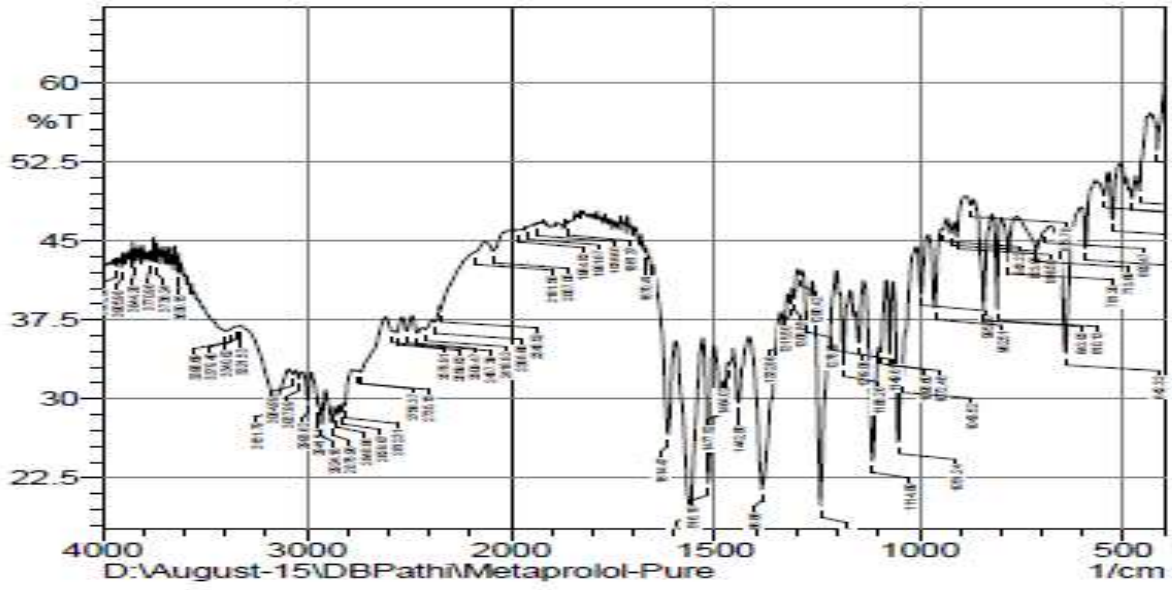

Figure 8: FT-IR spectrum of pure drug Metoprolol succinate

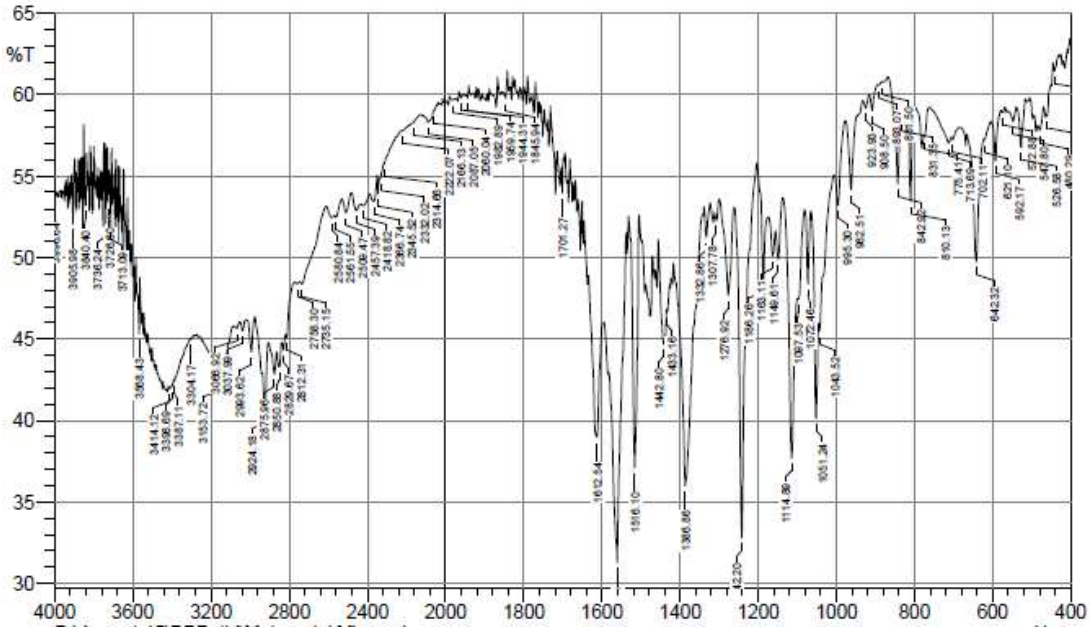

Figure 9: FT-IR spectrum of physical mixture (Metoprolol succinate+Sodium alginate+$CaCl_2$)

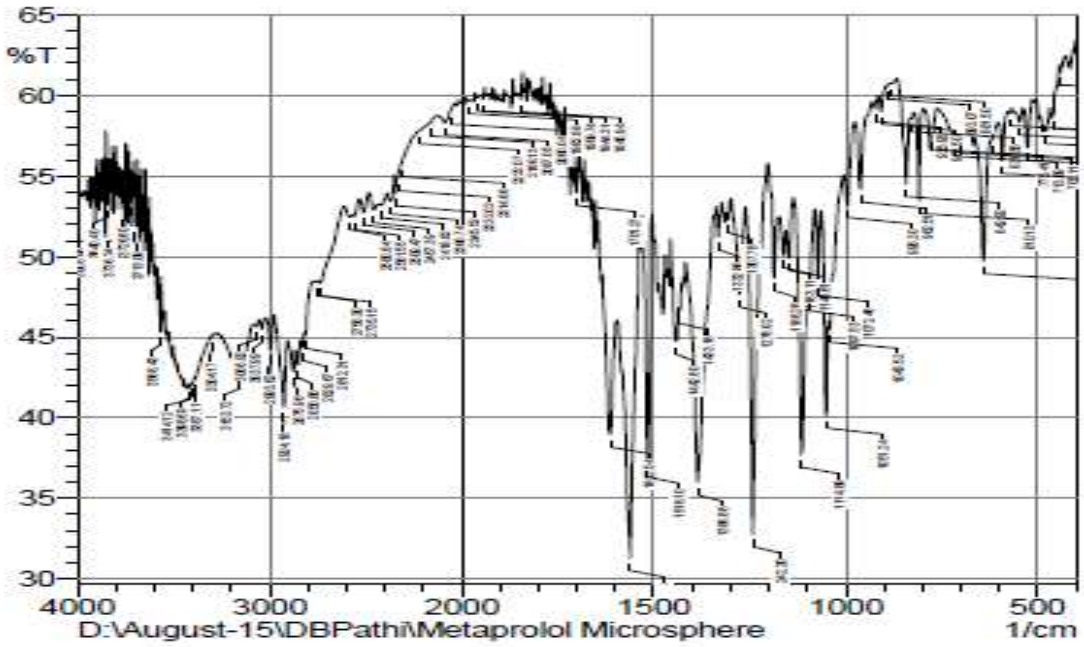

Figure 10: FT-IR spectrum of Metoprolol succinate optimized formulation S7

DSC Studies:

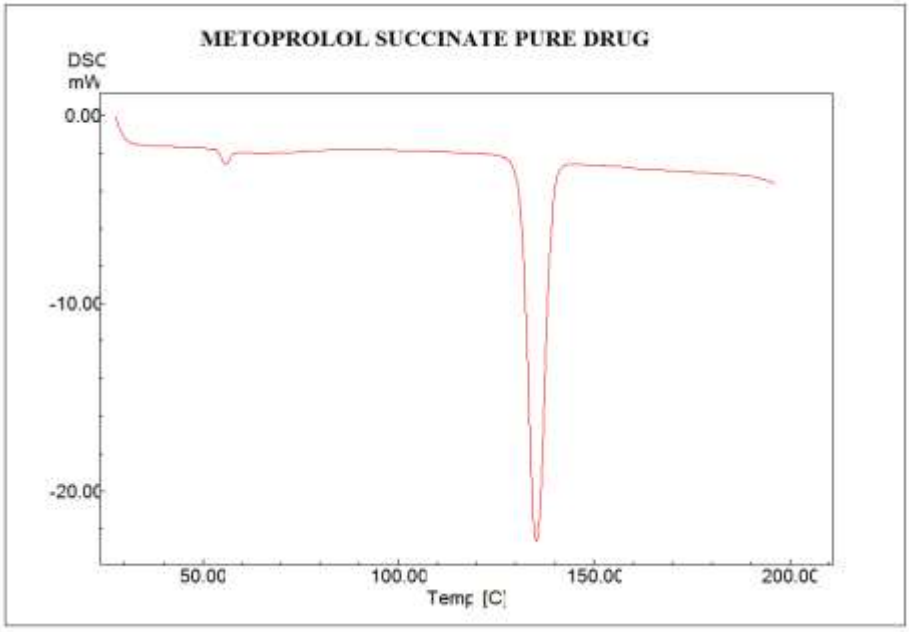

Figure 11:DSC thermogram of Metoprolol succinate pure drug

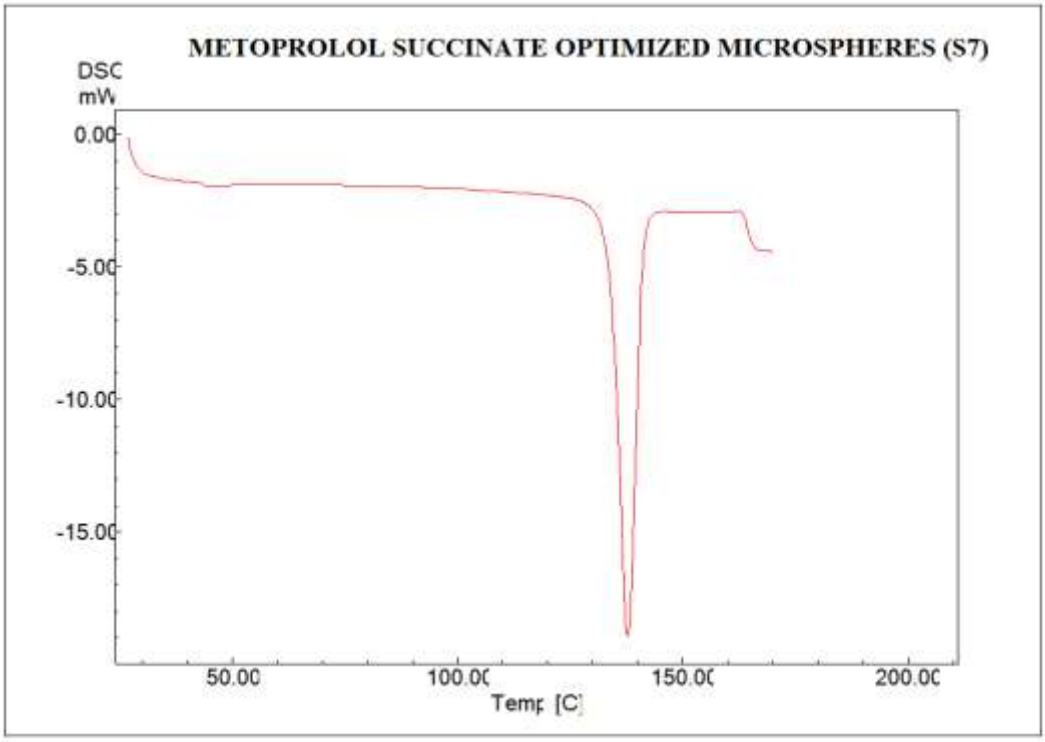

Figure 12: DSC thermogram of Metoprolol succinate optimized microspheres (S7)

DSC was used to detect interaction between Metoprolol succinate and excipients. The thermogram of pure Metoprolol succinate (Figure 11) exhibited a sharp endotherm melting point at 135 ^{0}C. The thermogram of microsphere loaded with Metoprolol succinate exhibited a sharp endotherm melting point at138 ^{0}C (Figure 12). There is no considerable change observed in melting endotherm of

drug in optimized formulation. It indicates that there is no interaction between drug & excipients used in the formulation.

SEM of Metoprolol Succinate microspheres

The external and internal morphology of controlled release microspheres were studied by Scanning Electron Microscopy.

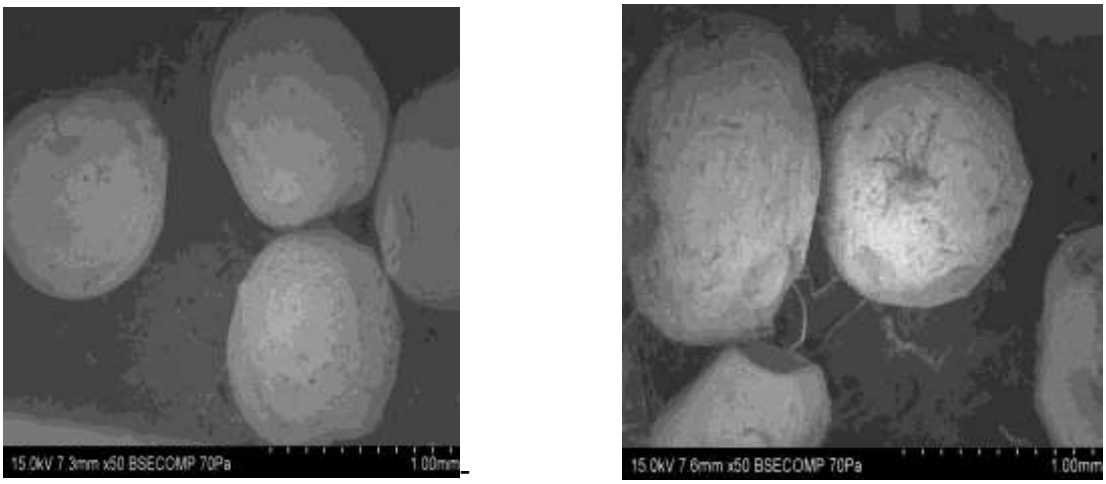

Figure 13: Scanning electron micrographs of Metoprolol succinate microspheres

Morphology of the various formulations of Metoprolol succinate microspheres prepared was found to be discrete and spherical in shape (Figure 13). The surface of the Metoprolol succinate microspheres was rough due to higher concentration of drug uniformly dispersed at the molecular level in the sodium alginate matrices. There are no crystals on surface which states that is drug is uniformly distributed.

Stability studies:

Optimized formulation S7 of Metoprolol succinate was selected for stability studies on the basis of high cumulative % drug release. Stability studies were conducted by performing Percentage yield, %Entrapment efficiency and *In-vitro* drug release profile for 6 months according to ICH guidelines. From these results it was concluded that, optimized formulation is stable and retained their original properties.

CONCLUSION

In the present study, an attempt was made to prepare Metoprolol succinate microspheres, which were characterized for particle size, scanning electron microscopy, FT-IR study, DSC, percentage yield, %drug entrapment, stability studies and found to be within the limits. Among all the formulations S7 was selected as optimized formulations based on the physico chemical studies and drug release studies. In the *in vitro* release study of formulation S7 showed 96.29% of drug release

after 12 h in a controlled manner, which is essential for disease like hypertension. The *in vitro* release profiles from optimized formulations were applied on various kinetic models. The best fit with the highest correlation coefficient was observed in Higuchi model, indicating diffusion controlled principle. FT-IR and DSC analyses confirmed the absence of drug-polymer interaction. It may be concluded from the result obtained from evaluation and performance study of Metoprolol succinate microspheres that system may be useful to achieve a controlled drug release profile suitable for peroral administration and may help to reduce the dose of drug, dosing frequency and improve patient compliance.

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