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FORMULATION AND EVALUATION OF ALGINATE MICROSPHERES OF METOPROLOL SUCCINATE

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ABSTRACT

The present research work was aimed to formulate and evaluate alginate microspheres of Metoprolol Succinate using hydrophilic swellable polymers. Different formulations of Metoprolol Succinate sustained release microspheres were prepared by ionic cross-linking technique (drop extrusion method) using various ratios of HPMC K4M, Carbopol 934P, Sodium Carboxy Methyl Cellulose and combination of both HPMC K4M and Carbopol 934P. Sodium Alginate and Calcium Chloride acts as cross-linking agents to form the microspheres. Preformulation studies like solubility, FTIR, DSC studies were performed. Total 12 batches of formulations were prepared by using ratios of 1:1, 1:2, 1:4 for each polymer. Microspheres prepared were evaluated for SEM analysis, drug loading, entrapment efficiency and percentage yield. Formulations were evaluated for the release of MS over a period of 24 hrs using USP type-I standard dissolution apparatus in 6.8 pH phosphate buffer at 37^{0}C. Among all the formulations studied, F7 containing Carbopol 934P (1:1) showed maximum release of drug for 24 h with cumulative percent release of 93.2%. No chemical interaction between drug and polymers was seen as confirmed by DSC and FTIR studies. In conclusion, the formulation F7 showed maximum drug release of alginate microspheres of Metoprolol Succinate by the diffusion mechanism. Further the efficacy of the developed formulations has to be assessed by pharmacokinetic studies in humans.

KEY WORDS

Metoprolol Succinate, Alginate Microspheres, Carbopol 934p, Sustained Release, SEM analysis

INTRODUCTION

Drugs that are having short half-life are eliminated quickly from the blood circulation, require frequent dosing. To avoid this problem most commonly used approach is formulation of sustained release formulations.

Sustained release, sustained action, prolonged action, controlled release, extended action, timed release, depot and repository dosage forms are terms used to identify drug delivery system that are designed to achieve prolong therapeutic effect by continuously releasing medication over an extended period of time after administration of a single dose.

In the present work Metoprolol Succinate (3 hrs half-life) is selected as the model drug to prepare the sustained release alginate microspheres where sustained or controlled drug delivery occurs while entrapment within a polymer that may be natural or semi-synthetic or synthetic in nature.

Metoprolol Succinate is Cardio selective beta1 adrenergic receptor blockers preferentially inhibit β1 receptors that are principally found in the myocardium, which acts as anti-hypertensive. It Decreases heart rate,

contractility and cardiac output, therefore decreasing blood pressure.

The aim of the present work is to formulate and evaluate alginate microspheres of Metoprolol Succinate with different ratios (1:1, 1:2, 1:4) of polymers like HPMC K4M, Carbopol 934P, Sodium CMC and in combination of both HPMC K4M and Carbopol 934P by Ionic Gellation method to achieve sustained release of drug from the dosage form, which acts as Anti-Hypertensive. This study also involves the study of hydrophilic swellable polymers on the drug loading, entrapment efficiency and drug release.

MATERIALS AND METHODS

Metoprolol Succinate drug was obtained as a gift sample from Therdose Pharma Pvt Ltd, Hyderabad, India; HPMC K4M, Sodium CMC from Qualikems Fine Chem Pvt Ltd, Delhi India; Carbopol 934P from Libraw Pharma Pvt Ltd, Delhi India; Sodium Alginate from Lobha Chemie Pvt Ltd, Mumbai, India. Calcium Chloride and Ethanol were purchased from Merck Pvt Ltd, Hyderabad, India.

Formulations

All ingredients were weighed accurately. The drug solution and the polymer solutions were prepared separately in beakers by adding small amount of buffer and these two are added to the sodium alginate and mixed well. The total drug-polymer and alginate mixture was kept on a magnetic stirrer for 1 hr to get a homogeneous mixture of desired viscosity to pass through the syringe dropper. The total mixture is taken in to the 5ml syringe with 21 guage and the viscous solution is dropped in to the CaCl2 solution with continues stirring to form alginate microspheres. The beads formed are allowed to stir for 2 hrs for strengthening. After curing time then the beads were filtered and washed with plenty of water and the obtained alginate microspheres were dried.

Table1: Formulation table of alginate microspheres of MS

Ingredients (mg)	Formulations											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Metoprolol Succinate	100	100	100	100	100	100	100	100	100	100	100	100
HPMC K4M	100	200	400	-	-	-	-	-	-	50	100	200
Sodium CMC	-	-	-	100	200	400	-	-	-	-	-	-
Carbopol 934P	-	-	-	-	-	-	100	200	400	50	100	200
Sodium Alginate	800	700	500	800	700	500	800	700	500	800	700	500
CaCl2 % W/V	10	10	10	10	10	10	10	10	10	10	10	10

EVALUATION OF ALGINATE MICROSPHERES

The prepared Alginate microspheres can be evaluated for various parameters like

SEM Analysis

Scanning electron microscopy has been used to determine particle size distribution, texture and to examine the morphology of fractured or sectioned surface. SEM studies were carried out by using JEOL JSM T-330A scanning microscope (Japan). Dry MS microspheres were placed on an electron microscope brass stub and coated with in an ion sputter. Picture of MS microspheres were taken by random scanning of the stub.

Percentage yield

The percentage yield was determined by weighing the microspheres and then finding out the percentage yield with respect to the weight of the input materials, i.e., weight of drug and polymers used.

Determination of Entrapment Efficiency

Known amount of beads (25 mg) were added to 10 ml of 6.8 pH phosphate buffer for complete swelling at 37^{o}C. The beads were crushed in a glass mortar with pestle, the solution was than for 2 hr to extract the drug completely and centrifuged to remove polymeric debris. The clear supernatant solution was analyzed for drug content using UV-visible spectrophotometer at 224 nm.

Determination of Drug Loading

Accurately weighed microspheres were pulverized and shaken well with 25ml of 0.1N HCl (pH 1.2) solution. The solution was filtered and then made necessary dilution with 6.8 pH buffer and absorbance was noted at 224nm using UV – VIS spectrometer. percentage drug loading is calculated as

(%) Drug Loading = Practical drug content/Weight of microspheres×100

***In-vitro* drug release**

In-vitro drug release of Alginate Microspheres Metoprolol Succinate was determined using USP Dissolution Apparatus I (Paddle type). The dissolution test was performed using 900 ml 6.8pH Phosphate buffer at $37^0C \pm 0.5^0C$. The speed of rotation of paddle was set at 50 rpm. 5 ml samples were withdrawn at time points of 1, 4, 8, 12, 16, 20 and 24 hrs and same volume was replaced with fresh media. Absorbance of solution was checked by UV spectrophotometer (Systronics UV-visible spectrometer) at a wavelength of 224nm nm and drug release was determined from standard curve. It was performed in triplicate.

RESULTS AND DISCUSSION

Analytical Methodology- Preformulation Studies

Solubility Profile studied according to USP Standards

DISCUSSION

The present investigation was undertaken to formulate and evaluate the Alginate Microspheres of Metoprolol Succinate by Ionic cross-linking method using hydrophyllic swellable polymers namely, HPMC k4M, Carbopol, Sodium CMC. Hydrophyllic swellable polymers are mainly used to attain sustained or controlled release of the drug over extended period of time throughout the GIT. On the other hand, Sodium Alginate and Calcium Chloride acts as the cross-linking agents in the formation of microbeads or microspheres. Selective β1 receptor blocker- Metoprolol Succinate was selected as a model drug. It was also selected for the proposed system, since it shows a short half-life, thus being rapidly eliminated from the organism and has less bioavailability. Therefore, alginate microspheres were formulated that allows drug release at the absorption site for a prolonged period of time would enhance drug bioavailability and reduce the frequency of administration.

A whole of 12 formulations of alginate microspheres of MS were prepared by taking 1:1, 1:2 and 1:4 ratio of polymers and evaluated for physical and analytical parameters. According to work plan, the microspheres were evaluated for their solubility, drug excipient interactions, size analysis, percentage yield, drug loading, entrapment efficiency and invitro drug release studies.

λ_{max} of Metoprolol Succinate

The analytical method development of Metoprolol Succinate was performed for the determination of its λ_{max} and quantification of the microsphere before proceeding for the experiment. Metoprolol Succinate was scanned in 6.8 pH phosphate buffer to determine the λ_{max}, by setting the wavelength in the range of 200 to 400 nm.

An absorption maximum of 224nm was obtained, which is considered as the absorption maximum in the present study.

Preparation of standard graph

Standard solutions in the range of 4, 8, 12, 16, 20 and 24 µg/ ml were prepared from the primary and secondary stock solutions and absorption values were recorded at 224nm against the blank solution (6.8 pH phosphate buffer). From this data, the standard curve of Metoprolol Succinate was obtained by plotting absorbance on Y-axis against concentration on X-axis.

The regression coefficient (R^2) value was found to be 0.999.

Solubility studies

The availability of literature on solubility profile of different substances according to USP was kept as a standard and the solubility of Metoprolol Succinate was estimated in different solvents. The solvents used are pH 6.8 phosphate buffer and water. Solubility study was confirmed by observing the solubility studies of Metoprolol Succinate practically by taking 1 gm of drug and gradually dissolving in increasing amounts of solvent that are taken for the study.

The drug showed more solubility in pH 6.8 buffer than when compared to water.

Drug-excipients compatibility study by FTIR

FTIR study was done by using BRUKER ALPHA T instrument to verify if there was any interaction between the pure drug and excipients which were used in the formulation. The various FTIR spectrums of drug and polymer alone and both of pure drug and excipients in combination were scanned by keeping the pellet in the instrument against KBr pellet which was taken as a blank.

Table1: Standard graph of metoprolol succinate

Concentration (µg/ml)	Absorbance
0	0
4	0.12
8	0.27
12	0.4
16	0.54
20	0.69
24	0.82

Table2: Solubility studies in water and pH 6.8 buffer

Solvent	Amount of Solvent Required (ml/gm)
Water	10
6.8 pH buffer	8.5

Table3: Evaluation of alginate microspheres

Formulation	% Entrapment	% Yield	% Drug Load
F1	32±2	83.1±2.5	6.28 ±1
F2	45.76±1.5	85.2±2	7.17±1.9
F3	56.70±1.5	87.4 ±1	7.28 ±1.3
F4	38.82±1.1	87.1±2	8.76±2
F5	53.52±1.5	89.2±1	9.44±1.2
F6	66.11±1	91.6±1	9.86±0.9
F7	42.11±2	89.9±2	9.97±1.6
F8	60 ±1.2	91.2±1	10.28±2
F9	77.05±2	94.5±2.5	10.16±1.8
F10	36.58±1.7	85.9±1.1	8.63±2
F11	50.82±2	87.7±1.8	9.48±2
F12	62.35±2	92.5±1	9.64±1.5

All values represent mean and standard deviation, n=3

Table 4: Dissolution profile of alginate microspheres of metoprolol Succinate (F1 to F6)

TIME (hrs)	CUMMULATIVE PERCENTAGE DRUG RELEASE					
	F1	F2	F3	F4	F5	F6
1	18.32±0.9	17.47±0.7	13.87±0.4	13.24±0.2	12.92 ±0.8	10.48 ±0.4
4	25.94±0.6	24.56 ±0.4	21.25±0.5	26.68±0.4	22.84±0.6	25.36 ±1.0
8	40.27±0.8	40.57±0.5	32.49±0.7	44.06±0.2	40.75±0.1	38.74 ±0.5
12	57.86 ±0.7	56.57 ±0.6	52.79±0.8	64.98±0.2	48.81±1.7	48.91±1.5
16	95.97±1.0	82.4 ±0.7	71.18±0.9	80.38±0.2	64.33±0.8	64.11 ±0.5
20	…	97.79±0.3	81± 0.6	90.35±1.1	79.4±0.8	75.79 ±1.0
24	…	…	91.61±0.8	…	87.96±1.2	85.7 ± 0.6

All values represent mean and standard deviation, n=3

Table 5: Dissolution profile of alginate microspheres of Metoprolol Succinate

TIME (hrs)	CUMMULATIVE PERCENTAGE DRUG RELEASE					
	F7	F8	F9	F10	F11	F12
1	16.94±0.8	15.78±1.3	14.29±0.6	9.95±0.6	9.11±0.5	8.47±0.8
4	24.55±0.4	24.97±0.7	22.63±0.4	20.38±0.7	18.16±0.6	17.84±0.6
8	41.38±1.1	41±0.8	39.06±0.9	37.54±0.5	39.86±0.7	28.1±0.7
12	58.27±1.1	57.63±0.4	56.75±0.3	48.13±0.5	43.85±1.1	46.36±0.7
16	70.56±1.1	69.38±0.7	67.23±0.7	60.46±1.1	58.32±1.1	57.73±0.9
20	81.85±0.8	78.66±0.5	76.39±0.7	70.75±1.2	66.58±0.8	63.34±1.2
24	93.2±0.8	89.78±0.9	86.12±1.2	85.11±0.9	81.24±0.7	77.11±0.7

(F7 to F12) All values represent mean and standard deviation, n=3

Table 6: Interpretation of FT-IR graph

S.No	Wave number in formulation (cm^{-1})			Characteristic Wave number range (cm^{-1})	Bond nature and bond attributed
	Pure drug	Polymer	Optimized formulation		
1	3300	-	3374	3400-3250	N-H stretching,O-H stretching secondary amines, alcohol
2	2977	2877	2973	3000-2850	C-H stretching Alkanes
3	-	1645	1648	1700-1300	C=O stretching Carbonyl
5	1467	1453	1487	1500-1400	C-C stretch in ring Aromatics

The respective FTIR spectrums which were obtained are interpreted in Table 7.

From the table, IR spectrum of pure drug, polymer and the formulation exhibit characteristic peaks at 3300, 2977 and 2877, 1645, 1467 and 1453, cm^{-1} due to N-H, C-H, C=O, C-C stretching respectively.IR spectrum of F7 formulation also showed characteristics peaks at 3374, 2973, 1648, 1487 $cm^{-1.}$ The presence of above peaks confirms undisturbed drug in the formulations. It was observed that, there was no disappearance or shift in peak position of metoprolol succinate in any spectra of drug and excipients, which proved that drug and excipients were

compatible. Hence, it can be concluded that drug can be used with the selected polymer without causing instability in the formulation.

Drug-excipients compatibility study by DSC

DSC study was done to verify if there was any interaction between the pure drug and excipients which were employed. The various DSC thermograms of pure drug and both drug and excipients in combination were mixed and the blend was formulated sealed in aluminum pans and scanned.

Sharp endothermic peaks of drug (at 130°C) was observed in both the graphs at the same point indicating no shift in the endotherms obtained.

This indicates that the drug and excipients used are compatible with each other.

SEM Analysis

The surface morphology of the alginate microspheres of MS was studied by SEM. SEM photographs of optimised formulation showed that the surface of the microspheres was smooth and plane which enable even release of drug from the polymer matrix.

The size of the alginate microspheres of optimised formulation was found to be 500 µm.

Percentage yield

The percentage yield for alginate microspheres of MS were 82.1%, 85.2% and 87.41% for formulation F1, F2 and AF3 respectively which are formulated by using the polymer Sodium CMC. The percentage yield for alginate microspheres of MS were 87.1%, 89.21% and 91.6% for

formulation F4, F5 and F6 respectively which are formulated by using the polymer HPMC K4M.

The percentage yield for alginate microspheres of MS were 89.9%, 91.2% and 94.5% for formulation F7, F8 and F9 respectively which are formulated using the polymer Carbopol 934P. The percentage yield for alginate microspheres of MS were 85.9%, 87.70% and 92.5% for formulation F10, F11 and F12 respectively which are formulated by using combination of HPMC K4M and Carbopol 934P.

This indicates that maximum yield of the alginate spheres were obtained, which complied with the limits of standard deviation.

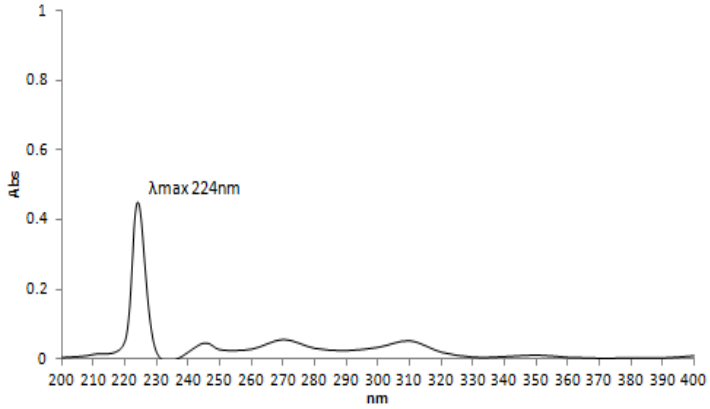

Figure 1: λ-max of metoprolol succinate- Abs-absorbance, nm-wave length

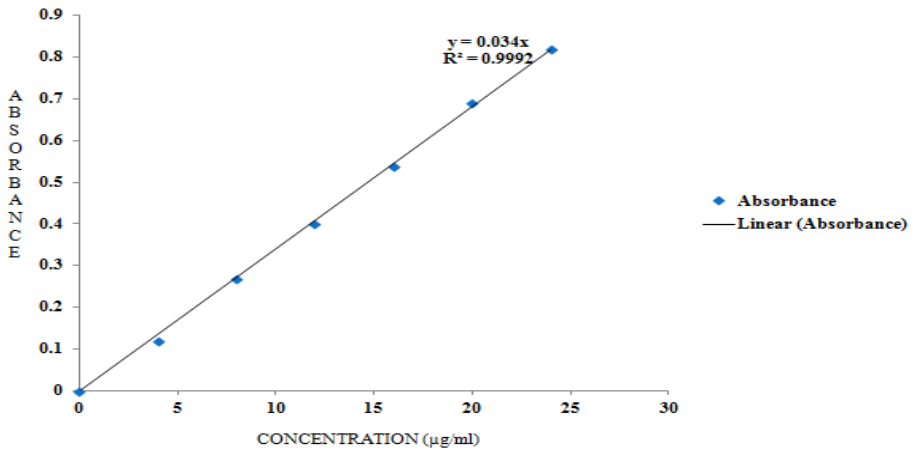

Figure2: Standard graph of metoprolol succinate

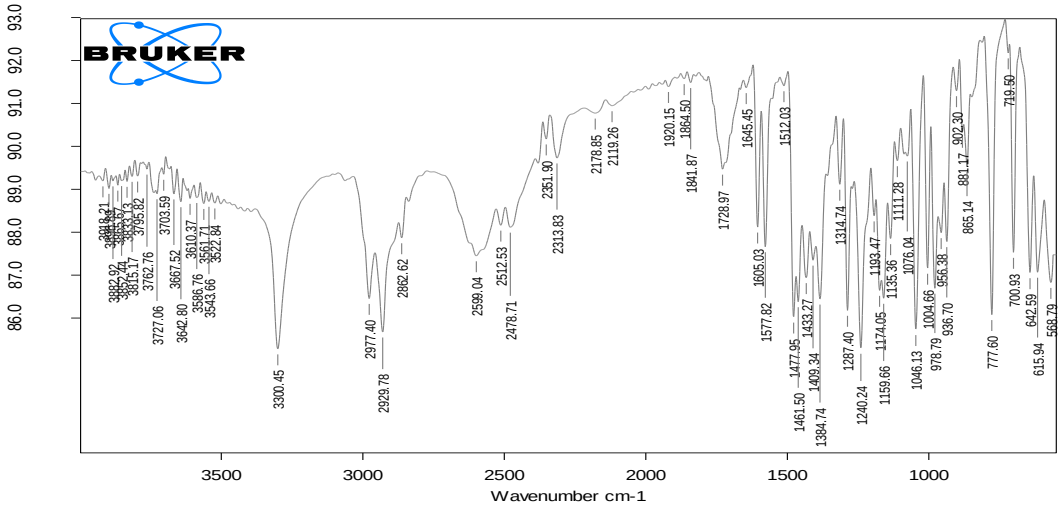

Figure3: FTIR spectrum of MS

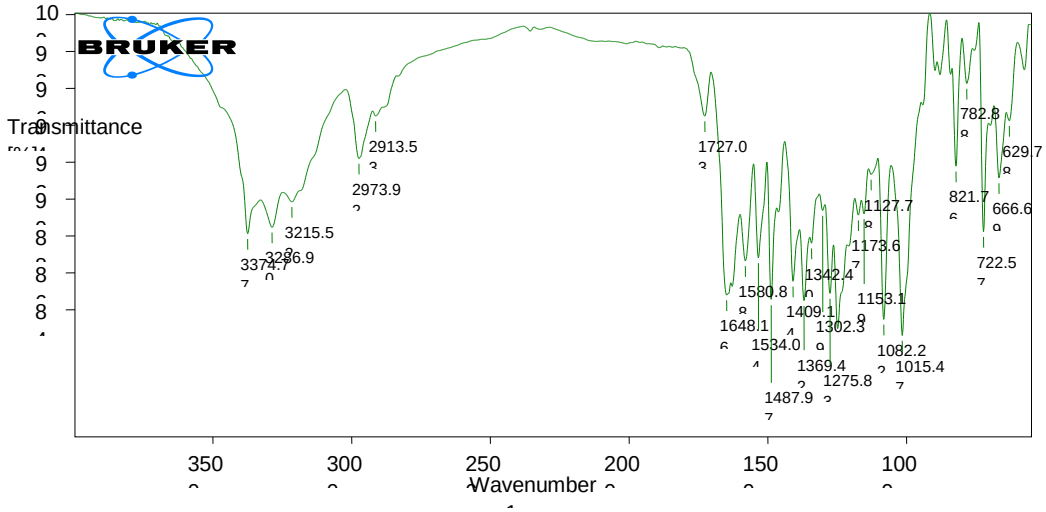

Figure4: FTIR spectrum of optimized formulation

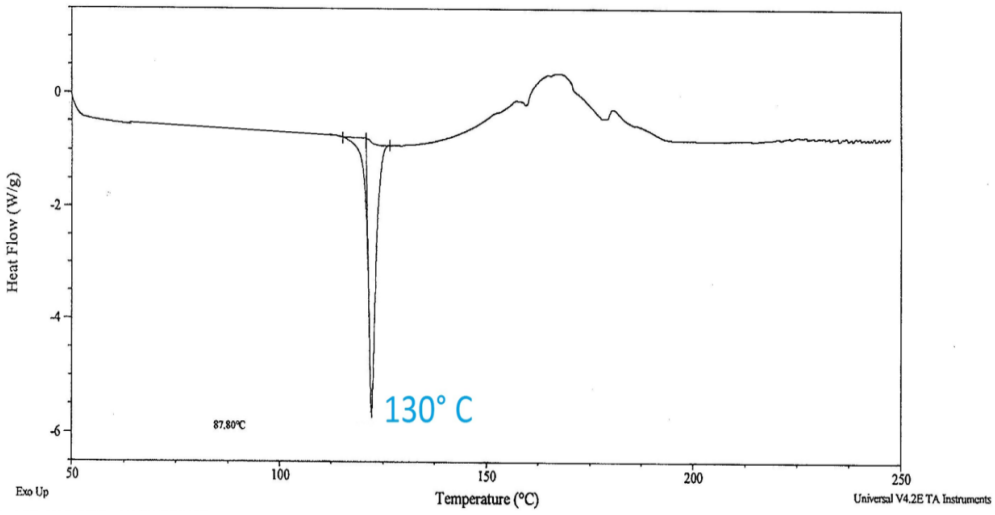

Figure5: DSC thermogram of metoprolol succinate

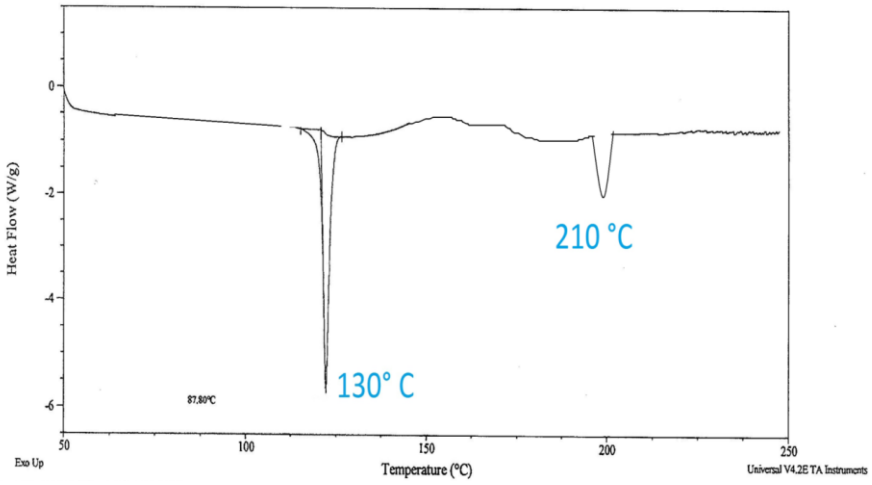

Figure6: DSC thermogram of optimized formulation

Evaluation Methods

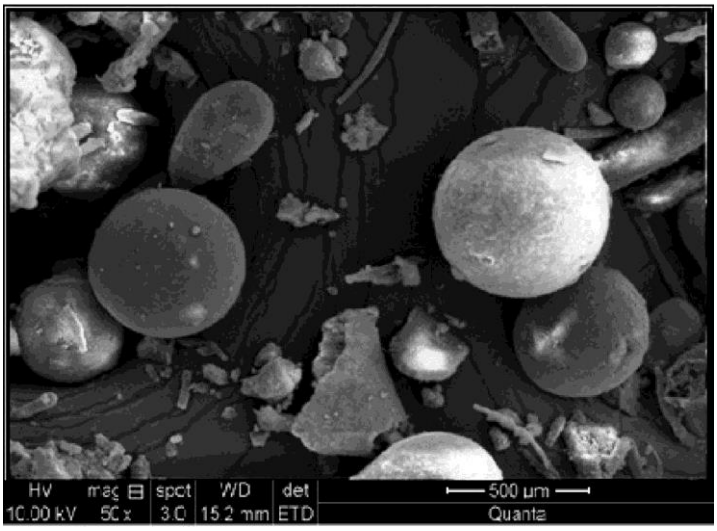

Figure7: SEM picture of optimized formulation.

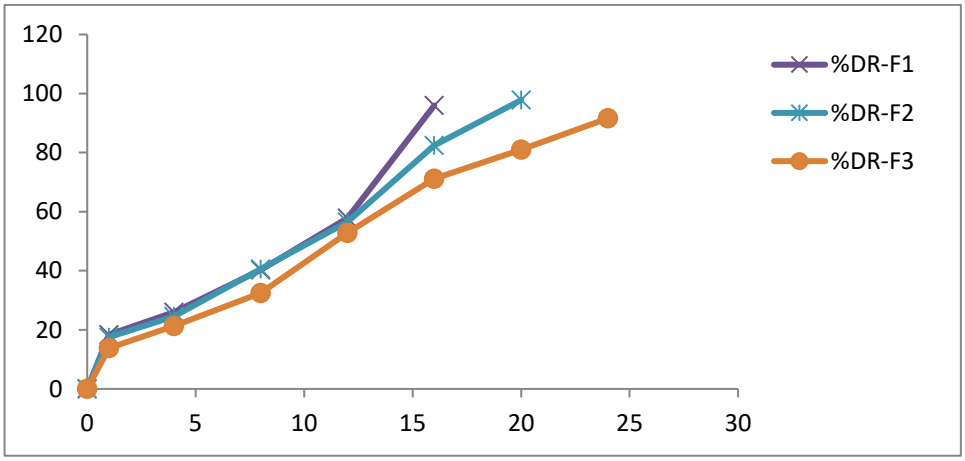

Figure8: Diss. profile of alg. microspheres of MS with Sod. CMC (F1-F3)

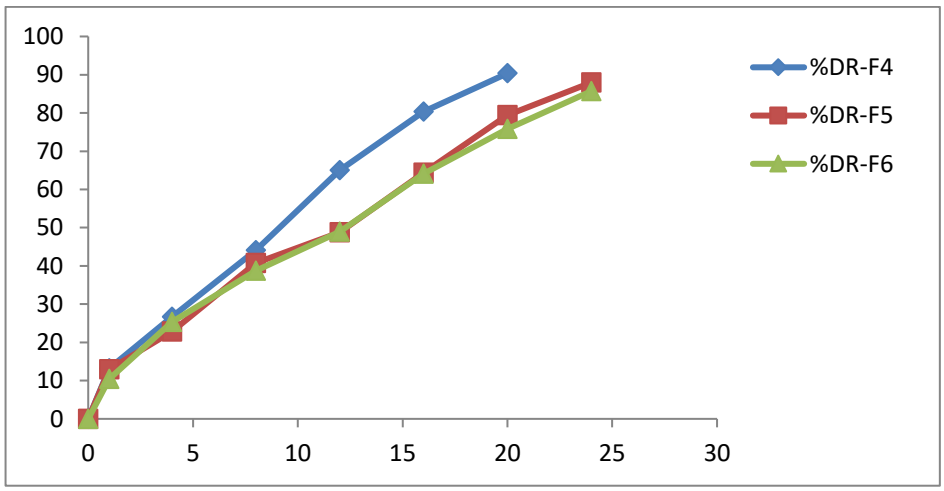

Figure9: Diss. profile of alg. microspheres of MS with HPMC k4M (F4-F6)

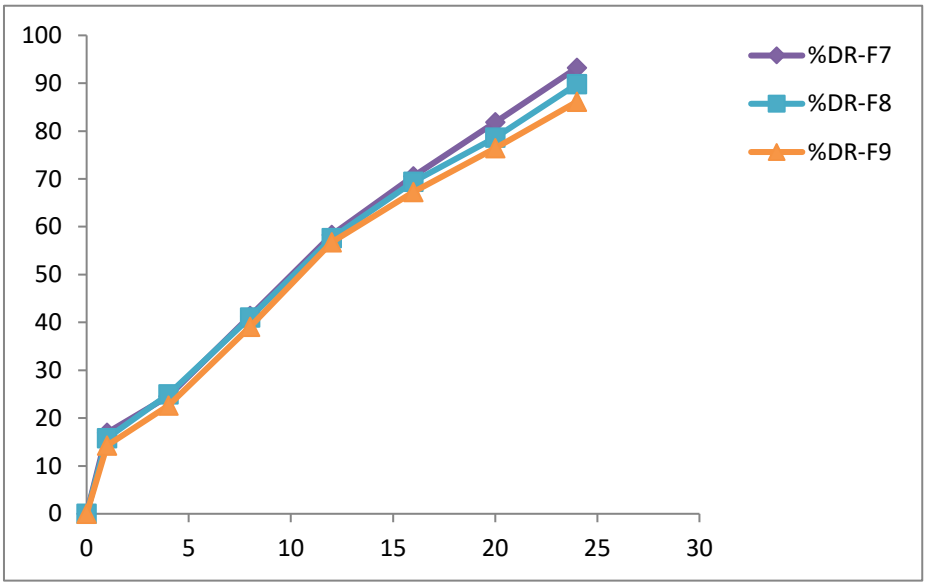

Figure 10: Dissolution profile of alginate microspheres of MS with Carbopol 934P (F7-F9).

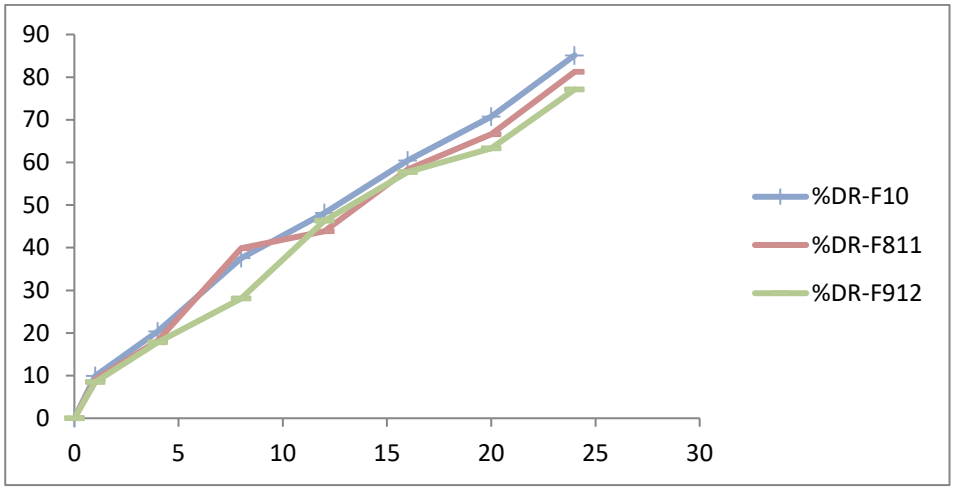

Figure11: Dissolution profile of alginate microspheres of MS with combination of HPMC k4M and Carbopol 934P (F10-F12)

Percentage drug loading and entrapment efficiency

Entrapment efficiency and drug loading was found to be increased with increase in the drug-polymer concentration from 1:1 to 1:4. From the results it can be inferred that there is a proper distribution of drug in the microspheres and the deviation is within the acceptable limits.

The percent of drug loading in Sodium CMC microsphere's formulations was found to be in the range of 6.28% to 7.28%. The percentage entrapment efficiency was found to be 32% to 56.70%. The percent of drug loading in HPMC k4M microspheres formulations was found to be in the range of 8.76% to 9.86%. The percentage entrapment efficiency was found to be 38.82% to 66.11%.

The percent of drug loading in Carbopol 934P microsphere's formulations was found to be in the range of 9.97% to 10.16%. The percentage entrapment efficiency was found to be 42.11% to 77%. The percent of drug loading in combination microsphere's formulations was found to be in the range of 8.63% to 9.64%. The percentage entrapment efficiency was found to be 36.58% to 62.35%.

A maximum of 77% drug entrapment efficiency was obtained in the F9 microspheres which were prepared by using Carbopol 934P. It was further observed that the drug entrapment was proportional to the MS: polymer

ratio and size of the microspheres. By increasing the polymer concentration, the encapsulation efficiency was increased.

***In vitro* drug dissolution studies**

The *in-vitro* performance of alginate microspheres of metoprolol succinate showed prolonged and sustained release of MS. The results of the in vitro dissolution studies of formulations F1 to F12 were shown in Table 4 and 5. The study indicated that the amount of drug release decreases with an increase in the polymer concentration. Among all the formulations, F7(Carbopol 934P) of ratio1:1 showed a maximum of 93.2% and F12(combination) of ratio 1:4 showed a minimum of 77.11% cumulative drug release after 24 hrs. So F7 is considered as the optimized formulation.

CONCLUSION

From the above experimental results, it can be concluded that preformulation studies like solubility and UV analysis of MS were complied with USP standards. The FTIR Spectra's revealed that, there was no interaction between polymers and MS. All the polymers used were compatible with the MS. The DSC data indicates that the MS is stable inside the microspheres, confirmed by the presence of sharp endothermic peaks. Surface smoothness of the MS microspheres and the size of the spheres were determined by SEM. The SEM was performed to the F7 optimized formulation. Entrapment efficiency increase with increase in the polymer concentration. From the results it can be inferred that there was a proper distribution of MS in the microspheres and the deviation was within the acceptable limits. The study also indicated that the amount of drug release decreases with an increase in the polymer concentration. The in-vitro performance of MS microspheres showed prolonged and sustained release of drug. The formulation F7 showed the maximum drug release of 93.2% from the prepared alginate microspheres. From the study it is evident that promising sustained release alginate microspheres of MS may be developed by ionic cross-linking technique by using polymers like Carbopol 934P, HPMC K4M and Sodium CMC.

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