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FORMULATION OF METOPROLOL SUCCINATE MICROSPHERES EMPLOYING NOVEL BIODEGRADABLA POLYMER "O-PALMITOYL PULLULAN" AS A POTENTIAL CARRIER IN THE EVALUATION AND STABILITY

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ABSTRACT

In the present investigation was attempts have been made to prepare metoprolol Succinate microspheres employing O-Palmitoyl pullulan (OPP) microspheres bearing ionotropic gelation method for sustained drug delivery. OPP was then synthesised from pullulan by chemically modifying with palmitoyl chloride and microspheres of metoprolol Succinate employing both OPP and sodium alginate were prepared. These prepared microspheres though gave a slow release, it was observed that more than 90% of drug released by 10-11 hrs itself or not uniformly spread throughout the expected 12 hrs. Formulation (F7) drug release was more controlled and the drug release was controlled up to 12 hours. Formulation (F7) followed zero order release kinetics and they showed Non-Fickian type of diffusion. If pharmaceutical preparations or new formulations are stored under normal conditions, their instabilities are detectable only after long storage periods. Such a method is time consuming and uneconomical. In an attempt to reduce the time required to obtain information about instabilities, various stress tests are undertaken. The most common stress conditions used are temperature, humidity and light. The stability studies conducted revealed that there was no change in the drug release profile after the products were stored at 37 ± 2^{0}C and 75 $\pm$ 5% RH and there was no significant drug loss after storage. Due to its biodegradable and non toxic nature OPP can be further used in different pharmaceutical formulations.

KEYWORDS: O-Palmitoyl pullulan (OPP), Microsphere, Entrapment efficiency, Swelling index, SEM.

INTRODUCTION

Metoprolol is a medication of the selective β_1 receptor blocker type. 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.[1] It is sold in formulations that can be taken by mouth or given intravenously. The objective of the present investigation is to study potential of O-Palmitoyl pullulan (OPP) as a vehicle for the preparation of microspheres. OPP containing drug delivery systems demonstrated that OPP can enhance the targeting capacity of the drug in the treatment of cancer, Alzheimer's disease. Earlier studies have restricted the usage of OPP only to liposomal formulations. The liposomal formulations are thermally unstable under normal environmental conditions and usually activate the reticulo-endothelial system leading to hypersensitivity reactions. Therefore in the present investigation intended to study the OPP as a carrier for preparation of microspheres. The OPP is a fluffy mass having very low density and high surface area. Upon standing and when exposed to atmosphere observed liquefaction which suggests that OPP in contrast to pullulan is hygroscopic. Also the viscosity of the 1% OPP solutions was found to be drastically higher; we expected it to act as a good co-polymer for the preparation of the various drug delivery systems. In the present investigation, we intend to prepare Sodium alginate – OPP interpenetrating polymeric microspheres and study its influence on the release profile of various hydrophilic drugs such as metoprolol succinate and sustain release upto 10 hrs. This work presents the release profile of Metoprolol succinate from Sodium alginate –OPP-copolymer matrix.[3]-[8]

MATERIALS AND METHODS

MATERIALS

Metoprolol succinate, A Gift sample from Dr reddy's Laboratory, Hyderabad.

Sodium alginate, A Gift sample from Loba Chemicals, Mumbai.

Calcium chloride, A Gift sample from Loba Chemicals, Mumbai.

Pyridine, A Gift sample from Loba Chemicals, Mumbai.

Palmitoylchloride, A Gift sample from Himedia, Mumbai.

Pullulan, A Gift sample from Sigma chem.

Dimethy formamide, A Gift sample from Merck(synthetic grade).

Ethanol A Gift sample from Merck(commercialgrade).

Diethyl ether, A Gift sample from Merck Ltd, Mumbai.

Guar gum.

Xanthan gum.

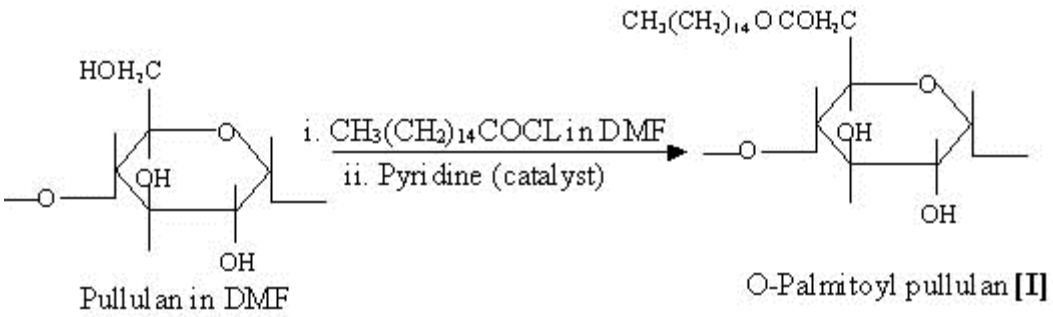
Gellan gum.

Other materials are procured from Commercial Grade.

METHODS

Preparation of O-palmitoyl Pullulan

O-palmitoyl pullulan (OPP) was prepared 1g pullulan was dissolved in 11 ml dry dimethylformamide at 60°C.[2] To the resulting solution add 1 ml of dry pyridine and 0.1 g palmitoyl chloride to dissolved in 0.24 ml dry dimethylformamide were added. The mixture was stirred at 60°C for 2 h followed by 1h at room temperature. This mixture was then slowly poured into 70ml absolute ethanol under stirring. The precipitate so formed was collected and washed with 80 ml absolute ethanol and 60 ml dry diethyl ether. The white solid material obtained was dried in vacuum at 50°C for 2 h synthesis shown in below chemical reaction.



Preparation of Microspheres

Metoprolol succinate microspheres using sodium alginate and copolymer OPP were prepared by orifice ionic gelation method. 0.5%, 1%, 1.5%, 2%, 3% and 4% sodium alginate solution and 1% OPP solution were prepared initially. Then, Metoprolol succinate was added to 1% OPP solution mixture and homogenized thoroughly with a magnetic stirrer to form a homogeneous dispersion. The drug-copolymer solution and sodium alginate were mixed in 2:1,1:1,1:1.5,1:2,1:3 and 1:4 ratios. The resulting bubble free dispersion was added drop wise manually with a 10 ml syringe fitted with an 18 gauge needle into 100 ml of (5% w/v)

calcium chloride ($CaCl_2$) solution kept under stirring in a 250 ml beaker. The gelation time of 15 min was allowed to complete the curing reaction and produce spherical rigid microspheres. The spheres so prepared were collected by decantation, washed with n-hexane and dried at less than 40^oC for 12h. The alginate solution is dripped, one drop at the time from a large syringe into solution of calcium chloride. The spheres are instantly formed in the $CaCl_2$ solution.

Evaluation of Formulated Microspheres

Percentage Yield

Percentage practical yield is calculated to know the efficiency of any method, thus it helps in selection of appropriate method of production. It was calculated as the weight of spheres recovered from each batch in relation to the sum of starting material. The percentage yield of prepared spheres was calculated by using the formula.

$$percentage\ yield = \frac{practical\ yield}{theoritical\ yield} \times 100$$

Particle Size Determination

50 microspheres size was measured by optical microscopy. The mean diameter was determined by measuring the number of divisions covered by microspheres using ocular micrometre previously calibrated using stage micrometre.

Swelling Index

Pre weighed metoprolol succinate microspheres (W_0) formulated with polymers by employing different ratios were placed in pH 7.4 phosphate buffer maintained at 37°C. After 8h. The microspheres were collected and blotted to remove excess water and weighed (W_t). The swelling index was calculated with the following formula.

$$\boldsymbol{swelling\ index = \frac{Wt - Wo}{Wo} \times 100}$$

Where W_t = weight of microspheres observed at the end of 3h and W_0 = the initial weight of microspheres.

Drug Entrapment Efficiency

5mg spheres of each batch were placed in 10ml phosphate buffer P^H7.4 and mechanically agitated on shaker at 200rpm for 24hrs. The resultant solution was filtered and analysed at

278nm using UV visible spectrophotometer. The percentage drug entrapment efficiency (% EE) of each sphere was calculated by using the following equation.

$$\%EE = {}^{AQ}/_{TQ} \times 100$$

Here AQ is actual drug content and TQ is theoretical drug content of drug present in each sphere.

Surface Morphology

In pharmaceutical industry, scanning electron microscopy (SEM) may be used as a qualitative tool for the analysis of drug substance and drug product in order to obtain information on the shape and surface structure of the material. SEM plays an important role in the characterization of nanoscale and substance micron particles. It has been used to determine surface topography, texture and to examine the morphology of fractured or sectioned surfaces. The examination of surface of polymeric drug delivery system can provide important information about the porosity and microstructure of device.

In Vitro Drug Release

In vitro drug release studies of metoprolol succinate microspheres was carried out using USP type II dissolution rate test apparatus (LABINDIA DS 8000) with a PADDLE stirrer at 50 rpm in 900 ml of 0.1N hydrochloric acid for first 2 hrs and in phosphate buffer of P^H7.4 for next 10 hrs and temperature maintained at 37 ± 0.5°C. Microspheres equivalent to 50mg of Metoprolol succinate were taken in the paddle. 5ml samples of the dissolution fluid was withdrawn at regular intervals and replaced with fresh dissolution medium. The samples were filtered, diluted and analyzed using UV-Visible Spectrophotometer (LABINDIA UV3000) at a wavelength of 278nm. (n=3).

Kinetics

Zero-order release rate kinetics

To study the zero-order release kinetics, the release rate data are fitted to the following equation: $Q=K_0$ t Where "Q" is the fraction of drug released, "K_0" is the zero order release rate constant and "t" is the release time.

First order rate kinetics

A first-order release would be predicted by the following equation.

$LogC = LogC_0 - [K_1 t / 2.303]$ Where C = amount of drug remaining at time "t", C_o = initial amount of the drug and K_1 = first-order rate constant (h^{-1}). The constant "K_1" can be obtained by multiplying 2.303 with the slope obtained in the log cumulative percentage drug remained versus time plot, which yield a straight line, indicating that the release follows first-order kinetics.

Huguchi Release Model

To study the Higuchi release kinetics, the release rate data were fitted to the following equation: $Q=K_H.t^{1/2}$ Where "Q" is the amount of drug released, "K_H" is the release rate constant, and "t" is the release time. Higuchi release rate constant K_H can be calculated by finding the slope of the cumulative drug released versus square root of time plot; which yield a straight line, indicating that the drug was released by diffusion mechanism.

Hixson–Crowell Model

Hixson and Crowell recognizing that the particle regular area is proportional to the cubic root of its volume derived an equation that can be described in the following manner: $W_o^{1/3} - W_t^{1/3} = K_s t$

Where, W_o is the initial amount of drug in the pharmaceutical dosage form, W_t is the remaining amount of drug in the pharmaceutical dosage form at time t and K_s is a constant incorporating the surface–volume relation.

Korsmeyer-Peppas Release Model

The release rate data were fitted into the following equation.

$Mt/M_\infty = Q = K.t^n$ Where $Mt/M\infty$ is the fraction of drug released, "K_{KP}" the release constant, "t" the release time and "n" is the diffusion exponent for the drug released that is dependent on the shape of the matrix dosage form. Korsmeyer Peppas release rate constant K_{KP} can be calculated by finding antilog of the Y intercept of the log % drug released versus log time plot, which yield a straight line with a slope equal to "n" which indicate drug release mechanism. When 'n' approximates 0.43, a Fickian/diffusion control release is implied; where $0.43<n<0.85$, it implies non-Fickian transport; and $n \geq 0.85$ for zero-order release.[9]-[10]

Short Term Stability Study for Selected Formulations

Optimized formulation of the microparticles was selected for stability studies formulations were packed in a screw capped bottle and studies were carried out for 90 days by keeping at 37 ± 2°C and 75 ± 5% RH. Samples were withdrawn on 30th, 60th and 90th day

and were analyzed for physical appearance, entrapment efficiency *and In-vitro* drug release.[11]

RESULTS AND DISCUSSIONS

The present investigation is to study potential of O-Palmitoyl pullulan (OPP) as a vehicle for the preparation of microspheres OPP containing drug delivery systems demonstrated that OPP can enhance the targeting capacity of the drug in the treatment of cancer, Alzheimer's disease. The melting point of Metoprolol Succinate sample was determined by capillary tube method and it was found to be 141^{o}C which is within the reported range 140- 144^{o}C. It complies with the purity of the drug sample. FTIR spectroscopy was carried out to study the compatability of pure drug Metoprolol Succinate with OPP as co-polymer and sodium alginate as polymer used in the formulation of spheres. The pure Metoprolol Succinate has characteristic IR peaks at 2990.26 cm^{-1} (methyl and methylene), 2967.23 Cm^{-1} (methoxy), and 2541.23 Cm-1 (protonated amine). From the FTIR spectra of the pure drug and the combination spectra of drug with the polymers, it was observed that all the characteristic peaks of Metoprolol Succinate with optimized formula were present in the combination spectra as well thus indicates the compatability of the drug with polymers used shown in Fig.1.

Percentage yield of all formulations were calculated the results are shown in the table.2. The microspheres obtained were evaluated for particle size diameter using optical microscope. The drug loaded microspheres are spherical in shape shown in table.3. Percentage drug entrapment in the spheres includes drug entrapped within the polymer matrices. The values were in the range of 28 to 85 for dried microspheres shown in table.4. Entrapment efficiency increased with increased ratios of polymer may be initially due to the hygroscopic nature of OPP entrapment was low and with increase in sodium alginate proportion the binding nature of OPP enhanced and its hygroscopic nature decreased.

In SEM photograph of formulation, a single sphere at 40x magnification was shown in Fig.2 and Fig.3. The figure produced microspheres with spherical conformation and heterogeneous size distribution. The swelling index was shown in range 4.2 to 10.5% shown in table.5. The drug release of metoprolol succinate from various formulations of o-palmitoyl pullulan and sodium alginate microspheres were studied in 0.1N hydrochloric acid for first 2 hours and then in phosphate buffer of pH 7.4 for the remaining period of 10 hrs. The drug release profile is shown in table.6, Fig.4 and Fig.5. These prepared microspheres though gave a slow

release and it was observed that more than 90% of drug released by 10-11 hrs itself or not uniformly spread throughout the expected 12 hrs. Then another formulation with natural gum was prepared (F7). In this formulation, drug release was more controlled and the drug release was controlled up to 12 hours. The Metoprolol Succinate release was found to be retarded from the matrix of microspheres. However the extend of retarding the release was dependent upon the nature of copolymer (OPP) and also the nature of the natural gum added keeping sodium alginate as constant. The various kinetic parameters of drug release are shown in table.7 and table.8. The data indicated a good linearity with significantly high correlation coefficient values for the first order release rate constants than for the zero order release constants. As the kinetics of release is affected by physico-chemical changes dissolution medium and processing variables so it is extremely difficult to obtain zero order kinetics for controlled release system to deliver the drug. For the further evaluation of drug release mechanism, a plot was drawn for square root of time vs. cumulative percent drug release as proposed by Higuchi and the plots were found to be linear in all cases and the correlation coefficient values are 0.8664, 0.8704, 0.8277, 0.9236, 0.9571, 0.9873, 0.9739 and 0.8195 for F1 to F8 respectively. So comparing both it was found that higuchi plots are more linear in all cases indicating diffusion matrix formulations. Korsemeyer peppas plot was also drawn taking log time vs. log percent drug release. The ‘n’ values (0.49-0.82) obtained in the peppas plot are shown in table.8. It indicates that the drug release is by Non Fickian diffusion mechanism.[12]-[18]

It is necessary to perform stability testing for optimizing formulation as per ICH guidelines to find out the extent of deterioration and to ensure the degradation has not exceeded an acceptable level assuring the safety of the patient and the activity of the product. Degradation of active ingredients in pharmaceutical formulations may occur by hydrolysis, oxidation, reduction, racemisation, photolysis, decarboxylation and isomerisation. Physical degradation of pharmaceutical products may occur due to loss of water, loss of volatile constituents, absorption of water, crystal growth, polymorphic changes and colour changes.

If pharmaceutical preparations or new formulations are stored under normal conditions, their instabilities are detectable only after long storage periods. Such a method is time consuming and uneconomical. In an attempt to reduce the time required to obtain information about instabilities, various stress tests are undertaken. The most common stress conditions used are temperature, humidity and light. Pharmaceutical preparations may often

exhibit physical or chemical reactions that may end in instability due to which the product gets deteriorated. This deterioration may lead to reduction in the activity of the product, formation of toxic products. Stability studies were carried out for all the six formulations as per ICH guidelines for 3 months data shown in table.9 there was no significant change in appearance, % entrappment efficiency and cumulative % drug release of the microspheres of best formulation (F6) at the end of 3months. It was found that the formulation was stable throughout the study period.

Table 1: Formulae of Prepared Microspheres (F1-F8) Employing OPP.

S.NO	INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8
1	Metoprolol Succinate (mg)	50	50	50	50	50	50	50	50
2	Sodium alginate(ml)	25	25	25	25	25	25	25	25
3	OPP%	1	2	2	2	2	2	2	2
4	Guar gum(mg)	-	-	200	400	-	-	-	-
5	Xanthan gum(mg)	-	-	-	-	200	400	-	-
6	Gellan gum(mg)	-	-	-	-	-	-	200	400

Table 2: Percentage Yield of Formulations F1 to F8.

Formulation code	% yield
F1	48.31
F2	56.72
F3	63.68
F4	67.72
F5	71.35
F6	68.43
F7	72.65
F8	67.72

Table 3: Particle Size of Formulations F1 to F8.

Formulation code	Particle size (mm)
F1	0.452±0.058
F2	0.475±0.069
F3	0.494±0.064
F4	0.492±0.054
F5	0.478±0.078
F6	0.475±0.069
F7	0.464±0.068
F8	0.475±0.062

Table 4: Entrapment Efficiency of F1 to F8 Formulations.

Formulation code	%EE
F1	85
F2	90
F3	86
F4	88
F5	88
F6	91
F7	92
F8	93

Table 5: Swelling Index Values of formulations F1 to F8.

Formulation Code	Swelling Index (%)
F1	4.2
F2	5.8
F3	7.2
F4	7.8
F5	9.2
F6	10.3
F7	10.4
F8	10.5

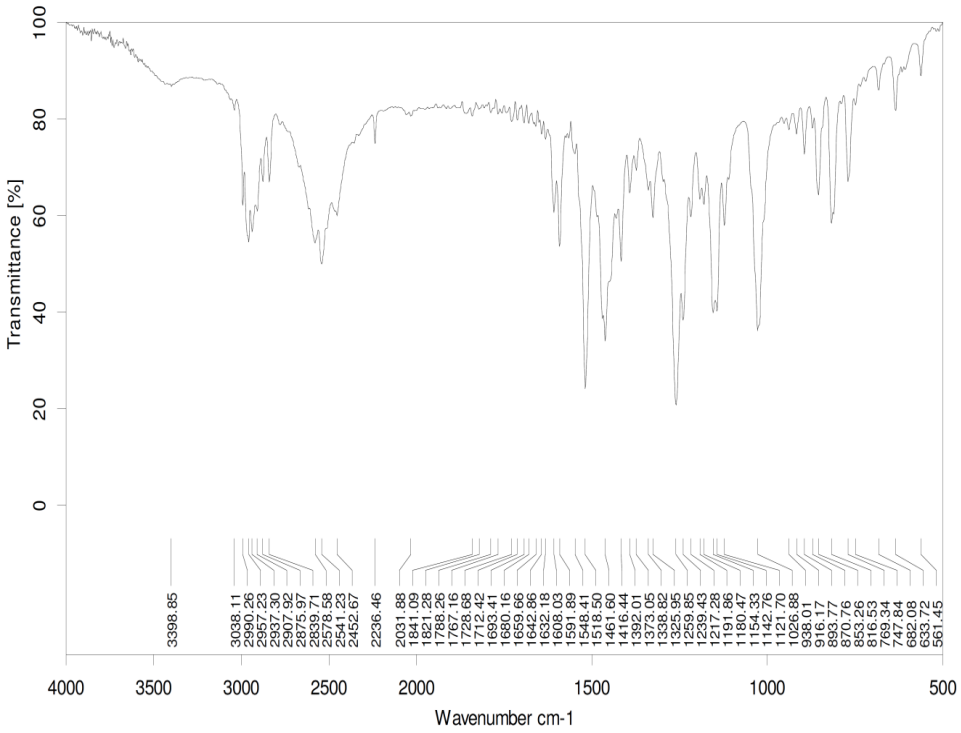


Fig 1: FTIR Spectrum Of F7 Formulation.

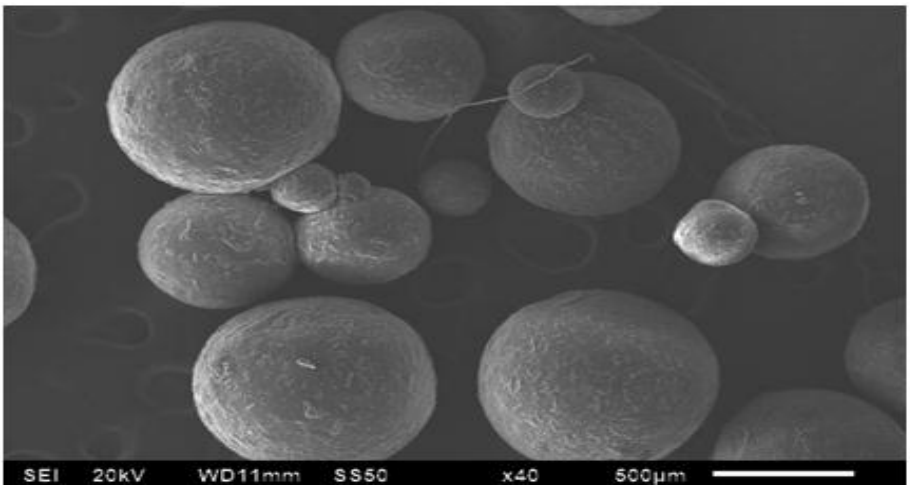


Fig 2: SEM Photograph of Formulation F7.

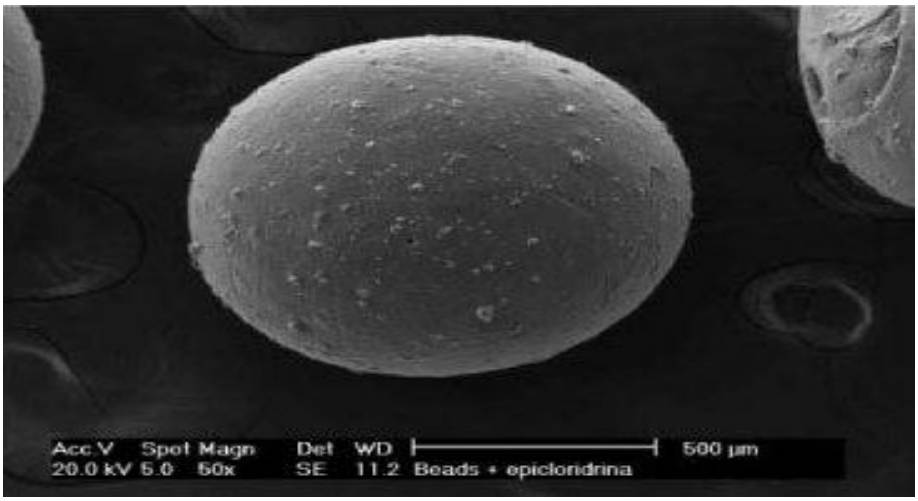


Fig 3: SEM Photograph of Formulation Microsphere.

Table 6: Percent Drug Release of F1 to F8 Formulations of Microspheres.

TIME (hrs)	Cumulative % Drug Release							
	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
0.5	0	0	0	0	6.2763	7.2100	6.7100	0
1	21.772	0.000	0.000	0.000	16.740	16.350	14.350	0.000
2	26.524	19.214	0.000	22.937	50.248	30.130	27.130	46.464
3	30.552	30.932	7.831	24.024	52.221	45.320	34.370	47.693
4	26.699	28.865	7.831	39.519	47.975	51.510	43.520	46.310
5	22.843	24.866	51.737	27.832	57.072	59.320	53.470	47.078
6	55.976	75.594	61.089	51.431	66.474	67.320	60.320	47.386
7	74.348	77.109	75.196	63.141	70.563	73.520	67.520	106.541
8	83.632	79.728	58.712	70.303	76.778	76.350	76.350	97.630
9	91.316	67.323	65.840	74.419	89.549	84.350	84.350	97.886
10	100.385	95.581	90.255	78.509	90.257	90.320	90.320	80.612
11	102.383	97.253	92.357	81.659	94.366	95.250	96.250	85.689
12	104.298	98.335	94.466	84.778	98.579	99.980	100.010	89.568

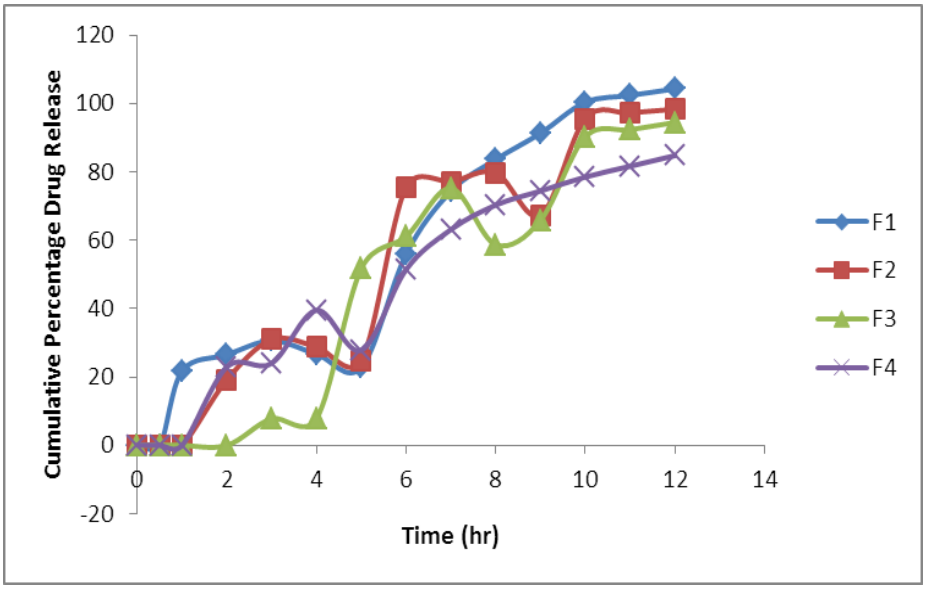


Fig 4: Cumulative Percentage of Drug released Formulations (F1-F4).

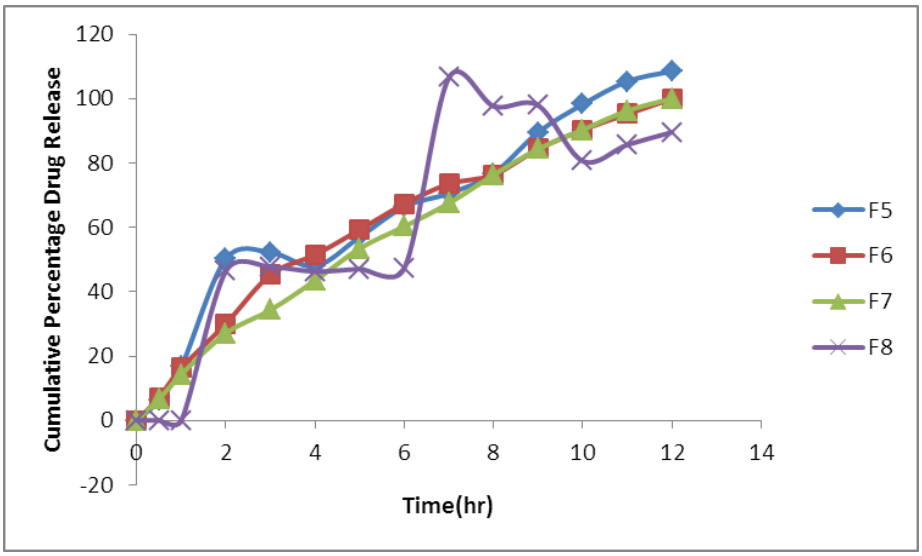


Fig 5: Cumulative Percentage of Drug released Formulations (F5-F8).

Table 7: Drug Release Kinetic Parameters of F1 to F6 Formulations of Microspheres (Zero order and First order).

S.NO	Formula	Zero order		First order	
		K_0	R^2	K_1	R^2
1	F1	9.3434	0.9364	-0.1013	0.8106
2	F2	9.1287	0.9162	-0.1386	0.8358
3	F3	9.1706	0.911	-0.1031	0.8655
4	F4	7.7601	0.9554	-0.0713	0.9689
5	F5	8.4135	0.9381	-0.1247	0.765
6	F6	8.042	0.9571	-0.1807	0.5904
7	F7	8.3372	0.9863	-0.1084	0.8957
8	F8	8.2184	0.7684	-0.1062	0.5836

Table 8: Drug Release Kinetic Parameters of F1 to F6 Formulations of Microspheres (Higuchi, Hixson crowell and Peppas).

S.NO	Formula	Higuchi		Hixson crowell		Peppas	
		K_H	R^2	K_S	R^2	N	R^2
1	F1	34.324	0.8664	-0.1095	0.813	0.5787	0.7416
2	F2	33.988	0.8707	-0.0634	0.9015	0.5802	0.8101
3	F3	33.387	0.8277	-0.0545	0.9056	0.9336	0.8627
4	F4	29.142	0.9236	-0.0416	0.7741	0.4957	0.8764
5	F5	32.458	0.9571	-0.1001	0.7728	0.7942	0.9222
6	F6	31.196	0.9873	-0.0626	0.9191	0.7876	0.9795
7	F7	31.627	0.9729	-0.0676	0.8733	0.8296	0.9948
8	F8	32.415	0.8195	-0.059	0.3808	0.288	0.587

Table 9: Stability Studies of Optimized Formulation (F7).

Time (Month)	Physical Appearance	% Entrapment Efficiency	Cumulative % Drug Release
0	Off white	92.36	100.01
1	Off white	92.28	99.98
2	Off white	92.2	99.95
3	Off white	92.0	99.9

CONCLUSION

1. In this work, attempts have been made to prepare the OPP-sodium alginate microspheres bearing Metoprolol Succinate by ionotropic gelation method for sustained drug delivery.
2. OPP was then synthesised from pullulan by chemically modifying with palmitoyl chloride and microspheres of metoprolol Succinate employing both OPP and sodium alginate were prepared.
3. These prepared microspheres though gave a slow release, it was observed that more than 90% of drug released by 10-11 hrs itself or not uniformly spread throughout the expected 12 hrs. Formulation (F7) drug release was more controlled and the drug release was controlled up to 12 hours.
4. Formulation (F7) followed zero order release kinetics and they showed Non-Fickian type of diffusion.
5. The stability studies conducted revealed that there was no change in the drug release profile after the products were stored at 37 ± 2^0C and 75 $\pm$ 5% RH and there was no significant drug loss after storage.
6. The controlled release products of metoprolol Succinate could be designed by ionic gelation method with OPP-sodium alginate and Gellan gum that is F7.

7. Due to its biodegradable and non toxic nature OPP can be further used in different pharmaceutical formulations.

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