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Formulation and evaluation of extended release microspheres loaded with labetalol

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Abstract

Microspheres loaded with Labetalol were prepared using chitosan as the polymer and TPP as the crosslinking agent. The formulation of micropsheres was done at various concentrations of the polymer and TPP. All the microsphere formulations (F1 to F6) were characterized for yield, entrapment efficiency, rheological properties (angle of repose, bulk density, tapped density, Hausner's ratio, Carr's index), and in vitro drug release. The pharmacokinetic release profile of drug from all the formulations was determined using zero order, Higuchi equation and Korsemeyer-Peppas release model.The particles were found to be in the range of 600-1100 µm and the percentage yield varied from 64-83%.The drug entrapment efficiencies of different formulations were in the range of 64.26 to 86.00%. All the formulated microspheres exhibit good flow properties with a value of angle of repose between 21°32' to 29°72'. The Carr's index of all the formulations was found in the range 5.88 to 14.57 % indicating good flow ability. The Hausner's ratio of the formulations was found in the range 1.062 to 1.170. The cumulative drug release was observed to be 61.774%, 63.061%, 36.035%, 34.380%, 60.855% and 57.178% for the formulations F1 to F6 respectively. Consequently, it can be concluded that the microspheres produced from chitosan and TPP using ion gelation method is an excellent delivery system that has good extended release behavior and this would be beneficial in decreasing the dosing frequency of Labetalol in treatment of hypertensive conditions.

Keywords: Labetalol, antihypertensive, extended release, microspheres, chitosan, TPP

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Introduction

Oral drug delivery, for decades, has been known as the most widely recommended route of administration among all the routes and can be classified as the immediate release systems and the modified release systems (Chen, 2005).The immediate release drug delivery systems allow for rapid disintegration and exhibit instant drug release. They are also associated with variations in plasma levels of the delivered drug, which in turn leads to reduction in effectiveness of the drug (Brahmankar and Jaiswal, 2005). Modified drug release systems, on the contrary to immediate release systems, not only lead to improved pharmacokinetic profiles of drugs and active pharmaceutical ingredients(APIs) but also the patient compliance,and are able to reduce the sideeffects. Extended release drug delivery systems are a type of modified release system that has the capability to attenuate the dose required for treatment of any disease while achieving the therapeutic effects. Polymeric microspheres as drug carriers present an excellent approach for the extended release of drug molecules (Klausnera et al, 2003).

Labetalol hydrochloride, 2-Hydroxy-5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl) amino] ethyl]-benzamide is a non selective α, β-adrenoceptor antagonist used in the treatment of hypertension (drugbank, 2021). It is appreciably soluble in lower and higher pH solutions, with minimum solubility between pH 6 to 10. The drug exhibits variable bioavailability ranging from 10-80% which can be attributed to its instability in alkaline pH and poor absorption due to precipitation. It is administered in doses ranging from 50-200 mg twice a day due to its shorter half life of 3-6 h suggesting the need for sustained release formulation. Some attempts have been made to extend the drug release duration of Labetalol formulating as floating tablets (Jain et al, 2016; Subhash Kumar et al, 2016), microspheres (Swathi et al, 2014), floating microbeads (Sunil Kumar et al, 2020). The drug release could be extended until a period of 12 h. Hence, the objective of the present investigation was to develop microspheres consisting Labetalol hydrochloride to achieve sustained release of Labetalol thereby resulting in reduction of the dosing schedule.

Material and Methods

Labetalol was obtained as gift sample from Ipca Pharmaceuticals Limited, Ratlam and was confirmed by IR spectroscopy. Acetone, liquid paraffin, Chitosan and Sodium alginate were procured form Thomas Baker, Himedia and S.D.Fine Chemicals limited and were used as obtained. All other chemicals and reagents used were of analytical grade and used as obtained.

Preformulation Studies (Martin, 1995)

The organoleptic features, solubility, loss on drying (LOD), FTIR, melting point and absorption maxima of the procured drug sample was determined. The calibration curve of labetalol was prepared in phosphate buffer pH 6.8 by UV spectrophotometry at 285nm.

Formulation of microspheres

Ionic gelation method (Chowdary et al, 2003; Shabaraya et al, 2003) was used to formulate the chitosan microspheres loaded with Labetalol. The composition of the different batches of microspheres is given in table 1.

Table 1 Composition of different batches of microspheres

Formulation Code	Conc. of chitosan (%)	Conc. of TPP (%)	Stirring speed (rpm)	Stiring Time (min)	Volume of chitosan-drug solution
F1	1	0.5	600	30	10
F2	1	1	600	30	10
F3	1.5	1	600	30	10
F4	1.5	2	600	30	10
F5	2	1	600	30	10
F6	2	2	600	30	10

TPP – sodium tripolyphosphate

Briefly, chitosan stock solution was prepared by dissolving chitosan in glacial acetic acid at room temperature. The drug Labetalol (1% w/v) was dissolved directly into the above prepared chitosan solution. Through a disposable syringe needle 10 ml of this bubble free solution was dropped into a gently agitating 100 ml of sodium tripolyphosphate solution. The dropping rate and falling distance were kept constant. The solution was magnetically stirred for half an hour followed by filtration and rinsing with distilled water. Gel like beads were achieved which air was dried for 24 hours followed by over drying for 6 hours at 50ºC.

Characterization and Evaluation of Formulations

Determination of Yield

The dried microspheres were collected and weighed accurately. The percentage yield was then calculated using formulae given below:

$$\% \text{ yield} = \frac{\text{Mass of the dried microspheres obtained} * 100}{\text{Total Weight of drug and polymer}}$$

Determination of particle size of microspheres

The particle size of the microspheres was determined by using an Olympus microscope, employing the calibrated eye piece and stage micrometer method.

Determination of drug entrapment in the microspheres (Stithit et al, 1998)

The various formulations of the microspheres were subjected to drug content analysis. 50 mg of the microspheres of each batch were accurately weighed and crushed. The powdered microspheres were dissolved in 10 mL methanol, in a 100 mL volumetric flask and the volume was made up to the mark with PBS pH 6.8. The solution was filtered through Whatman filter paper No. 44. After filtration 10 mL of this solution was pipette out and diluted up to 100 mL with PBS pH 6.8. 2 mL of this solution was then diluted up to 10 mL with PBS pH 6.8 and the absorbance was measured at 285 nm against PBS pH

6.8 as blank. The percentage drug entrapment was calculated as follows:

$$\% \text{ Drug Entrapment} = \frac{\text{Calculated Drug Concentration} * 100}{\text{Theoretical Drug Concentration}}$$

Rheological Properties

Angle of repose, Carr's Index, Bulk density, Tapped density and Hausner's ration were determined to assess the flow ability of the prepared microspheres.

In vitro release study

USP type II dissolution apparatus (paddle type) was performed at 50 rpm in 900 mL PBS pH 6.8. 5 mL of the sample was withdrawn at a predetermined interval and the volume of dissolution medium was maintained by adding equal volume of fresh dissolution medium. The absorption of the withdrawn sample was measured spectrophotometrically with suitable dilution and the corresponding concentration was determined from the calibration curve. The temperature was maintained at 37°C throughout the study.

Results and Discussion

Physical characterization of the drug was performed according to the reported procedure and the results obtained were compared with that of the standard specifications (Table 2).

The calibration curve was obtained in phosphate buffer pH 6.8 and the equation for the regression line was found to be Absorbance = 0.066 (concentration) – 0.009 with and regression coefficient (R^2) of 0.998 (Figure 1). The calibration curve equation was used for calculation of drug concentration wherever required.

Table 2 Physical characterization of Labetalol

S No	Parameter	Observation	Solubility
1	Physical appearance	White to off-white crystalline powder	Soluble in water, methanol, ethanol and DMSO
2	Odour	Odourless	
3	Melting Point	178-181°C	

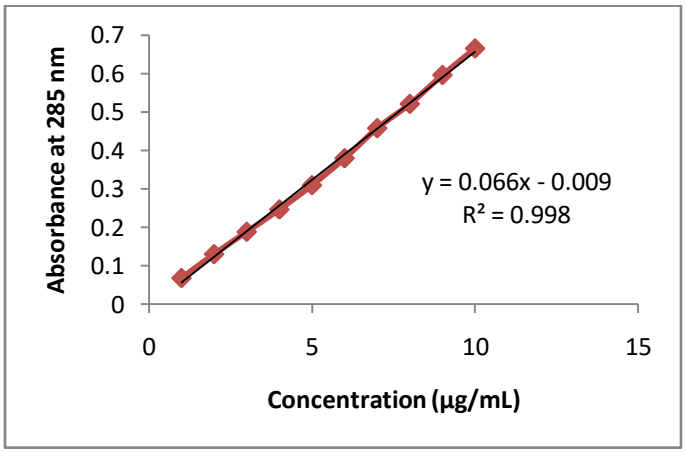

Figure 1 Calibration curve of labetalol in phosphate buffer pH 6.8

Formulation of microspheres

Microspheres of Labetalol were prepared using chitosan as the polymer and TPP as the crosslinking agent by ion gelation method by varying the concentration of the polymer and the crosslinking agent. The success of formulation depends upon its

yield, drug content and entrapment efficiency. The drug concentration was kept fixed at 1% w/v of the polymer solution.

Particle Size, shape and yield of microspheres

The various formulations of the prepared microspheres were evaluated for the percentage process yield. The percentage yield varied from 64-83%. (Table 3) The results show that increasing the concentration of the polymer had mixed effect on the yield of the microspheres.

The particle size of various formulations was determined by stage micrometer and eye piece method. The average particle size of the microspheres obtained from the various compositions revealed that the particles size increased with increasing the concentration of chitosan and further increased with increasing TTP concentration. The particles were found to be in the range of 650-1034 µm.

Table 3 Particle size, shape and yield of various formulations

Formulation Code	Yield (%)	Particle Size (µm)	Shape
F1	83	650	Spherical
F2	76	710	Spherical
F3	72	782	Spherical
F4	64	854	Spherical
F5	81	896	Spherical
F6	72	1034	Spherical

Entrapment of drug in the microspheres

The drug entrapment of various formulations of Labetalol was carried out as per the procedure and performed in triplicate. The drug entrapment efficiencies of different formulations were in the range of 64.26 to 86.00% (Table 4). Drug entrapment slightly increases with increase in chitosan and TPP concentration. This is due to the permeation characteristics of chitosan that could facilitate the diffusion of part of entrapped drug to the surrounding medium during the formulation of the microspheres.

Table 4 Drug entrapment efficiency of various formulations

Formulation Code	Drug entrapment (%)
F1	64.26
F2	72.5
F3	73.36
F4	76
F5	81
F6	86

Flow properties

All the formulated microspheres exhibit good flow properties with a value of angle of repose between 21°32' to 29°72'. All the formulations had an angle of repose of less than 40° thereby suggesting excellent rheological property thereof. The bulk density was found in the range 0.350 to 0.571 g/cm^3 and the tapped density from 0.390 to 0.666 g/cm^3.

The Carr's index of all the formulations was found in the range 5.88 to 14.57 % indicating good flow ability. The percentage compressibility value of less than 20 is an indicator of excellent flow property of the powder. The Hausner's ratio of the formulations was found in the range 1.062 to 1.170. a ratio of less than 1.25 indicates good flow properties.

Table 5 Rheological properties of microspheres

Batch Code	Angle of repose (°)	Bulk Density	Tapped Density	Carr's Index	Hausner's ratio
F1	24.33	0.444	0.487	8.82	1.096
F2	21.32	0.408	0.465	12.25	1.139
F3	27.54	0.416	0.487	14.57	1.17
F4	24.32	0.35	0.39	10.25	1.114
F5	27.86	0.571	0.666	14.26	1.166
F6	29.72	0.5	0.571	12.43	1.142

In vitro drug release study

The *in vitro* drug release study of the microspheres was evaluated in PBS pH 6.8. The % release, % cumulative release and % log cumulative release was calculated. The data was statistically evaluated using Higuchi and Korsemeyer-Peppas model to determine the best fit explaining the release of the drug Table 6.

In vitro drug release study

The *in vitro* drug release study of the microspheres was evaluated in PBS pH 6.8. The % release, % cumulative release and % log cumulative release was calculated. The data was statistically evaluated using Higuchi and Korsemeyer-Peppas model to determine the best fit explaining the release of the drug Table 6.

Table 6 *In vitro* release data of F1-F6

Time (h)	% cumulative release					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	6.251	32.358	4.412	3.861	5.332	4.964
2	20.775	43.205	14.892	15.444	9.56	9.376
3	27.578	54.42	25.004	24.636	15.811	12.87
4	32.358	59.016	27.945	28.681	23.165	17.65
5	38.425	59.936	31.622	29.784	29.968	24.085
6	47.066	60.671	32.358	31.439	36.954	32.358
7	52.03	61.774	33.829	32.542	47.066	44.492
8	57.546	62.142	35.116	33.277	52.03	48.169
9	59.2	62.693	35.483	33.829	58.649	52.214
10	61.774	63.061	36.035	34.38	60.855	57.178

The in vitro release data from different formulations were studied in phosphate buffer ph 6.8 for 10 h using USP-II type dissolution apparatus. From the release data it was observed that the percentage cumulative release and the release rate depended upon the type and amount of polymer used.

For the formulations F1 to F6 it was found that the release of Labetalol significantly decreased with increasing the amount of chitosan and TPP. This is due to the fact that when higher concentration of polymer is used the density of the polymer matrix is increased thereby increasing the diffusion path length. The cumulative drug release was observed to be 61.774%, 63.061%, 36.035%, 34.380%, 60.855%

and 57.178% for the formulations F1 to F6 respectively.

In order to establish the mechanism of drug release, different kinetic models are used. The drug release data were subjected to various mathematical kinetic model including zero order release kinetics (plot of cumulative percent release vs time), Highuchi's equation (plot of cumulative percentage of drug release vs square root of time) and Korsmeyer-Peppas equation (log cumulative percentage release vs log time) (Figure 2-4). The Korsmeyer model is widely used when the release mechanism is not well known or when more than one type of release phenomena could be involved. The interpretation of data was based on the value of the resulting regression coefficients.

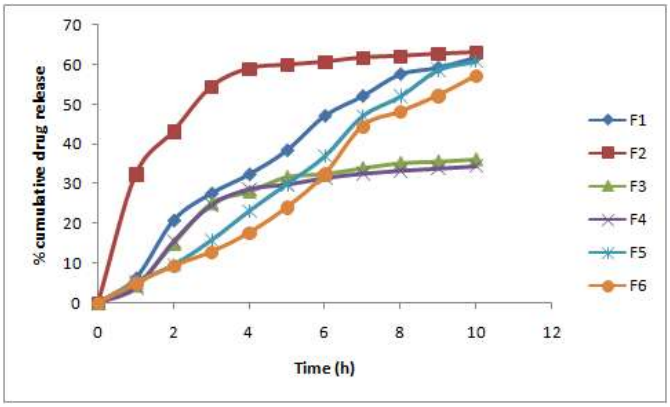

Figure 2 Zero order release profile of labetalol from formulations

For all the formulations, the values of R^2 of zero order, Higuchi and Peppas model were calculated. It was clearly observed that for most of the formulations, the value of resulting regression coefficient is highest for Higuchi model which shows that all the formulations predominantly followed the Higuchi square root kinetics. The corresponding n values of maximum formulations were below 0.5 which indicates that the formulations released drug though Fickian diffusion mechanism.

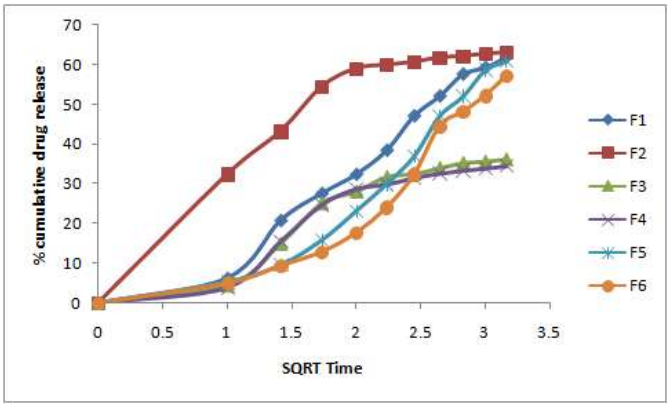

Figure 3 Higuchi model kinetic release of labetalol from formulations

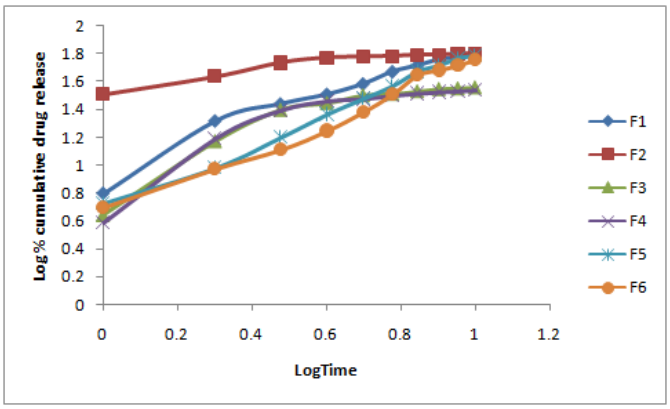

Figure 4 Korsemeyer-Peppas model kinetic release of labetalol from formulations

On the bases of all the evaluation parameters it was found that formulations F3, F4 and F6 were the best formulations with good drug entrapment and extended drug release profile.

Conclusion

In the present study, microspheres loaded with Labetalol were prepared using ion gelation method using chitosan and TPP. The results obtained showed that this methodology was able to produce reproducible microspheres and for extended release of drug from the formulations. Consequently, it can be concluded that the microspheres produced from chitosan and TPP using ion gelation method is an excellent delivery system that has good extended release behavior and this would be beneficial in decreasing the dosing frequency of Labetalol in treatment of hypertensive conditions.

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