Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2018, 10 [4]: 86-99
[http://scholarsresearchlibrary.com/archive.html]

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Development and Evaluation of Controlled Release Microspheres Containing Labetalol

Swathi K.N., Ashok Kumar P* and Suresh V. Kulkarni

Department of Pharmaceutics, Sree Siddaganga College of Pharmacy, Tumkur, B. H. Road, Karnataka

***Corresponding author:** Ashok K, Department of Pharmaceutics, Sree Siddaganga College of Pharmacy, Tumkur, B. H. Road, Karnataka, India. E-mail: ashokkumarscp@yahoo.com*

ABSTRACT

The aim of the study was to develop and evaluate microspheres containing Labetalol in order to achieve extended release of drug and thereby to enhance the patient compliance. Microspheres were prepared by using solvent evaporation method. The release microspheres were formulated using different polymers Eudragit RSPO, Eudragit RLPO and Ethyl cellulose with different concentrations and combination of microspheres with different polymers. The prepared microspheres were characterized for their particle size, drug entrapment efficiencies (90.26% - 94.20%), studied by FT-IR and scanning electron microscopy. The prepared microspheres were in the micrometer range, good entrapment efficiency and Scanning electron microscopy study revealed that the microspheres have rough surface and spherical in shape. The IR spectra showed stable properties of Labetalol in mixture of polymers used and revealed the absence of interactions between drug and selected polymers. The in vitro release studies were performed in pH 6.8 Phosphate Buffer which showed a release of 93.26% at the end of 10 hours in case of best formulation. Fitting the in vitro release data to korsmeyer peppas equation indicated that diffusion along with erosion is the mechanisms of drug release.

Keywords: Labetalol, Eudragit RSPO, Eudragit RLPO, Ethyl cellulose microspheres, Solvent evaporation method.

INTRODUCTION

The oral route of drug administration is traditionally the most preferred for systemic drug delivery by physicians and patients. The population of patient with chronic diseases has recently been increasing. So there is necessary of taking drug for a long period and/or multiple doses of same and/or different medicines simultaneously, which can lead to increase in non-compliance. The drugs with short half-life could be a problem for administration. So a method to find a dosage form to release the drug gradually is necessary to solve these kinds of problems.

Controlled release dosage form cover a wide range of prolonged action formulations that provide continuous release of the active ingredients at a predetermined rate and for a predetermined time [1]. The term microcapsule is defined as "a spherical particle with size varying 1 µm to 1000 µm, containing core substance." However, the term microcapsules and microspheres are often used synonymously [2,3]. Labetalol is a medication used to treat high blood pressure. It can be given intravenously in severe hypertensive situations, or by mouth for long term hypertension management. Its dose and use is limited by its main side effect postural hypotension, where there is a substantial drop in blood pressure when standing up. Labetalol's dual alpha and beta adrenergic antagonism has different physiological effects in short- and long-term situations. In short-term, acute situations, labetalol decreases blood pressure by decreasing systemic vascular resistance with little effect on stroke volume, heart rate and cardiac output. During long-term use, labetalol can reduce heart rate during exercise while maintaining cardiac output by an increase in stroke volume. It has been used in patients suffering from hypertension and used to treat high blood pressure. Its chemical formula is $C_{19}H_{24}N_2O_3$ and it is freely soluble in organic solvents such as methanol, Acetone and sparingly soluble in water (117 mg/L (at 25°C), The bioavailability is highly variable average is 25% and maximum dose of Labetalol is 100 mg and it is usually taken 3 or 4 times per day. Half-life is 6-8 hours; Protein binding is 50% [4]. Hypertension is a worldwide health problem and can cause serious complications as well as significant costs to society. Expenditure on the prevention or early identification and subsequent treatment of hypertension, both with and without medication, are thus well worthwhile ways to reduce the overall risk of cardiovascular morbidity and mortality [5-7]. Cardiac output and total peripheral resistance are the factors which finally determine blood pressure [8]. Hypertension is chronic disease and needs medication for long term. Therefore, conventional dosage form administration for hypertension lacks patient compliance. Hence, development of microspheres, reduce dosing frequency and improves patient compliance. Sustain release of drug from microspheres reduces fluctuation in drug concentration of blood. Hence, there is a need to develop microsphere of labetalol [9]. The aim of this research work was to prepare of microspheres of Labetalol to treat hypertension in view to sustain the drug release, reduce the frequency of administration, improved patient compliance and therapeutic action.

The specific objective of this research includes:

1) To develop Labetalol loaded microspheres.

2) To evaluate the formulated dosage form by official *In vitro* studies.

3) To estimate the drug in the formulation by analytical method.

MATERIALS AND METHODS

Materials

Labetalol, Eudragit RSPO, Eudragit RLPO, Ethyl cellulose were purchased from yarrow chemicals tumkur, all other reagents and chemicals used were for analytical grade.

Preparation of the microspheres

Preparation of microspheres loaded Labetalol using Eudragit RSPO, Eudragit RLPO and Ethyl cellulose.

Solvent evaporation method

The microspheres were prepared by solvent evaporation technique. The Polymer Eudragit RLPO, Ethyl cellulose, Eudragit RSPO in various ratio was dissolved in the mixture of Methanol and Acetone having different ratios like (1:1, 1:2,1:3). The drug was dispersed in above solution of polymers for 10 minutes with stirring. The resulting dispersion was poured slowly under stirring into distilled water (using dispersion medium) containing 0.01% of tween 80. The stirring speed was maintained at 1600rpm, stirring was continued for 4 hours and allow evaporating completely. After evaporation the microspheres formed were collected by filtration using filter paper, then washed 3 to 4 times with Petroleum ether and dried at room temperature for 24 hrs. After that, subsequently stored in a desiccator. Blank microparticles were prepared by following the similar procedure by without drug (i.e., Labetalol).

Evaluation of microspheres

1) Percentage yield of microspheres

The prepared microspheres were collected and weighed from different formulations. The measured weight was divided by total amount of drug and polymers which were used for the preparation of the microspheres to obtained percentage yield.

$$\%Yield = \frac{(Weightof\,Microspheres)}{(Weight\ of\ \ drug + \ Weight\ of\ \ polymer)} \times 100$$

2) Drug entrapment efficiency

To determine entrapment efficiency, 20 mg accurately weighted microspheres were crushed and dissolved in 100 ml pH 6.8 Phosphate Buffer. The microspheres were kept to soak for overnight. After that the solution was filtered through whattman filter. After appropriate dilution with pH6.8 Phosphate Buffer the drug content was determined spectrophotometrically at 305 nm.

$$\%Drug\ \ entrapment\ \ efficiency = \frac{(Calculated\ \ drug\ \ concentration)}{(Theoreticaldrug\ \ concentration)} \times 100$$

3) Fourier-Transform Infrared Spectroscopy (FT-IR)

Drug-polymer interactions were studied by FTIR spectroscopy. Pure drug and excipients were subjected to FT-IR studies. The samples were intimately mixed with dry powder potassium bromide. The powder mixture was taken in a diffused reflectance samples and the spectra recorded by scanning in the wavelength of 500-4000 cm-1 in a FT-IR spectrophotometer.

4) *In vitro* drug release study

The *in-vitro* release study of the microsphere was carried out using USP rotating basket method at 50 rpm at 37°C. Dissolution study was performed in Phosphate buffer pH 6.8 taking 900 ml for each study. 100 mg of the microsphere was placed in the dissolution medium and test samples were taken from the medium at predetermined time intervals over a period of 10 hours and the samples were analyze Labetalol content in UV spectrophotometer [10].

5) Drug release kinetics

Data obtained from *in vitro* release studies were fitted to various kinetic equations to find out the mechanism of drug release from the microsphere. The kinetic models used were:

i. Zero-order equation

(Cooper and Gunn, 1986) $Q = k_0 t$ where, Q is the amount of drug released at time t, and k_0 is the release rate.

ii. First-order equation

$\text{Log } Q = \text{Log } Q_0 - k_1 t / 2.303$

where, Q is the amount of drug un-dissolved at t time, Q_0 is drug concentration at t = 0 and k_1 is the release rate constant.

iii. Higuchi's equation

$Q = k_2 t^{1/2}$

where, Q is the percent of drug release at time t, and k_2 is the diffusion rate constant.

6) Stability studies

Stability studies of pharmaceutical products were done as per ICH guide lines. These studies are designed to increase the rate of chemical or physical degradation of the drug substance or product by using exaggerated storage conditions.

Methods

The optimized formulation sealed in vial with rubber cap and kept in humidity chamber maintained elevated temperatures such as 25°C ± 2°C / 60% ± 5% RH, 30°C ± 2°C / 65% ± 5% RH and 40°C ± 2°C / 75% ± 5% RH for 3 months. After 30, 60 and 90 days samples retrieved and analyzed for the drug content and *in vitro* drug release.

RESULTS AND DISCUSSION

The microspheres of Labetalol was prepared by solvent evaporation using polymers like Eudragit RSPO, Eudragit RLPO and Ethyl cellulose for sustain the drug release. Therefore the main objective of the study to formulate and evaluate the controlled release microspheres of Labetalol by using Eudragit RSPO, Eudragit RLPO, Ethyl cellulose were achieved. Labetalol shows maximum absorbance at 305 nm with pH 6.8 Phosphate Buffer as solvent. This value is used for further analysis and calculations (Figures 1 and 2, Tables 1-4).

Table 1: Drug and polymer combination in different concentrations.

Formulation code with ratio	Labetalol(mg)	Eudragit RS +EC(mg)	Eudragit RL +EC(mg)	Eudragit RS+ Eudragit RL(mg)
F1 (1:1)	100	50+50	-	-
F2 (1:2)	100	100+100	-	-
F3 (1:3)	100	150+150	-	-
F4 (1:1)	100	-	50+50	-
F5 (1:2)	100	-	100+100	-
F6 (1:3)	100	-	150+150	-
F7 (1:1)	100	-	-	50+50
F8 (1:2)	100	-	-	100+100
F9 (1:3)	100	-	-	150+150

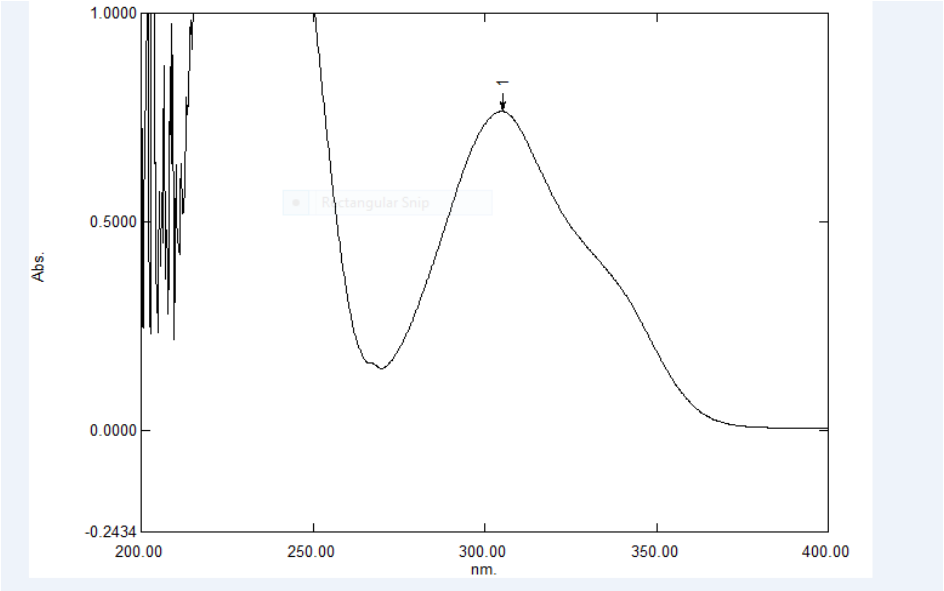

Figure 1: λ-max of labetalol in ph 6.8 phosphate buffer.

Table 2: Calibration curve of labetalol in PH 6.8 phosphate buffer.

Concentration (µg/ml)	**Absorbance at 221 nm**
10	0.0725
20	0.1436
30	0.2209
40	0.3089
50	0.3811
60	0.4664

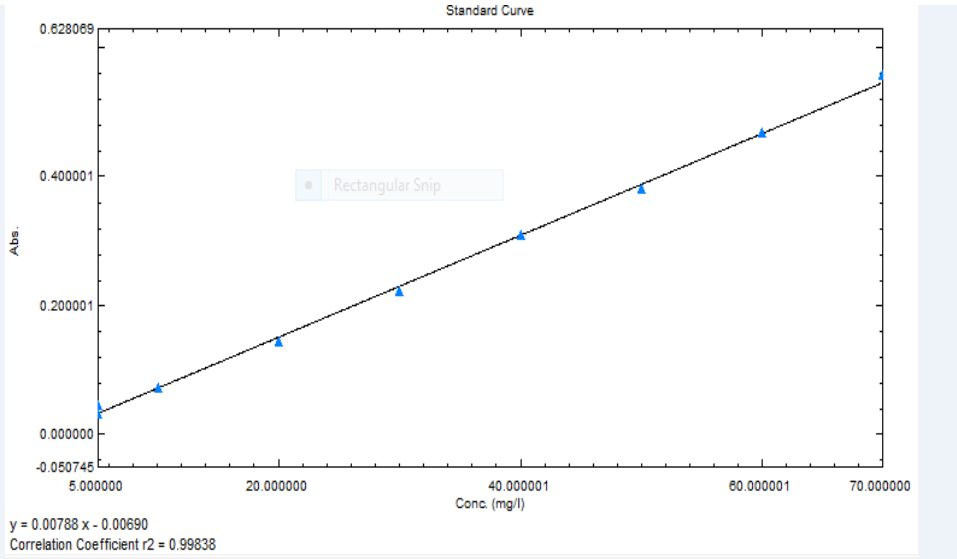

Figure 2: Showing calibration curve of labetalol in pH 6.8 phosphate buffer.

Table 3: Percentage yield of microspheres from F1 to F9.

S. No of formulation	Formulation Percent Yield (%)
F1	94.00
F2	91.60
F3	89.50
F4	90.25
F5	95.83
F6	94.30
F7	93.00
F8	90.16
F9	93.00

Table 4: Percentage drug entrapment efficiency of formulation from F1 to F9 (DEE).

Sl. No of formulation	Formulation DEE (%W/W)
F1	90.29
F2	92.15
F3	92.72
F4	94.70
F5	95.65
F6	94.23
F7	91.08
F8	94.52
F9	94.2

The microspheres were evaluated for the particle size, percentage yield, percentage drug entrapment efficiency & *In vitro* drug release. From the results it was found that the formulations showed particle size in the micrometer range, good percentage yield, good entrapment efficiency and retarded release of the drug.

Compatibility studies (FT-IR spectroscopic studies)

Drug-polymer interactions were studied by FTIR spectroscopy. Pure drug and excipients were subjected to FT-IR studies. The samples were intimately mixed with dry powder potassium bromide. The powder mixture was taken in a diffused reflectance samples and the spectra recorded by scanning in the wavelength of 500-4000 cm^{-1} in a FT-IR spectrophotometer. Figure 3, demonstrate the FTIR spectra of Labetalol characteristic peaks at 3351.88cm^{-1}(-OH,-NH_2) stretch, 2075.02 cm^{-1}, (Aliphatic –CH) stretch. Figure 4 demonstrate the FTIR spectra of optimized formulation characteristic peaks at 3358.43 cm^{-1} (-OH,-NH_2) stretch, 3193.54 cm^{-1}, (Aliphatic –CH) stretch, 1377.89 cm^{-1} CH_3 bend. FT-IR spectrophotometry is a useful analytical technique utilized to check the chemical interactions between the drug and other excipients used in the formulations as shown in Figures 4 and 5. All prominent peak of pure drug Labetalol is seen in spectra. The weak peak of Ar-CH has merged with –OH_2 peak. The polymer used for physical mixture is found to be compatible.

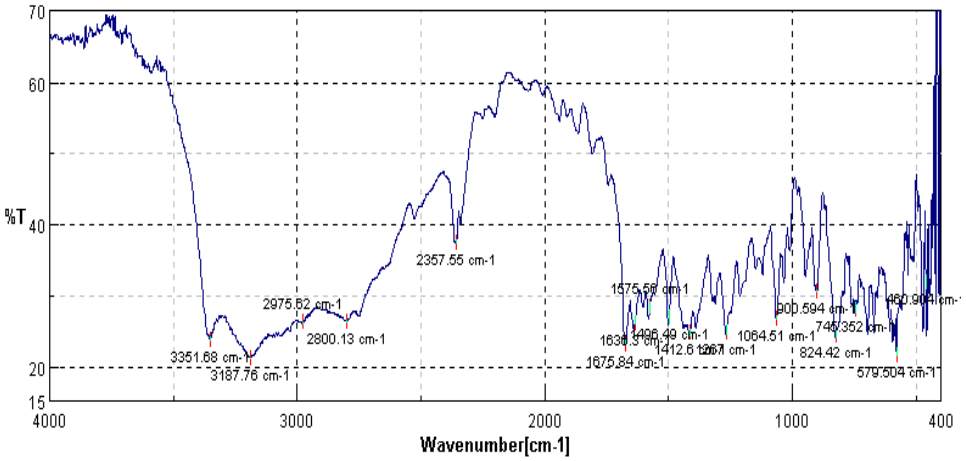

Figure 3: FT-IR Spectrum of pure drug labetalol.

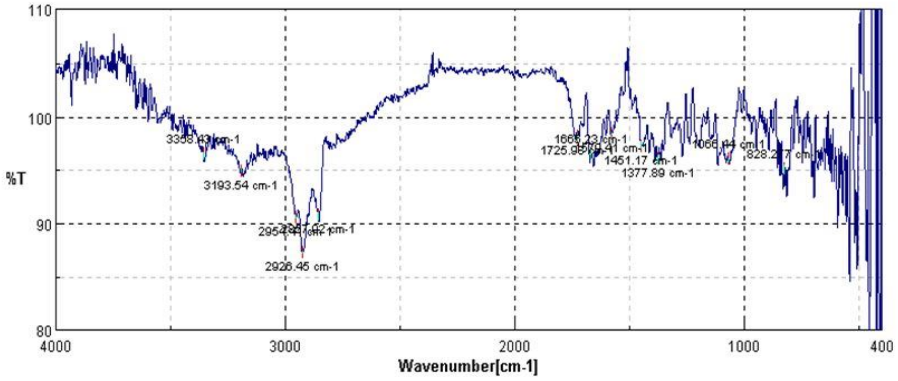

Figure 4: IR Spectroscopy of Optimized formulation of F5

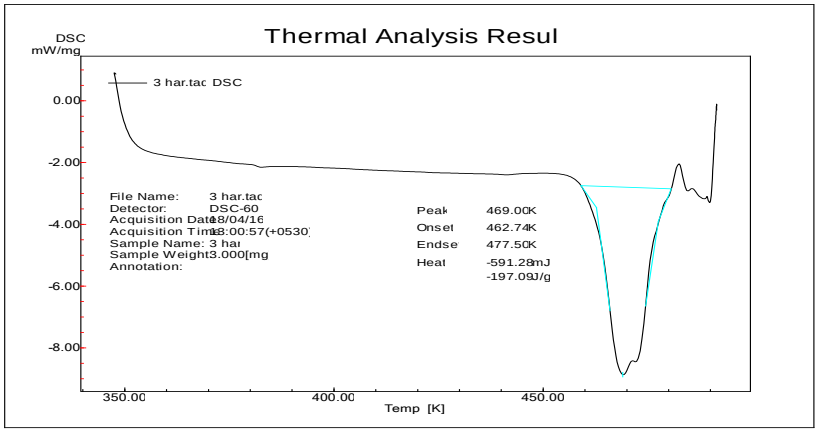

Figure 5: DSC thermogram of pure drug labetalol.

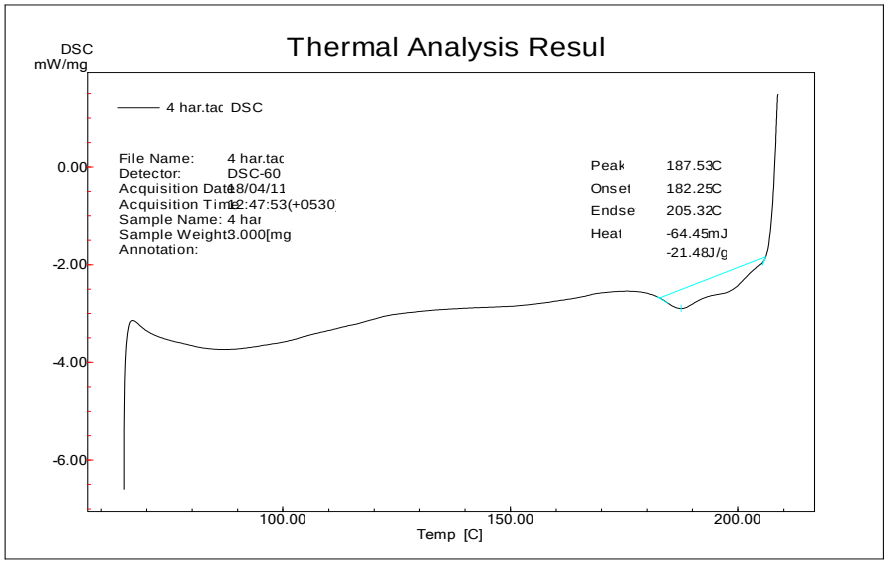

Figure 6: DSC thermogram of optimised formulation.

In DSC studies Thermogram of Labetalol is shown in Figure 5 Which indicates of pure drug 196°C is melting peak of optimized formulation (F5) is 187°C, was observed in the Figure 6, change in temperature is due to various concentrations of drug and other excipient in physical mixture. This shows that there is no interaction between drug and optimal formulation F-5. DSC studies revealed that there was no much shift in the melting point of a drug in the physical mixture compared to the pure drug this indicates that there is no interaction between drug and matrix materials (Figures 7 and 8, Tables 5 and 6).

Table 5: Showing % CDR of labetalol.

Time in hrs	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1	17.19	17.19	18.1	26.24	19	25.34	18.09	17.1	29.86
2	18.19	17.25	20.91	35.44	20.92	30	21.79	18.19	36.32
3	19.2	18.29	23.74	38.35	21.94	32.88	23.71	20.1	37.42
4	19.3	30.15	24.78	44.89	22.96	34.87	22.04	27.45	43.03
5	26.65	40.27	25.82	51.47	29.42	40.49	32.96	37.56	45.06
6	30.41	49.54	34.1	55.37	37.73	46.14	38.54	45.91	51.61
7	38.72	57.05	55.1	59.29	51.51	50.92	56.75	47.97	58.19
8	44.36	58.27	58.12	68.66	62.65	57.53	63.36	50.94	68.4
9	61.8	62.2	59.34	72.65	75.66	66.89	68.2	57.55	74.17
10	88.38	89.69	88.62	89.33	93.26	91.68	90.29	91.35	89.05

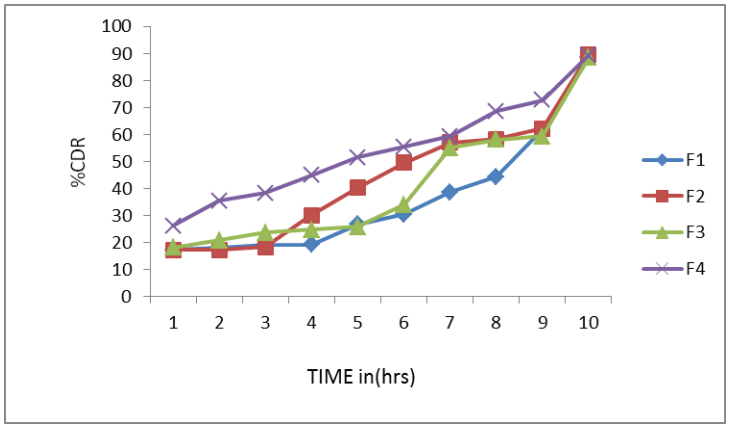

Figure 7: *In vitro* release of labetalol microspheres of formulation F1 to F4

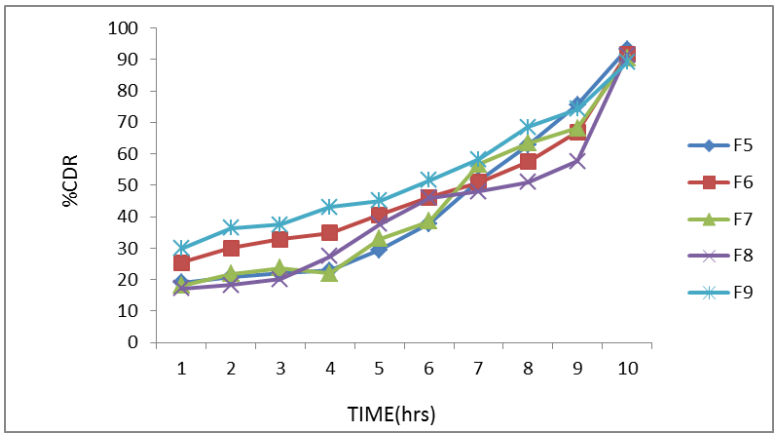

Figure 8: *In vitro* release of labetalol microspheres of formulation F5 to F9

Table 6: Kinetic model fitting for optimized formulation (F5).

Time in Hours	$\sqrt{T}$	**log (*T*)**	**%CDR**	**%CDR unreleased**	**Log(%CDR)**	**Log(%CDR unreleased)**
1	1	1	19	81	1.2787	1.9084
2	1.414	0.3010	20.92	79.08	1.3205	1.8980
3	1.732	0.4771	21.94	78.06	1.3412	1.8924
4	2	0.6020	22.96	77.04	1.3609	1.8867

5	2.236	0.6989	29.42	70.58	1.4686	1.8486
6	2.449	0.7781	37.73	62.27	1.5766	1.7942
7	2.645	0.8450	51.51	48.49	1.7118	1.6856
8	2.828	0.9030	62.65	37.35	1.7969	1.5722
9	3	0.9542	75.66	24.34	1.3863	1.3863
10	3.162	1	93.26	6.74	1.8286	1.8286

Table 7: Physical appearance of optimized formulation after stability studies

Temperature and Relative Humidity	**Formulation F-5**			**Parameters**
	Days			
	30	60	90	
25°C ± 2°C / 60% ± 5% RH	No change			Physical appearance
30°C ± 2°C / 60% ± 5% RH				
40°C ± 2°C / 60% ± 5% RH				

Table 8: Percentage of drug release at 10 hours and drug content of optimized formulation after stability studies

No. of Days	**Formulation F-5**					
	% Drug release			**Drug content (%)**		
	25°C /60% RH	**30°C /65% RH**	**40°C /75% RH**	**25°C / 60% RH**	**30°C /65% RH**	**40°C /75% RH**
0	85.65	--	--	97.44	--	--
30	80.06	79.97	74.75	93.76	96.69	92.65
60	79.55	71.25	65.10	87.53	87.45	86.39
90	68.57	67.23	53.19	82.33	80.21	79.15

In kinetic release profile the release data was fitted to various mathematical models to evaluate the kinetics and mechanism of drug release. The kinetic data of all formulation F1 to F9 could be best expressed by Zero order equation as the plots showed highest linearity (R2:0.792 to R2:0.970), than First order release kinetics (R2: 0.462 to R2: 0.834), The n values obtained from

Korsmeyer Peppa's range from (R2: 0.725 to R2: 0.953) indicates that mechanism of release of formulations F-1 to F9 was Quasi-Fickian diffusion to Anomalous diffusion (non-Fickian).

Stability studies

Stability studies were carried out on selected formulation F-1 as per ICH guidelines.There was not much variation in integrity of the microspheres at all the temperature conditions. There was no significant changes in drug content, physical stability,drug release (Tables 7 and 8) for the selected formulation F-1 after 90 days at 25°C ± 2°C / 60% ± 5% RH, 30°C ± 2°C / 60% ± 5% RH,40°C ± 2°C / 60% ± 5% RH.

***In vitro* dissolution study**

The release of drug (Labetalol) from the microspheres varied according to different ratio of polymer. As a concentration of Eudragit RSPO, Eudragit RLPO and Ethyl cellulose is increased, the release of drug decreased. F5 batch showed maximum drug release (93.26%) at the end of 10 hrs because of Eudragit RSPO and Ethyl cellulose. Thus, Eudragit RSPO and Ethyl cellulose has proved to be efficient carrier for oral drug delivery of Labetalol. The stability studies of F5 formulation showed that negligible change in percentage drug entrapment efficiency and *in vitro* drug release profile. Therefore, in the present investigation, promising results of microspheres of Labetalol are achieved in case of formulation F5 which contains Eudragit RSPO with Ethyl cellulose and as release retarding matrices. Therefore, formulation F5 is considered as best formulation in this study.

CONCLUSION

UV- Spectrophotometric method was developed for the determination of Labetalol in pH6.8 Phosphate Buffer at 305 nm. FT-IR spectrum of pure drug, pure polymer and drug-polymer mixture revealed no chemical interaction between them. Microspheres of Labetalol were prepared by solvent evaporation technique using Eudragit RSPO and Eudragit RLPO and Ethyl cellulose as polymers. Various physicochemical properties like % Yield, % DEE and particle size were evaluated. *In vitro* drug release studies were also performed. Percentage yield for the formulation of F1 to F9 varied from 89.50% to 95.83%. Percentage drug entrapment efficiency for the formulation of F1 to F9 varied from 90.29% to 95.65%. Particle size for the Optimized formulation of F5 varied from 10 µm and 100 µm. Release of drug from Formulation F5 followed the Korsmeyer-peppas model with desired release as well as having good percentage drug entrapment efficiency.

REFERENCES

1. Ninan, M., and Lu, X., Development and evaluation of new sustained-release floating microspheres. *Int Journal of pharmaceutics*, **2008.** 358: 82-90.

2. Chaturvedi, G., and Saha, RN., A review on microsphere technology and its application. *Birla Inst., Tech. Sci*, **2009.** 56-58.
3. Alagusundaram, M., and Madhu Chetty, C., *Int Journal of Pharmaceutics*, **2009.** 1(3): 526-53.
4. Mohamed, SE., Clinical guidelines for the management of hypertension, EMRO Technical publications series-29. Editor Oussama MNK.
5. Bhatt, JD., et al. Effect of abana, an ayurvedic preparation on ethinyl estradiol-induced
6. Hypertension in Rats, *Ind J Pharmacology* **1998.** 30: 399-403.
7. Mascher, D., et al. Ethanolic extract of *Spirulina* maxima alters the vasomotor reactivity of aortic rings from obese rats. *Archives of Medical Research* **2006.** 37: 50-57.
8. Chobanian, AV., et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and treatment of high blood pressure: The JNC 7 report. JAMA **2003.** 289: 2560-2572.
9. https://en.wikipedia.org/wiki/Labetalol
10. Katzung, BG., Basic and clinical pharmacology. 9th edn. New Delhi, India, **2004.** 1: 160.