AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Development and Evaluation of Floating Microspheres Containing Candesartan Cilexetil

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ABSTRACT

The aim of the present study is to develop and evaluate floating microspheres containing candesartan cilexetil in order to achieve extended release of drug and thereby to enhance the patient compliance. Floating microspheres were prepared by using solvent evaporation method. The microspheres were formulated using different polymers like ethyl cellulose, HPMC K4M and eudragit RSPO 100 in different concentrations and combinations. The prepared floating microspheres were characterized for their percentage yield (95.44 - 98.52%), drug entrapment efficiencies (71.52 - 97.87 %) and percentage buoyancy (93.45 - 98.66%). The FTIR and DSC studies revealed absence of interactions between drug and selected polymers. *In vitro* release studies were performed in 0.1 N HCl which showed a drug release of 97.62 % at 24 hour in case of formulation (F7). Fitting the *in vitro* drug release data to Korsmeyer equation indicated that *Fickian* diffusion is the mechanism of drug release.

Keywords: Candesartan cilexetil, Floating Microspheres, FTIR, Ethyl cellulose, HPMC K4M, Eudragit RSPO 100.

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Received 03 April 2019, Accepted 15 May 2019

Please cite this article as: Manjunath K *et al*., Development and Evaluation of Floating Microspheres Containing Candesartan Cilexetil. American Journal of PharmTech Research 2019.

INTRODUCTION

The population of patient with chronic diseases such as hypertension has recently been increasing. So, there is necessary of administering drug for a long period or multiple doses of medicines, which can lead to increase in non-compliance. 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.

Oral release dosage form have been the field of focus to the fact of overweighed benefits despite its limitation of unsuitability of drugs that are easily absorbed from the GIT and have short life are eliminated quickly from the blood circulation. Such dosage forms require frequent dosing[1]. Floating drugs delivery system (FDDS) possess lower bulk density than the gastric fluid exerting buoyancy in the stomach leading to slow drug release in an extended manner before it reaches absorption window. In the present study, dual benefits of buoyancy as well as prolonged action are tried to achieve with an intension to maintain the study state of drug release[2].

Microspheres can be defined as solid, approximately spherical particle with particle size ranging from 1 to 1000 µm, containing core substance. Multiple unit dosage forms are dispersed in gastro intestinal system more homogeneously than the single unit dosage forms. This enables prolonged and continuous input of the drug to stomach and upper part of GIT and reduces absorption differences. Microspheres are one of the multiple unit polymeric drug delivery systems able to protect drug from degradation and hence these have been widely preferred for the controlled release of drugs[3].

Hypertension is a major health problem throughout the world because of its high prevalence and its association with increased risk of cardiovascular disease. It is a major risk factor and a powerful predictor of cardiovascular morbidity and mortality with proven benefits after treatment[4]. Moreover, the risk of excessive morbidity and mortality are present even among persons with so called ‘mild’ hypertension[5].

Candesartan cilexetil, an ester form pro drug of candesartan belongs to a group of drugs known as Angiotensin-II receptor antagonists or Angiotensin converting enzyme inhibitors. It is used in the treatment of hypertension[6]. Candesartan cilexetil is hydrolyzed to candesartan during absorption from gastrointestinal tract. The use of a pro drug form increases the bioavailability of candesartan despite absolute bioavailability is relatively poor[7]. Candesartan cilexetil is Class II drug under BCS classification that is poorly water soluble. It has molecular weight of 610.67 g/mol and molecular

formula of $C_{33}H_{34}N_6O_6$. Its half life is 5.1 – 10.5 hrs. Candesartan cilexetil is metabolized completely by esterase in the intestinal wall during absorption to the active candesartan moiety.

Development of Candesartan cilexetil microspheres may reduce fluctuation of drug blood concentration and provides better treatment for hypertension. Hence, there is a need to develop candesartan cilexetil floating microspheres. In the present study, we tried to develop candesartan cilexetil floating microspheres using different three polymers and their combinations.

MATERIALS AND METHOD

Materials

Candesartan cilexetil was purchased from Yarrow chemicals, Mumbai and polymers like Eudragit RS-100, HPMC K4M were purchased from HiMedia laboratories Pvt Ltd. Other ingredients like Ethyl cellulose, ethanol, Acetone were purchased from SD FineChem. Ltd and liquid paraffin was purchased from Merck Pvt Ltd.

Methods

Determination of λmax Candesartan Cilexetil

A dilute solution of candesartan cilexetil in methanol was prepared and scanned between UV range i.e. 200 to 400 nm (Lab India 3000+ spectrophotometer, Japan). Candesartan cilexetil showed maximum absorbance at 254 nm.

Preparation of Microspheres

Floating microspheres of candesartan cilexetil were prepared by solvent evaporation technique[8]. Polymers ethyl cellulose, HPMC K4M and eudragit RS 100 were weighed and dissolved in the mixture of ethanol (15 ml) and acetone (15 ml) as shown in Table 1. Drug was dispersed in above solution of polymers under stirring at 200 rpm for 10 minutes. The resulted dispersion was poured slowly under stirring into 200 ml liquid paraffin (dispersion medium) containing 0.01% of tween 80. The stirring speed and temperature were maintained at 1600 rpm and 60 – 70 °C, respectively for 3 hour and allowed evaporation of ethanol and acetone completely. After evaporation the microspheres formed were collected by filtration using whatman filter paper, then washed 3 to 4 times with petroleum ether and dried at room temperature for 24 hours and stored in desiccators. Quantities mentioned in the Table 1 are sufficient for preparation of ten doses of candesartan cilexetil.

Table 1: Composition of candesartan cilexetil microsphere prepared using three polymers and their combinations.

Formulation code	Candesartan cilexetil (mg)	EC (mg)	HPMC K4M (mg)	Eudragit RS 100 (mg)
F1	300	300	---	---
F2	300	600	---	---
F3	300	---	300	---
F4	300	---	600	---
F5	300	---	---	300
F6	300	---	---	600
F7	300	150	---	150
F8	300	150	150	---
F9	300	---	150	150

Percentage Yield of Microspheres

The prepared floating microspheres of different formulations were collected and weighed. Percentage yield was calculated using the following formula.

$$Percentage\ Yield = \frac{\text{Weight of floating microspheres obtaianed}}{\text{Weight of drug} + \text{ weight of polymer}} \times 100$$

Drug Entrapment Efficiency

Accurately weighted microspheres (20 mg) were crushed and placed in 100 ml 0.1 N HCl for overnight. Then subject for filtration using Whatman filter paper. After appropriate dilution with 0.1 N HCl the drug content was determined spectrophotometrically at 254 nm.

$$Percentage\ \text{Drug entrapment Efficiency} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

Percentage Buoyancy

Microspheres were placed in 900 ml 0.1N HCl using USP XXII dissolution apparatus type II. The medium is to be agitated with a paddle rotating at 100 rpm for 24 hrs. The floating and the settled portions of microspheres were recovered separately. The microspheres were dried and weighed. Buoyancy percentage was calculated as the ratio of the mass of the microspheres that remain floating to the total mass of the microspheres.

Fourier-transform infrared spectroscopy (FTIR) study

Drug – polymer interactions if any were studied by FTIR spectroscopy. Pure drug and physical mixtures were subjected to FTIR 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 frequency of 500 - 4000 cm^{-1} using FTIR spectrophotometer. The following are the samples analyzed by FTIR spectroscopy.

1. Pure drug
2. Physical mixture of drug and ethyl cellulose
3. Physical mixture of drug and HPMC K4M
4. Physical mixture of drug and eudagit RS-100
5. Optimized formulation F7

DSC Study

Differential scanning calorimetry was used to measure enthalpy changes due to changes in the physical and chemical properties of material as a function of temperature.

***In vitro* Drug Release study**

In vitro drug release studies were carried out for all nine formulations using USP Type II dissolution test apparatus. The dissolution medium was 900 ml of 0.1N HCl solution, temperature maintained was 37 ± 0.5 °C and rpm was 100. Microspheres containing 100 mg of drug was used for dissolution study. 5 ml of the aliquot was withdrawn at predetermined intervals. Required dilution were made with 0.1 N HCl solution, filtered and analyzed for the drug content spectrophotometrically at 254 nm against suitable blank. Equal volume of fresh dissolution medium was replaced in the vessel after each with drawls to maintain sink condition.

Surface Morphology

Floating microspheres of candesartan cilexetil were observed under optical microscope at 45× magnification to study surface characteristics.

RESULTS AND DISCUSSION

Standard plot of Candesartan cilexetil in methanol

Standard solutions (2-18 µg/ml) of candesartan cilexetil were prepared in methanol in triplicate and absorbance was measured at 254 nm using UV- Spectrophotometry. The standard plot of candesartan cilexetil was as shown in (Figure 1). The correlation coefficient obtained was 0.996. The obtained regression equation y = 0.0476 x + 0.0044 was used to calculate the concentration of unknown samples of *in vitro* studies.

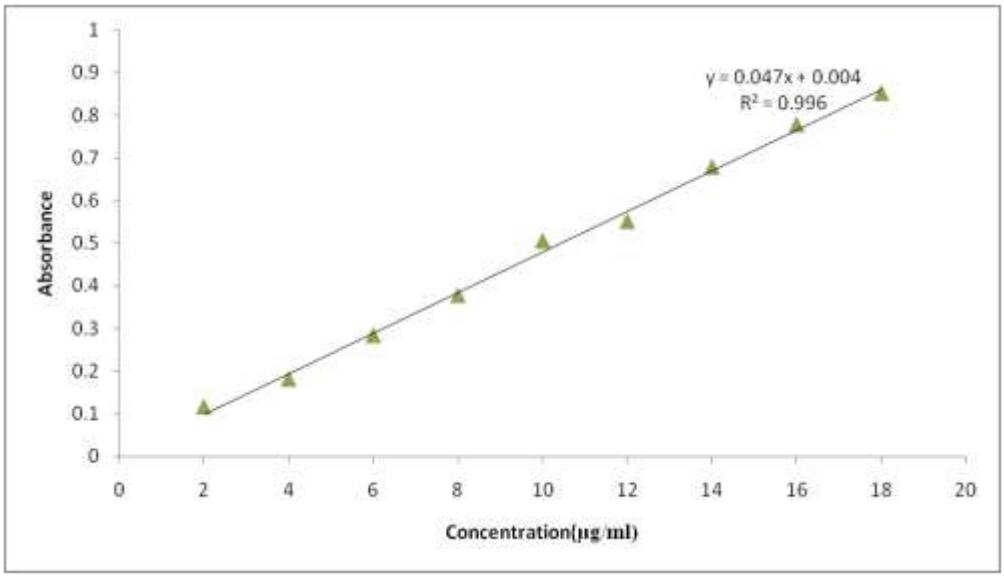
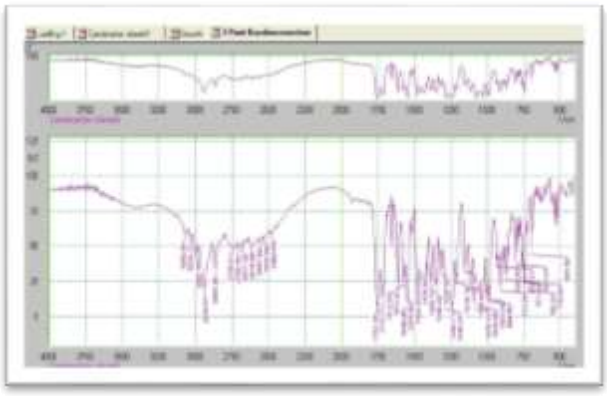


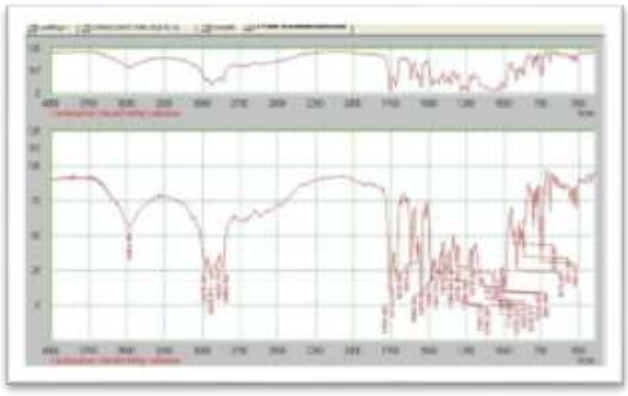
Figure 1: Standard plot of candesartan cilexetil in methanol

Evaluation of Drug Polymer Interaction by FTIR

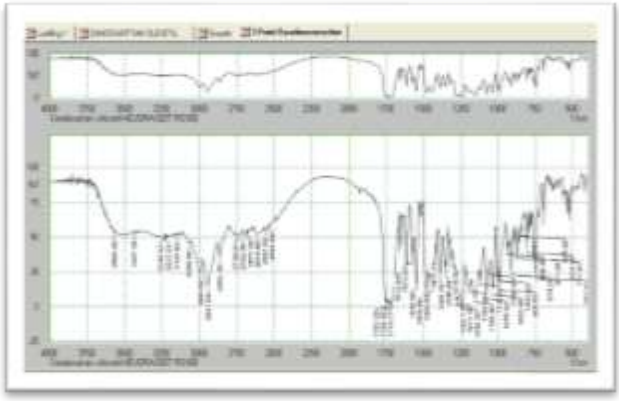
Drug excipients compatibility was confirmed by FTIR spectroscopy. Pure drug showed the characteristic peaks similar to literature values shown in Table 2 which indicates the purity of the drug. All the absorption peaks of candesartan cilexetil were retained in the physical mixtures of drug with various polymers (ethyl cellulose, HPMC K4M and eudragit RSPO 100) and optimized formulation F7 .The spectra of physical mixtures and optimized formulation F7 did not show any shift of vibration bands of candesartan cilexetil can be observed in Figure 2. It indicates that there was no chemical interaction between the drug and selected excipients.



A) Pure drug

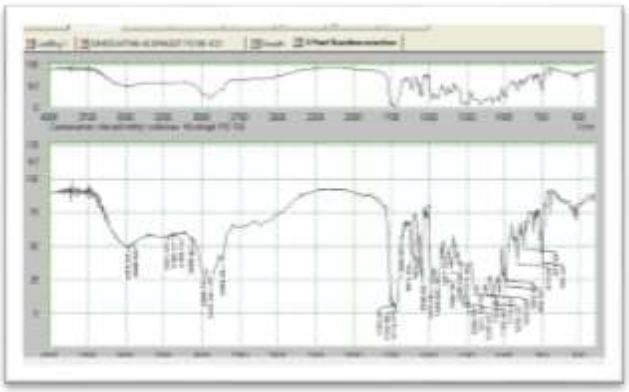


B) Physical mixture of drug and ethyl cellulose



C) Physical mixture of drug and Eudagit RS-100

D) Physical mixture of drug and HPMC K4M



E) Optimized formulation

Figure 2: FTIR spectra of (A) candesartan cilexetil; (B) Physical mixture of drug and ethyl cellulose; (C) Physical mixture of drug and Eudagit RS-100; (D)Physical mixture of drug and HPMC K4M; (E) Optimized formulation F7.

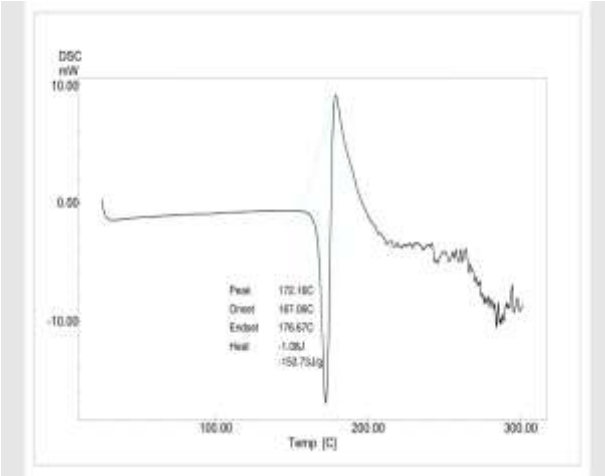
Table 2: Functional group and their characteristic peak values of candesartan cilexetil and optimized formulation (F7) obtained by FTIR studies.

Functional group	Wave number (cm^{-1})		
	Literature Values	Obtained values	
		Pure Drug	Optimized Formulation (F7)
Aromatic CH stretch	2941.24	2939.61	2941.54
C=O stretching	1755.1	1753.35	1753.49
C-N stretching	1614.31	1612.54	1612.54
C-O stretching	1244	1240.27	1242.20
O-substitution	750.26	750.33	750.33

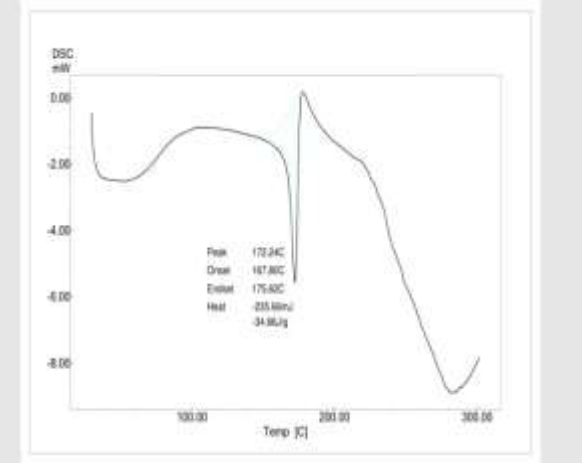
Characterization by DSC

DSC thermograms of pure drug and optimized formulation (F7) were shown in Figure 3. The melting point of pure drug was at 172.16 °C whereas melting point of drug in optimized

formulation was at 172.24 °C. There is no change in the melting point of drug in final optimized formulation. Sharp melting peak of drug was retained in optimized formulation F7 indicating the crystalline nature of the drug in the formulation. Hence, it can be concluded that there was no interaction between the drug and the excipients. Even adopted preparation method of microspheres i.e. solvent evaporation method did not cause any changes to the crystallinity of the drug.



A) Thermogram of pure drug

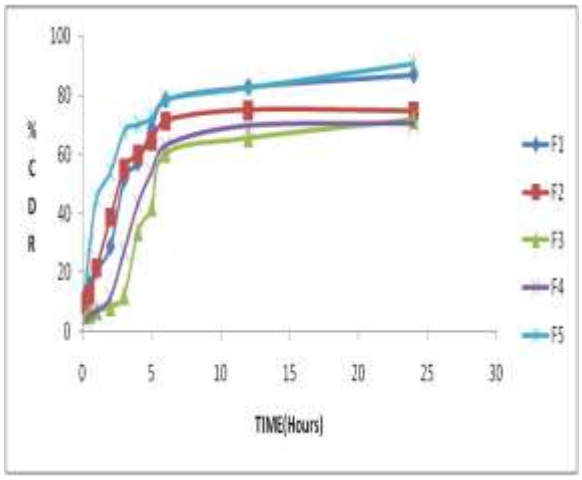


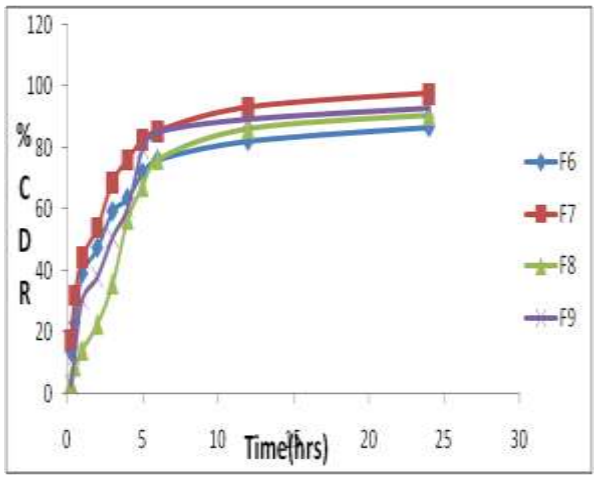
B) Thermogram of optimized Formulation F7

Figure 3: DSC thermogram of (A) candesartan cilexetil; (B) optimized formulation F7.

Characterization of floating microspheres

Amount of the candesartan cilexetil selected for the preparation of floating microspheres was 300 mg equivalent to 10 doses (dose of candesartan cilexetil is 30 mg per day). To achieve controlled release of drug three different polymers (ethyl cellulose, HPMCK4M and eudragit RS-100) were selected at 1:1 and 1:2 drug to polymer ratios. Six formulations F1 to F6 were prepared using individual polymers. Further, three formulations (F7 to F9) were prepared using combination of two polymers (Table 1).





A) drug release profiles of F1-F5 B) drug release profiles of F6-F9

Figure 4: *In vitro* drug release profiles of candesartan cilexetil microsphere formulations prepared with three different polymers.

The prepared drug loaded floating microspheres were studied to know percentage yield, percentage drug entrapment efficiency and percentage buoyancy and the values were given in the Table 3. Percentage yield ranged from 95.44 to 98.52 % for formulations F1 to F9 showing the good yield. Drug entrapment efficiency range was found to be from 71.52 to 97.87 %. The percentage buoyancy of formulation F1 to F9 were in the range of 93.45 to 98.66 %. It indicates that all nine formulations were floated sufficiently for 24 hours in the selected dissolution medium i.e. 0.1 N hydrochloric acid solution. Hence, the developed formulations may float in the stomach while oral administration.

Table 3: Characterization of of candesartan cilexetil microspheres of nine formulations.

Formulations	Percent Yield (%)	Drug Entrapment Efficiency(% w/w)	Percentage Buoyancy (%)
F1	96.41	88.88	93.61
F2	96.66	75.75	96.13
F3	97.00	76.5	97.00
F4	97.25	71.52	94.75
F5	97.62	91.15	95.01
F6	96.90	94.33	97.00
F7	98.52	97.87	98.66
F8	95.44	90.34	93.45
F9	96.33	91.71	95.67

***In vitro* drug release profile**

Further to optimize, developed floating microspheres of candesartan cilexetil were subjected to *in vitro* drug release studies. *In vitro* drug release studies of F1 and F2 formulations showed the percentage drug released was 86.82 and 74.59 %, respectively at 24 hours. As the amount of ethyl cellulose increased, the drug release was decreased. The similar trend was observed in case of floating microspheres prepared with other two polymers HPMCK4M and eudragit RS-100.

Both the formulations i.e. drug to polymer ratios 1:1 and 1:2 exhibited controlled release, where as formulations prepared with 1:1 shown higher percentages of drug release at 24 hours. Hence, for further formulations 1:1 drug to polymer ratio was fixed but two polymers were taken in combination in equal amounts. Formulation F7 containing ethyl cellulose and eudragit RS 100 showed better drug release of 97.62 % among all nine formulations (Figure 4). Hence, it is finally considered as optimized formulation.

To understand the release pattern of the drug from the floating microspheres, *in vitro* release data obtained was processed and plotted as zero, first order, Higuchi and Korsmeyer - Peppas model. The regression values (R^2) obtained were tabulated in the Table 4. By considering correlation coefficients, the drug release from floating microspheres of candesartan cilexetil was able to follow Korsmeyers - Peppas model with *Fickian* diffusion[9].

Table 4: Drug release kinetic of candesartan cilexetil floating microspheres

Formulation code	Zero order R^2	First order R^2	Higuchi Model R^2	Peppas Model	
				R^2	Slope(n)
F1	0.7781	0.9555	0.6931	0.9162	0.6836
F2	0.7757	0.7669	0.6951	0.9674	0.6086
F3	0.8619	0.9297	0.7297	0.9483	0.7588
F4	0.8746	0.8581	0.6581	0.8866	0.4612
F5	0.8692	0.9691	0.6691	0.9624	0.7995
F6	0.7939	0.9639	0.7639	0.8934	0.5427
F7	0.8752	0.9868	0.7821	0.9738	0.3754
F8	0.8697	0.9734	0.7196	0.9631	0.5336
F9	0.7864	0.9158	0.7799	0.9711	0.4881

Candesartan cilexetil floating microspheres of formulation F7 prepared with ethyl cellulose and eudragit RS 100 were subjected for microscopic analysis using optical microscope. Images at magnification of 45× were shown in Figure 5. It was observed that selected solvent evaporation method leads to the formation of almost spherical shaped floating microspheres.

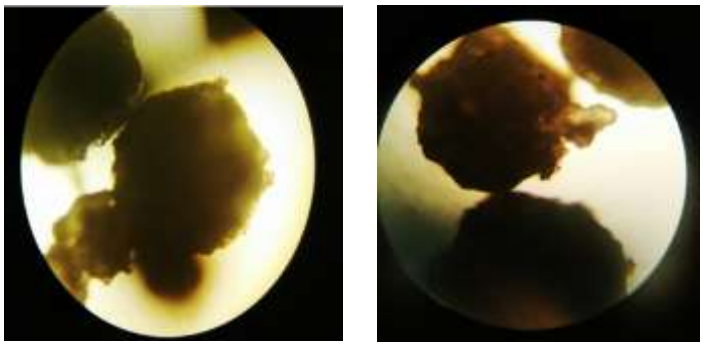


Figure 5: Images of candesartan cilexetil microsphere (formulation F7) obtained by optical microscopy

CONCLUSION

1. Floating microspheres containing candesartan cilexetil was prepared successfully using three polymers ethyl cellulose, HPMC K4M and eudragit RSPO 100. *In vitro* release studies confirmed that the suitable percentage of drug released in the formulation F7 which contained ethyl cellulose and eudragit RSPO 100 at 25 %w/w levels respectively. Thus

developed floating microspheres may reduce frequency of dosing, thereby minimizing the occurrence of side effects and fluctuation in blood drug concentration. Thus developed floating microspheres also increase the gastric retention time to improve the bioavailability and to achieve better drug therapeutic activity.

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