WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 16, 430-443. Research Article ISSN 2277– 7105

DESIGN FORMULATION AND EVALUATION OF FLOATING MICROSPHERES OF TIMOLOL

Ch. Taraka Ramarao*[1] and T. Yugandhar[2]

Department of Pharmaceutical Technology, Sri Venkateswara College of Pharmacy, Etcherla, Srikakulam, Andhra Pradesh, India-532410.

Article Received on
14 October 2017,

Revised on 05 Nov. 2017,
Accepted on 25 Nov. 2017

DOI: 10.20959/wjpr201716-9838

***Corresponding Author**
Ch. Taraka Ramarao
Department of Pharmaceutical Technology, Sri Venkateswara College of Pharmacy, Etcherla, Srikakulam, Andhra Pradesh, India-532410.

ABSTRACT

The goal of present investigation attempt was made to prepare microspheres of timolol employing by using various polymers like HPMC K15M, Eudragit S100 and ethyl cellulose to achieve an oral controlled release of the timolol. In the present study nine formulations were formulated by using HPMC K15M, Eudragit S100 and EC in various proportions (1:1, 1:2, 1:3). All the formulations were subjected to (%) Percentage yield, drug content, buoyancy time and entrapment efficiency, *in vitro* dissolution and release kinetics shown satisfactory results. Entrapment efficiency was increased with increased polymer concentration. On the basis of release data and graphical analysis formulation F6 shown good controlled release profile with maximum entrapment efficiency because of high polymer concentration. The co-efficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism. The diffusion exponent 'n' values of Korsemeyer- Peppas model was found to be in the range of more than 1 for the Timolol floating microspheres prepared with drug and Eudragit indicating super case II transport diffusion mechanism of drug through timolol floating microspheres. The floating microsphere is possible to formulate promising controlled release timolol. The results it can be concluded that the drug release from the floating microspheres diffusion controlled. The formulations showed best appropriate balance between buoyancy and in vitro drug release. The floating microspheres are stable during shelf life.

KEYWORDS: Micro spheres**;** HPMC K 15M; Buoyancy; Entrapment efficiency; Percentage yield.

INTRODUCTION

Gastro retentive systems can remain in the gastric region for several hours and hence significantly prolong the GRT of drugs. Prolonged gastric retention improves bioavailability, reduces drug waste and improves solubility for drugs that are less soluble in a high pH environment. It has applications also for local drug delivery to the stomach and proximal small intestine. Gastro retention helps to provide better availability of new products with new therapeutic possibilities and substantial benefits for patients.[1,27] Timolol is a beta1 and beta2 (non-selective) adrenergic receptor blocking agent that does not have significant intrinsic sympathomimetic, direct myocardial depressant, or local anesthetic (membrane-stabilizing) activity. Timolol has a half life of (3-4 hrs) and it reaches a peak plasma concentration after 1hr. To formulate Timolol floating microspheres using polymers like HPMC K15 M, EUDRAGIT S 100 and EC for controlled delivery of anti-hypertensive drug Timolol.

MATERIALS AND METHODS

MATERIALS

Timolol, A Gift sample from Glenmark Pharmaceuticals LTD

Hydroxy propyl methyl cellulose K 15M, A Gift sample from Spectrum pharmalabs Hyderabad

Ethyl cellulose, A Gift sample from Spectrum pharmalabs Hyderabad

Eudragit S100, A Gift sample from Shreeji chemicals, Mumbai

NAHCO3 S D, A Gift sample from fine chemical Ltd, Mumbai

Dichloromethane, A Gift sample from S D fine chemical Ltd, Mumbai

Potassium dihydrogen phosphate, A Gift sample from S D fine chemical Ltd, Mumbai

Ethanol, A Gift sample from Central drug house (p) Ltd, Bombay

Sodium hydroxide pellets, A Gift sample from S D Fine chemical Ltd, Mumbai

METHODS

Preparation of Timolol Floating Microspheres

Method used**:** Emulsification – solvent evaporation method

Floating microspheres were prepared by solvent evaporation technique accurately weighed drug, HPMC K 15M, Eudragit S 100 and EC were dissolved in ethanol and dichloromethane (1:1) to form a homogenous polymer solution. This solution is poured in 250 ml water

containing 0.01% tween 80 maintained at 30-40^{0}C subsequently stirred at ranging agitation speed for 30 min to allow the volatile liquid to evaporate. The microspheres formed were filtered, washed with water and dried in vacuum. The microspheres were then stored in a desiccator over fused calcium chloride.

Evaluation of Timolol Floating Microspheres

Percentage yield

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

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Buoyancy percentage

Fifty milligrams of the floating microspheres were placed in 0.1N hydrochloric acid, 100 ml containing 0.02 w/v% Tween 20. The mixture was stirred at 100 rpm in a magnetic stirrer. After 12 hrs, the layer of buoyant microspheres was pipette and separated by filtration. Particles in the sinking particulate layer were separated by filtration. Particles of both types were dried in a desiccator until constant weight. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles.

$$\text{Buoyancy}(\%) = \frac{Wf}{Wf + Ws} \times 100$$

Where Wf and Ws are the weights of the floating and settled microspheres, respectively.

Percentage Drug Entrapment (PDE)

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment as per the following formula.

$$\text{PDE} = \frac{\text{Practical drug loading}}{\text{theoretical drug loading}} \times 100$$

Theoretical Drug Content

Theoretical drug content was determined by calculation assuming that the entire Timolol present in the polymer solution used gets entrapped in Timolol floating microspheres and no loss occurs at any stage of preparation of Timolol floating microspheres.

Practical Drug Content

Practical drug content was analyzed by using the following procedure, weighed amount of Timolol floating microspheres equivalent to eight mg of Timolol floating microspheres was dissolved in 100 ml of 6.8 phosphate buffer. This solution was kept overnight for the complete dissolution of the Timolol floating microsphere in 6.8 phosphate buffer. This solution was filtered and further diluted to make a conc of 10 µg/ml solution. The absorbance of the solutions was measured at 294nm using double beam UV-Visible spectrophotometer against 6.8 phosphate buffer solution as blank and calculated for the percentage of drug present in the sample.

In vitro dissolution studies

The release rate of Timolol floating microspheres was determined by employing USP XXIII apparatus by rotating basket method. The dissolution test was performed using 900 ml 6.8 phosphate buffer in 37 ± 0.5°C at 50 rpm. Timolol floating microspheres equivalent to 8 mg were placed in a basket to avoid floating of microspheres. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus hourly for 12 hrs and the samples were replaced with fresh dissolution medium. The samples were passed through whatman filter paper and the absorbance of these solutions was measured at 294nm Using UV Spectrophotometric method.

Drug Kinetics

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing zero order (Q v/s t), first order [Log(Q_0-Q) v/s t], Higuchi's square root of time (Q v/s $t^{1/2}$) and Korsemeyer Peppas double log plot (log Q v/s log t) respectively, where Q is the cumulative percentage of drug released at time t and (Q_0-Q) is the cumulative percentage of drug remaining after time t. In short, the results obtained from *in vitro* release studies were plotted in four kinetics models of data treatment as follows. Cumulative percentage drug release Vs. Time (zero order rate kinetics), Log cumulative percentage drug retained Vs. Time (first order rate kinetics), Cumulative percentage drug

release Vs. √T (Higuchi's classical diffusion equation), Log of cumulative percentage drug release Vs. log Time (Peppas exponential equation).

Stability Studies

The stability studies were carried out as per ICH guidelines at 25ºC/60%RH, 30ºC/65% RH and 40ºC/75% RH for 90 days.

RESULTS AND DISCUSSIONS

The present investigation to formulate and characterize the floating microspheres of Timolol by solvent evaporation technique with HPMC K15M, EC, Eudragit S100 as polymers in different concentration of polymer. Total nine formulations were prepared and the detailed composition is shown in Table.1. The prepared Timolol floating microspheres were then subjected to (%)Percentage yield, drug content, entrapment efficiency, *in vitro* dissolution, and scanning electron microscopy shown in Table.2, Table.3, Table.4, Table.5 and Fig.1 and Fig.2.

Buyounancy percentage

The microspheres floated for prolonged time over the surface of the dissolution medium without any apparent gelation. As the polymer concentration increases the buoyancy time increases. Percentage buoyancy of the microspheres was in the range 47.63% to 92.98% after 12 hrs shown in Table.2.

Percentage yield

The percentage yield for Timolol floating microspheres were in the range of 72.65 to 89.52%.

Percentage drug entrapment efficiency

Entrapment efficiency increases with increase in the polymer concentration. From the results it can be inferred that there is a proper distribution of Timolol in the microspheres and the deviation is within the acceptable limits. The percent of drug content in the formulations was found to be in the range of 91.10% to 99.55%. The percentage entrapment efficiency was found to be 89.11% to 99.25%. The results obtained are given in table. And their histograms shown in Fig.1. A maximum of 99.25% drug entrapment efficiency was obtained in the Timolol floating microspheres which were prepared by using Eudragit 1:3 ratio. By increasing the polymer concentration, the encapsulation efficiency was increased.

***In vitro* dissolution studies**

The *in vitro* performance of timolol floating microspheres shown in Table.4, Table.5 and Fig.3, Fig.4 and Fig.5. The formulations F1 shows drug release of 95.12%, formulations F2 shows drug release of 98.79%, formulations F3 shows drug release of 99.01%, formulations F4 shows drug release of 96.44%, formulations F5 shows drug release of 97.21%, formulations F6 shows drug release of 99.90%, formulations F7 shows drug release of 98.42%, formulations F8 shows drug release of 96.68% and finally formulations F9 shows drug release of 97.85% at the end of 12 hours by comparing all the formulations, formulation (F6) containing drug and Eudragit (1:3) ratio shows best drug release of 99.90% is selected as optimized formulation.

Release kinetics of Timolol Floating Microspheres

The plots of cumulative percentage drug release V/s. time, cumulative percent drug retained V/s. root time, log cumulative percent drug retained V/s. time and log cumulative percent drug release V/s. log time were graphically drawn. The slopes and the co-efficient of determinations (r^2) were listed in Table.6. The co- efficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism. The diffusion exponent 'n' values of Korsemeyer-Peppas model was found to be in the range more than 1 for the Timolol floating microspheres prepared with drug and Eudragit indicating super case II transport diffusion mechanism of drug through Timolol floating microspheres. Stability studies were conducted for optimized formulation (F6) which indicates there are no physical changes observed indicating it passes stability test results shown in Table.7.

Table 1: Formulation Design for Timolol Floating Microspheres using Different ratios of Drug and Polymers.

Name of Ingridens	F1	F2	F3	F4	F5	F6	F7	F8	F9
Timolol (mg)	10	10	10	10	10	10	10	10	10
HPMC K15M (mg)	100	200	200	-	-	-	-	-	-
Eudragit S100(mg)	-	-	-	100	200	300	-	-	-
E.C.(mg)	-	-	-	-	-	-	100	200	300
Methonol (ml)	5	5	5	5	5	5	5	5	5
Dichloro Methane (ml)	5	5	5	5	5	5	5	5	5
Tween80 (ml)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Water(ml)	250	250	250	250	250	250	250	250	250

Table 2: Buyounacy Percentage of Timolol Floating Microsphere.

Formulation	% Buoyancy time
F1	47.63±0.36
F2	56.65 ±0.20
F3	69.24 ±0.78
F4	71.15±0.98
F5	80.32 ±0.75
F6	92.98 ±0.11
F7	52.45±0.23
F8	61.14 ±0.78
F9	77.28 ±0.55

Table 3: Percentage Entrapment Efficiency, Drug content and Percentage Yield of Prepared Formulations (F1 to F9).

Formulation	Percentage yield	Drug content (%)	Entrapment efficiency (%)
F1	72.65	91.10	89.11
F2	75.42	92.63	90.24
F3	79.11	94.31	93.74
F4	80.71	97.11	94.89
F5	82.74	98.70	96.28
F6	89.52	99.55	99.25
F7	78.41	94.96	93.82
F8	80.44	97.85	95.89
F9	83.68	98.23	97.63

Table 4: *In vitro* release data of Timolol floating microspheres of different Formulations (F1-F6) ± SD (n=6).

Time (h)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	23.56± 0.18	18.48 ± 0.36	15.46 ± 0.08	20.74 ± 0.14	16.56± 0.11	12.78 ± 0.31
2	32.42 ± 0.06	28.52 ± 0.15	22.76 ± 0.14	38.84 ± 0.21	25.71 ± 0.16	23.70 ± 0.22
3	40.45 ± 0.42	36.48 ± 0.33	31.81 ± 0.06	44.71 ± 0.10	37.81 ± 0.12	35.45 ± 0.16
4	53.25 ± 0.05	47.71 ± 0.15	43.79 ± 0.14	56.80 ± 0.04	46.87 ± 0.29	42.45 ± 0.16
5	62.28 ± 0.41	58.29 ± 0.29	51.98± 0.10	65.98 ± 0.25	57.84 ± 0.23	49.54 ± 0.13
6	75.61 ± 0.21	69.81 ± 0.03	63.85 ± 0.25	76.89 ± 0.41	70.45 ± 0.25	56.87 ± 0.17
7	89.03± 0.32	78.75± 0.40	72.54 ± 0.09	87.81 ± 0.16	79.89 ± 0.22	65.74 ± 0.33
8	95.12 ± 0.39	86.92 ± 0.33	83.41 ± 0.27	96.44 ± 0.08	85.72 ± 0.12	74.25 ± 0.26
9		92.58 ± 0.30	89.63 ± 0.15		90.96 ± 0.25	81.41 ± 0.15
10		98.79 ± 0.28	94.76 ± 0.14		97.21 ± 0.27	87.49 ± 0.24
11			99.01 ± 0.06			92.45 ± 0.42
12						99.90 ± 0.36

Table 5: *In vitro* release data of Timolol floating microspheres of different Formulations (F7-F9) ± SD (n=6).

Time (h)	F7	F8	F9
0	0	0	0
1	25.86± 0.18	22.15 ± 0.36	19.54 ± 0.08
2	34.72 ± 0.98	31.89 ± 0.89	28.78 ± 0.36
3	45.12 ± 0.88	42.82 ± 0.54	36.93 ± 0.06
4	58.66 ± 0.45	53. 89± 0.69	44.71 ± 0.14
5	65.32 ± 0.36	61.02 ± 0.77	51.86± 0.10
6	75.94 ± 0.12	72.45 ± 0.82	57.44 ± 0.25
7	89.56± 0.19	78.11± 0.90	66.12 ± 0.09
8	98.42 ± 0.09	87.21 ± 0.47	74.65± 0.27
9		92.47 ± 0.88	82.92 ± 0.15
10		96.68 ± 0.63	89.14 ± 0.14
11			94.59± 0.06
12			97.85 ± 0.06

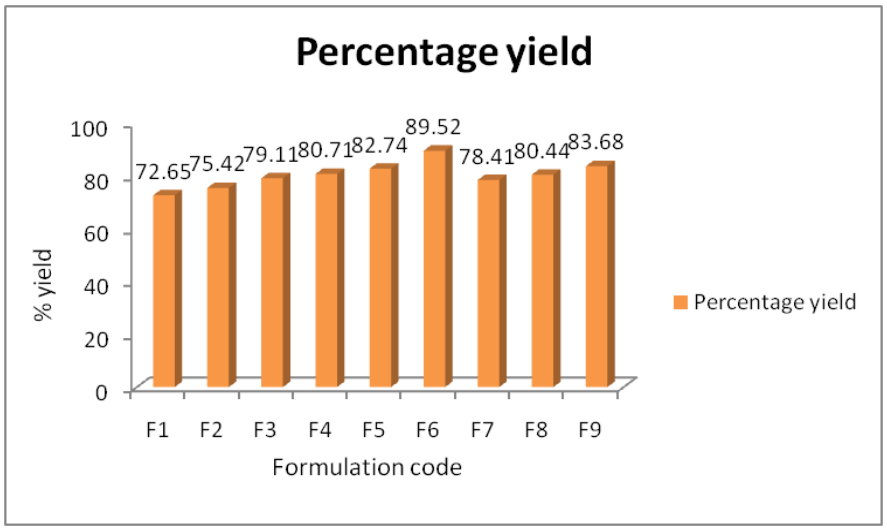

Fig 1: Percentage yield of Timolol floating microspheres.

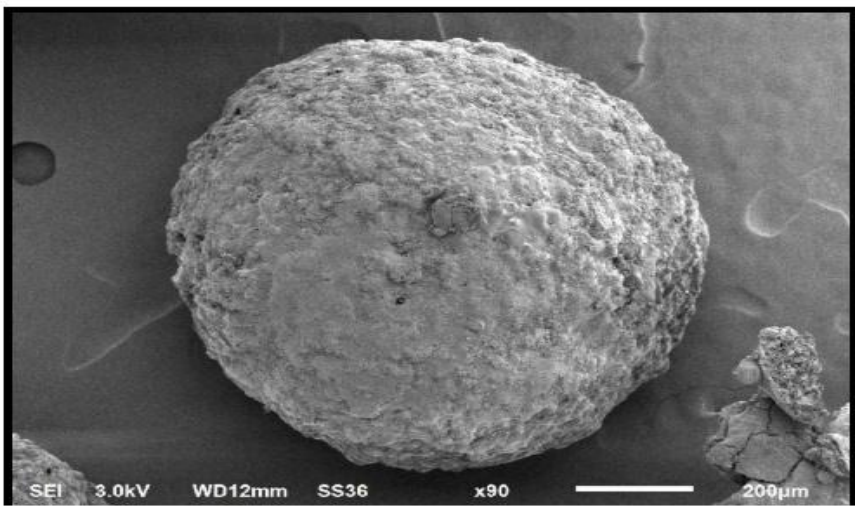

Fig 2: Scanning Electron Microscopy F6.

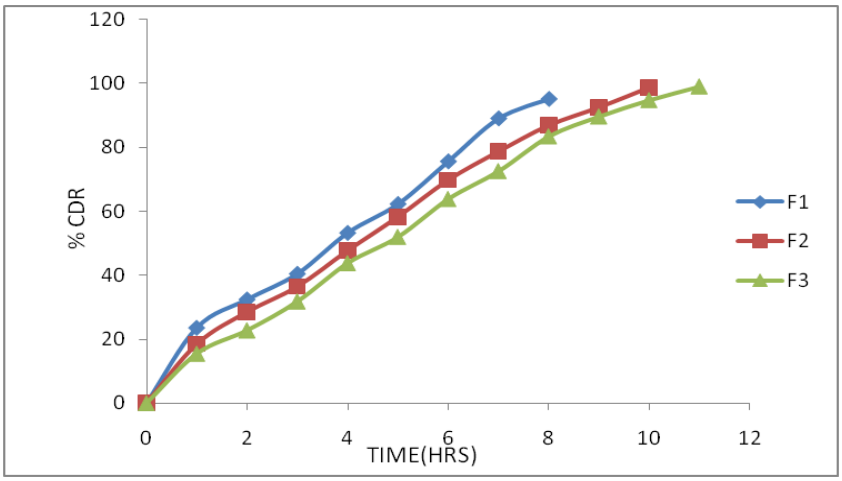

Fig 3: Cumulative Percentage Drug release (%CDR) profile of Timolol Floating microspheres Formulations (F1-F3).

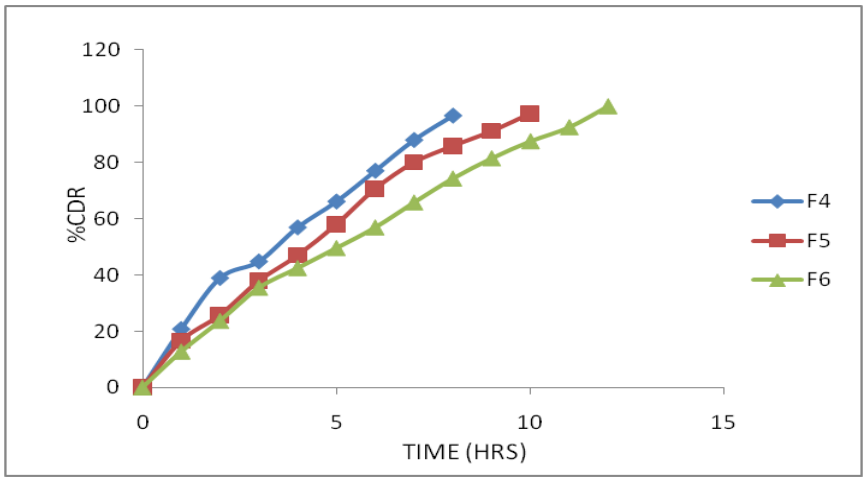

Fig 4: Cumulative Percentage Drug release (%CDR) profile of Timolol Floating microspheres Formulations (F4-F6).

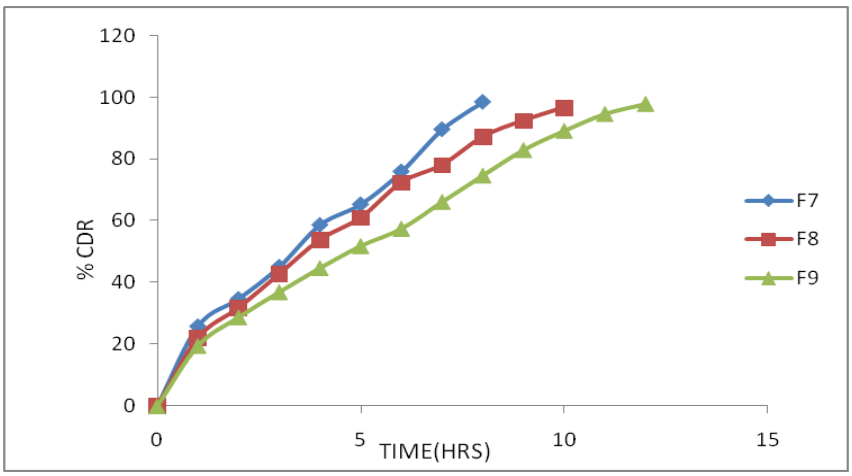

Fig 5: Cumulative Percentage Drug release (%CDR) profile of Timolol Floating microspheres Formulations (F7-F9).

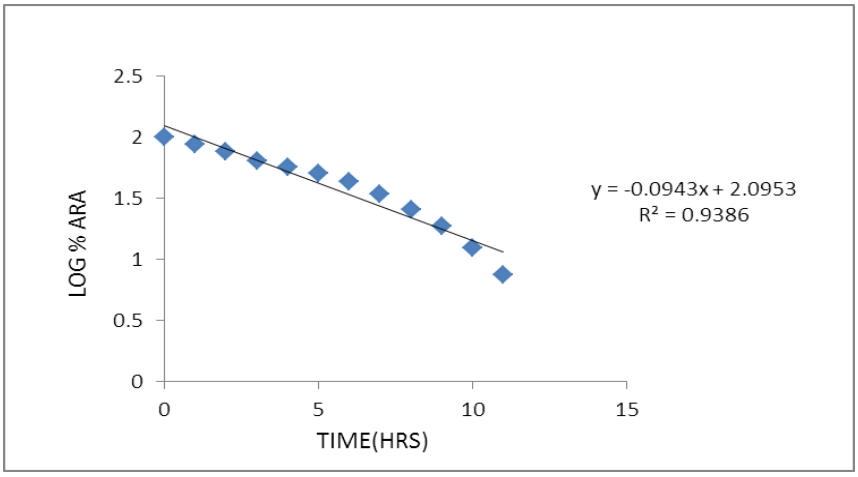

Fig 6: First order Drug Release Profile of Timolol Optimized Formulation (F6).

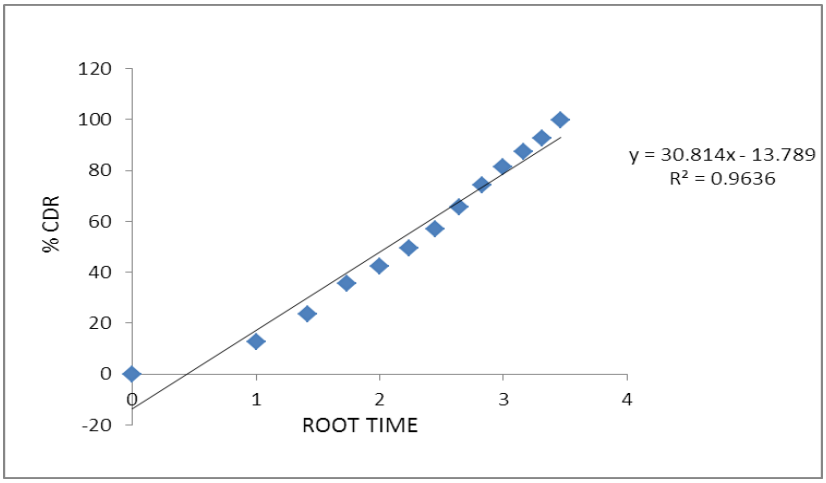

Fig 7: Higuchi Model Drug Release kinetics profile of Timolol Optimized Formulation (F6).

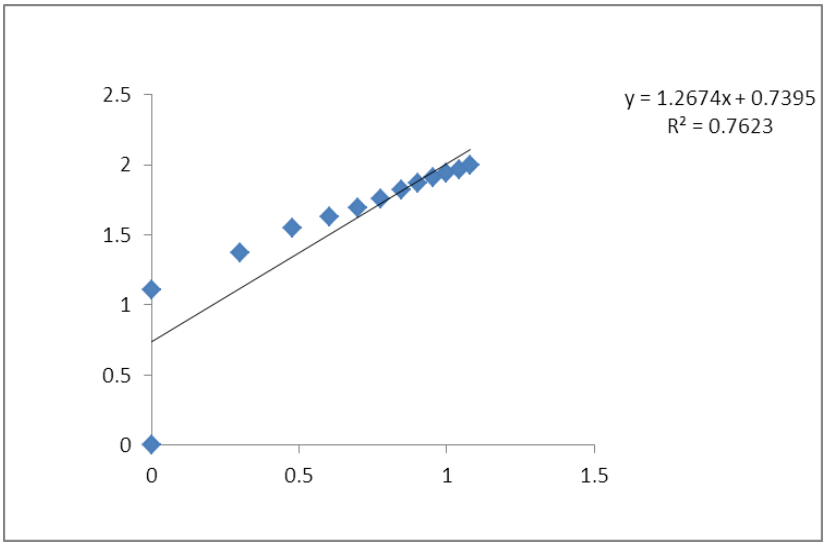

Fig 8: Peppas Model Drug release kinetics profile of Timolol Optimized formulation (F6).

Table 6: Correlation co-efficient (r^2) values Timolol floating microspheres Optimized Formulation(F6).

Formulation	Zero order	First order	Higuchi Matrix	Peppas plot	
				R^2 value	'n' value
F6	0.989	0.938	0.963	0.762	1.267

Table 7: Stability studies of Optimized Formulation (F6) Before storage and After storage.

Time(days)	% Drug content		
	25 ^{0}C/60%RH	30^{0}C/65% RH	40^{0}C/75% RH
0	99.55	99.55	99.55
15	99.48	99.50	99.47
45	99.53	99.51	99.49
90	99.51	99.53	99.52

CONCLUSION

1. The goal of present work is to provide a therapeutic amount of (Timolol) to the proper site in the body and also to achieve and maintain the desired Timolol concentration.
2. An attempt was made to prepare microspheres of Timolol floating o/w emulsion solvent evaporation techniques by using polymers like HPMC K15M, Eudragit S100 and EC achieve an oral controlled release of the Timolol.
3. In the present study nine formulations were formulated by using HPMC K15M, Eudragit S100 and EC in various proportions (1:1, 1:2, 1:3).
4. In pre formulation study, estimation of Timolol was carried out by Shimadzu UV spectrophotometer at λ_{max} 294 using 6.8 buffer as buffer, which had a good reproducibility and this method was used in entire study.
5. All the formulations were subjected to (%) Percentage yield, drug content, buoyancy time and entrapment efficiency, *in vitro* dissolution and release kinetics shown satisfactory results.
6. Entrapment efficiency was increased with increased polymer concentration. From the results it can be inferred that there was a proper distribution of Timolol in the microspheres and the deviation was within the acceptable limits.
7. On the basis of release data and graphical analysis formulation F6 shown good controlled release profile with maximum entrapment efficiency because of high polymer concentration.
8. The co-efficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release

mechanism. The diffusion exponent 'n' values of Korsemeyer- Peppas model was found to be in the range of more than 1 for the Timolol floating microspheres prepared with drug and Eudragit indicating super case 2 transport diffusion mechanism of drug through Timolol floating microspheres.

9. The above obtained data it can be summarized that it is possible to formulate promising controlled release floating microspheres of timolol by solvent evaporation technique using polymers like HPMC K 15 M, Eudragit and EC.

REFERENCES

1. Patel MG, Patel RH, Patel M, Patel KJ. Floating drug delivery system: An innovative approach to prolong gastric retention. Available at www.pharmainfo.net.aug.2010.
2. Garg R, Gupta GD. Progress in controlled gatroretentive delivery systems. Tropical J Pharm Res., 2008; 7(3): 1055- 66.
3. Ashford M. The gastrointestinal physiology and drug absorption. In: Pharmaceutics the science of dosage form design. Aulton ME, editor. Pharmaceutics the science of dosage form design. 2nd ed. Edinburgh: Churchill living stone. 2004; 220.
4. Wilson KR, Waugh A. Anatomy and physiology in health and illness, 9th ed. London: Churchill Livingstone, 1996.
5. Wilson CG, Washington N. The stomach: its role in oral drug delivery. Physiological Pharmaceutical: Biological Barriers to Drug Absorption. Chichester, UK: Ellis Horwood, 1989: 47-70.
6. Wong DSL. Prolonged release active agent dosage form adapted for gastric retention. US Patent, 2000; 15th Sept: 120-803.
7. Yuveraj Singh T, Naruk PS, Ojha GR. Development and evaluation of floating microspheres of Verpamil Hydrochloride. Braz J pharma sci., 2007; 43(4): 529-34.
8. Gattani YS, Kawtikwar PS, Sakarka DM. Formulation and evaluation of gastro retentive multiparticulate drug delivery system of Aceclofenac. Int J Chem Tech Res., 2009; 1(1): 1-10.
9. Yeole PG, Khan S, Patel VF. Floating drug delivery systems: Need and development. Indian J pharma Sci., 2005; 67(3): 265-72.
10. Timmermans J, Moes AJ. Floating dosages forms: A Review. Int J Pharm, 1990; 62: 207-16.

11. Garima, Chawla, Piyush. Gupta, Vishal. Koradia, Bansal KA. Gastro retention: A Means to address regional variability in intestinal drug absorption. Pharm tech, 2003; 27(2): 50-68.
12. R.Talukder and R.Fissihi. Gatroretentive Delivery Systems: A Mini review. Drug Dev Ind Pharmacy, 2004; 30(10): 1019-28.
13. Xu.WL, Tu.XD, Lu.ZD. Development of Gentamycin sulfate sustained-release tablets remaining-floating in stomach. Yao Hsueh Pao, 1991; 26(7): 541-45.
14. Srivastava AK, Narayanarao D. Floating microspheres of Cimetidine: formulation, characterization and in- vitro evaluation. Acta Pharm, 2005; 55: 277-85.
15. Fursule RA, Patra CN, patil GB, Kosalge SB, patil PO, Deshmukh PK. Study of multiparticulate floating drug delivery system prepared by emulsion gelation technique. Int J Chem Tech Res., 2009; 1(2): 162-7.
16. Kumaresh SS, Tejraj MA, Sunil AA, Nadagouda NM, Padmakar VK. Effect of coexcipient on drug release and floating property of Nifedipine hollow microspheres: a novel gastro retentive drug delivery system. J Appl Polym Sci., 2006; 100: 486-94.
17. Jain AK, Jain CP, Tanwar YS, Naruka, PS. Formulation characterization and in vitro evaluation of floating microspheres of Famotidine as a gastro retentive dosage form. Asian J Pharm, 2009; 3(3): 222-6.
18. Gronia R, Heun G. Oral dosage forms with controlled gastrointestinal transit. Drug Dev Ind Pharm, 1984; 10: 527-39.
19. Atyabi F, Sharma HL, Mohammad H, Fell JT. In vivo evaluation of a novel gatroretentive formulation based on ion exchange resins. J Cont Rel., 1996; 42: 105- 13.
20. Klausner A, Lavy E, Friedman M, Hoffman A. Expandable gatroretentive dosage form. J Cont Rel., 2003; 90: 143-62.
21. Singh BN, Kim HK. Floating drug delivery systems: An approach to oral controlled drug delivery via gastric retention. J Cont Rel., 2000; 63: 235-7.
22. Whitehead L, Collett JH, Fell JT. Amoxycillin release from a floating dosage form based on alginates. Int J Pharm, 2000; 210: 45-9.
23. Arora S, Ali J, Ahuja A, Khar RK, Baboota S. Floating drug delivery systems: A review. AAPS Pharm Sci Tech, 2005; 6: 372-90.
24. Mayavanshi AV, Gajjar SS. Floating drug delivery systems to increase gastric retention of drugs: A Review. Res J Pharm Tech, 2008; 1(4): 345-48.
25. Sunil KJ, Govind PA, Narendra KJ. Evaluation of porous carrier-based floating Orlistat microspheres for gastric delivery. AAPS Pharm Sci Tech, 2006; 7(4): 90.

26. Oth M, Franz M, Timmermans J, Moes A: The bilayer floating capsule: a stomach directed floating drug delivery system for Misprostal. Pharma Res., 1992; 9: 298- 302.
27. Menon A, Ritschel WA, Sakar A. Development and evaluation of a monolithic floating dosage form for Furosemide. J Pharm Sci., 1994; 83: 239-45.