Volume 5, Issue 6, November-December2016
ISSN No.: 2319-7536
Available Online at www.gpublication.com/crbps

Current Research in Biological and Pharmaceutical Sciences

RESEARCH ARTICLE

FORMULATION AND EVALUATION OF MUCOADHESIVE MICROSPHERE OF NIFEDIPINE BY USING CARBOPOL AND RLPO

Priyanka Dangi , Mahesh Kumar Gupta, Rohit Singhal, Neetesh Kumar Jain
Department of Pharmacy, Oriental University, Indore (M.P.)-India
Email: raj.sims18@gmail.com

Abstract
The present study was carried out to develop Mucoadhesive drug delivery System in the form of microsphere dosage form of Nifedipine by using Carbopol and RLPO and thereafter formulating the formulation. From the study it was observed that formulation act as prolonged dosage form. As the stirring speed increased the size of microsphere decreases and increases the released rate drug. The prepared microsphere of Nifedipine also gave good Micrometrics result, percent yield, drug entrapment and in-vitro release. In dissolution study of all formulations it was observed that change in process variables during the formulation of microspheres like stirring speed (RPM) and stirring time significantly affect the release rate of drug. The microspheres of F7 batch were found to be satisfactory in terms of percent yield, percent drug entrapment and in-vitro release; Surface morphology by stereomicroscope gives smooth surface of all batches.

Keywords
Nifedipine, Mucoadhesive, Microsphere

INTRODUCTION:

Microspheres are defined as spherical particles having size less than 200µm and made up of polymer matrix in which therapeutic substance is dispersed throughout the matrix at the molecular or macroscopic level[1-5]. The rationale of developing mucoadhesive microsphere drug delivery system lies behind the fact that the formulation will be 'held' on a biological surface for localized drug delivery. The drug will be released close to the site of action with a consequent enhancement of bioavailability. Mucoadhesive microspheres include microparticles and microcapsules (having a core of drug) of 1- 1000µm in diameter and consisting either entirely of a Mucoadhesive polymer or having an outer coating of it, respectively. Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery; but coupling of bioadhesive properties to microspheres has additional advantages e.g. efficient absorption and bioavailability of the drugs due to high surface to volume ratio, a much more intimate contact with the mucous layer, specific targeting of drugs to the absorption site. Bioadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in eye, nasal cavity. Mucoadhesive microspheres have advantages like efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site.[6-10]. Nifedipine has a short biological half-life of 2.5 h and is eliminated rapidly and its antihypertensive effect lasts only for few hours. As such controlled release products are needed for Nifedipine to prolong its duration of action and to improve patient compliance.[8]

Our objective to develop safe, effective and sustained release formulation of Mucoadhesive microspheres of nifedipine will ensure the maintenance of effective plasma concentration over prolonged period of time by extending the release of drug. These carrier systems will also increase the residence time of the drug in the gastrointestinal tract. Mucoadhesive drug delivery is a promising area for systemic delivery of orally inefficient drugs as well as an attractive alternative for noninvasive delivery of potent peptide and perhaps protein drug molecules.[9-15]

MATERIALS & METHODS

Nifedipine was procured from the F&D department of RANBAXY Laboratories Ltd. Dewas M.P, India. Excipients such as Eudragit RLPO, Carbopol, Light liquid paraffin, Ethanol were obtained from Local market of Indore (M.P).

Identification of Nifedipine

1. Melting point Determination

A small quantity of powder was placed into a fusion tube. That tube is placed in the melting point determining apparatus containing Silicon oil. The temperature of the castor oil was gradual increased automatically and read the temperature at which powder started to melt and the temperature when all the powder gets melted. Results show in given table no. 2. [14].

2. Solubility Studies

Drug (powder) was added in excess in conical flasks which contained 10 ml of the medium. Each solubility value was determined in triplicate, at least. The flasks were placed in orbital shaker incubator at 25.0 ± 0.5°C agitated at 60 rpm, for 24 h. After this period, sample was withdrawn, filtered and drug concentration was determined at specified wavelength in UV spectrophotometer against an appropriate blank. Solubility is determined in different solvents like: water, methanol, 0.1 N HCL, Ethyl Alcohol, and Chloroform. Results show in given table no. 3. [16].

***3.* Determination of $\lambda_{max.}$**

Accurately weighed 10 mg of Nifedipine was dissolved in 100 ml of distilled water in a 100 ml volumetric flask. Then, 1 ml of this stock solution was pipetted into a 10 ml volumetric flask and volume made up to the mark with 0.1 N HCL. The resulting solution was scanned between 200-400 nm using UV/Vis double beam spectrophotometer. The l_{max} was found to be 238 nm shows in given spectra.

4. Preparation of calibration graph of nifedipine

A spectrophotometric method based on the measurement of absorbance at 238 nm in a **0.1 N HCL** was used in the present study for the estimation of nifedipine in the formulations and in vitro studies.

5. Drug Excipient Compatibility Studies

The IR spectra of Nifedipine and physical mixture of drug with previously mentioned polymers were obtained by KBr pellet method employing Bruker alpha FTIR spectra. Data shows in given IR spectras.

6. Preparation of Mucoadhasive Microsphere of Nifedipine

All the ingredients along drug and with additional ethanol are dissolved, solution were sequentially dropped into appropriate quantity into light liquid paraffin. Light liquid paraffin was stirred with a mechanical stirrer at 1000 rpm at 50^0C temperature for 45 min. The mucoadhesive microspheres were gradually hardened and the hardened microspheres were collected by filtration. They were washed several times with petroleum ether and dried in vacuum oven at ambient temperature for 24 hr. The yield was calculated.

Table No. 1 Formulations of the Mucoadhasive Microspheres Prepared

Sr. No	Formulation Code	Nifedipine (mg)	RLPO (mg)	Carbopol (mg)
1.	F_1	50	50	-
2.	F_2	50	100	-
3.	F_3	50	150	-
4.	F_4	50	200	-
5.	F_5	50	-	50
6.	F_6	50	-	100
7.	F_7	50	-	150
8.	F_8	50	-	200
9.	F_9	50	25	25
10.	F_{10}	50	50	50
11.	F_{11}	50	75	75
12.	F_{12}	50	100	100

EVALUATION OF MICROSPHERES

A. Particle size analysis

The mean size of the microspheres was determined by Photo Correlation Spectroscopy (PCS) on a submicron particle size analyzer **(Horiba Instruments)** at a scattering angle of 90°. A sample (0.5mg) of the microspheres suspended in 5 ml of distilled water was used for the measurement. The average diameter were calculated by using formula.

B. Determination of zeta potential

The zeta potential of the drug-loaded microspheres was measured on a zeta sizer by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25 °C in triplicate.

C. Percentage Yield

The prepared microspheres with a size range of 609-874 µm were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres.

% Yield = Actual weight of product x 100
Total weight of drug and polymer

D. Drug Entrapment

The various formulations of the Mucoadhasive microspheres were subjected for drug content. 50 mg of Mucoadhasive microspheres from all batches were accurately weighed and crushed. The powdered of microspheres were dissolved with 10ml ethanol in 100ml volumetric flask and makeup the volume with 0.1 N HCl. This resulting solution is than filtered through whatmann filter paper No. 44. After filtration, from this solution 10 ml was taken out and diluted up to 100 ml with 0.1 N HCl. Again from this solution 2 ml was taken out and add 2 ml of Methylene orange and Extracted with Chloroform and the absorbance was measured at 554.0 nm against blank.

E. In-vitro Release Studies

The drug release rate from Mucoadhasive microspheres was carried out using the USP type II (Electro Lab.) dissolution paddle assembly. A weighed amount of Mucoadhasive microspheres equivalent to 100 mg drug were dispersed in 900 ml of 0.1 N HCI (pH 1.2) maintained at 37 ± 0.5°C and stirred at 100 rpm. One ml sample was withdrawn at predetermined intervals and filtered and equal volume of dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. The collected samples were treated with Methylene orange and analyzed spectrophotometrically at 554 nm to determine the concentration of drug present in the dissolution medium.

F. Drug Release Kinetic Data Analysis

Several kinetic models have been proposed to describe the release characteristics of a drug from matrix. The following three equations are commonly used, because of their simplicity and applicability. Equation 1, the zero-order model equation (Plotted as cumulative percentage of drug released vs time); Equation 2, Higuchi's square-root equation (Plotted as cumulative percentage of drug released vs square root of time); and Equation 3, the Korsemeyer-Peppas equation (Plotted as Log cumulative percentage of drug released vs Log time).

RESULT AND DISCUSSION:

S. No.	Onset	Complete	Melting Point
1	174^0C	176^0C	174 ± 2^0C
2	173^0C	175^0C	
3	173^0C	175^0C	

Table No. 2. Melting Point Range of Nifedipine

2. Solubility Study:

A solubility study of Nifedipine has been done in various solvent such as water, Chloroform, Ethanol, Methanol, and 0.1N HCL solution. We were found that a solubility of Nifedipine is good in 0.1N HCL solution.

S. No.	Solvent used	Solubility
1.	Water	Slightly soluble
2.	0.1 N HCL	Slightly soluble
3.	Ethanol	Soluble
4.	Methanol	Freely Soluble
5.	Chloroform	Sparingly soluble

Table No. 3. Solubility studies of Nifedipine in different solvent

3. Determination of λ_{max} by UV-visible spectroscopy:

Accurately weighed 10 mg of Nifedipine separately and dissolved in 10 ml of 0.1N HCL. The spectrum of this solution was run in 200-400 nm range in U.V spectrophotometer.

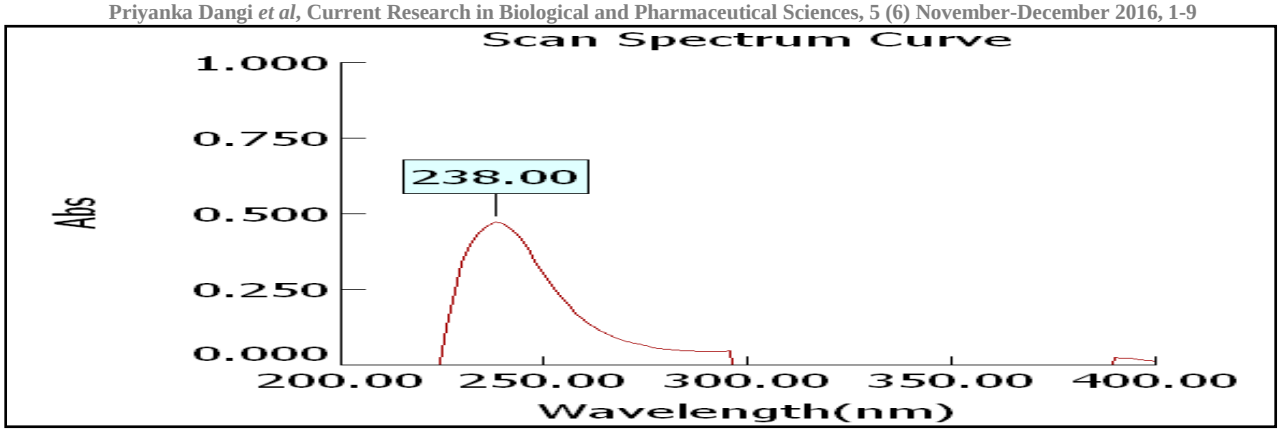

Fig No. 1. Determination of λmax of Nifedipine

S. No.	Concentration (µg/ml)	Absorbance
1	5	0.155
2	10	0.325
3	15	0.485
4	20	0.659
5	25	0.826

Table No. 4. Calibration curve of Nifedipine in 0.1 N HCl

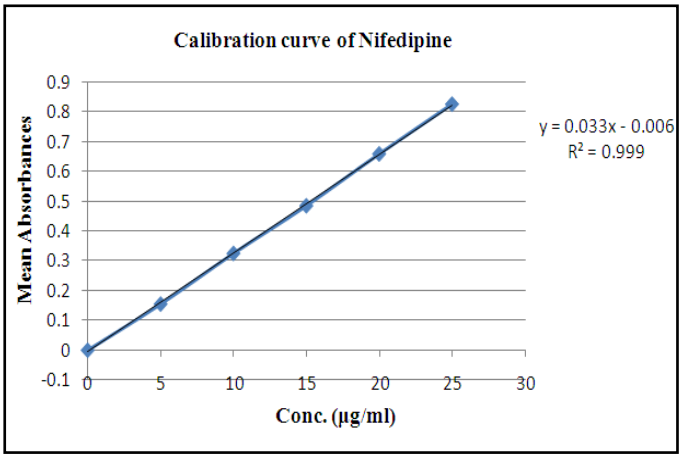

Fig No. 2. Calibration curve of Nifedipinein in 0.1 N HCl

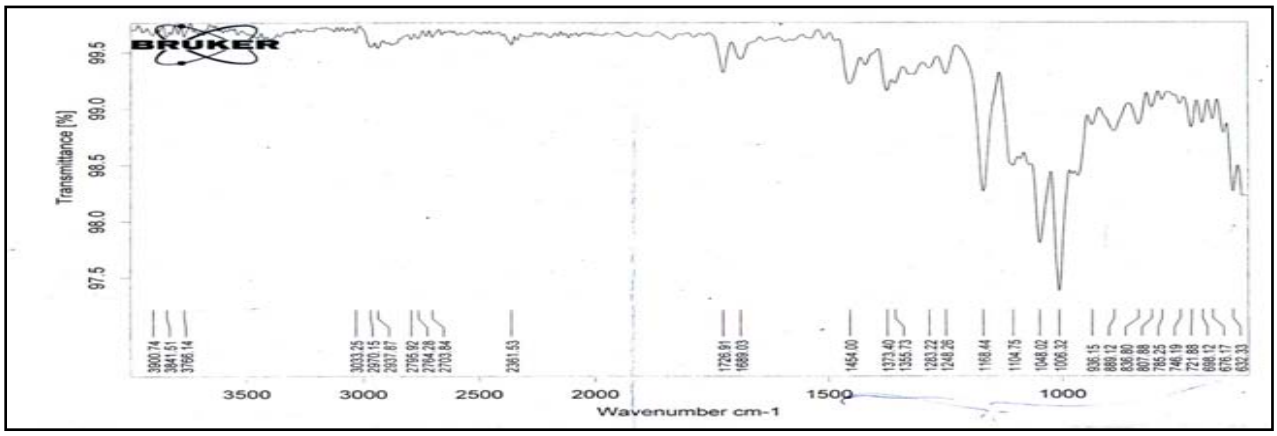

Fig No. 3. FT-IR Spectrum of Pure Drug (Nifedipine)

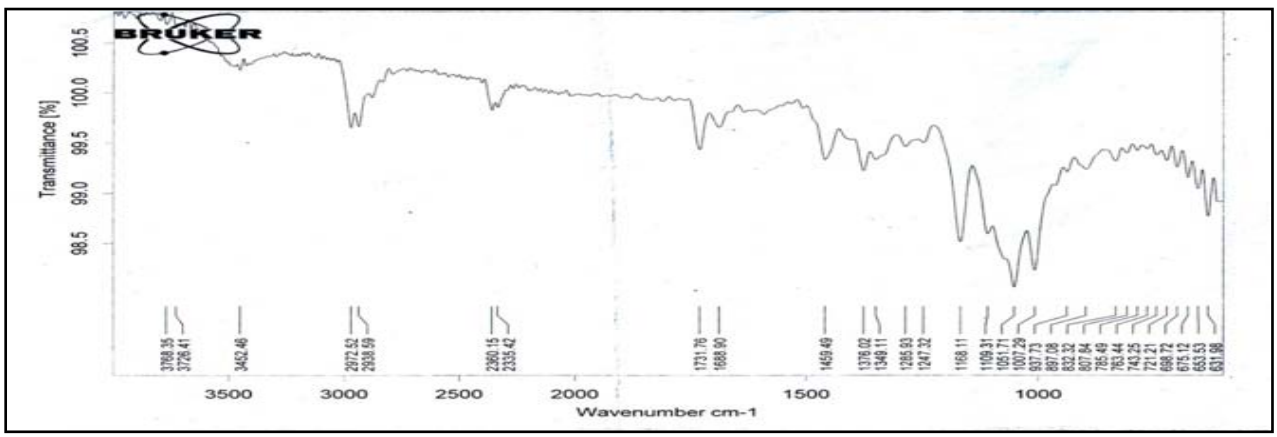

Fig No. 4. FT-IR Spectrum of Drug + Excipients

EVALUATION OF NIFEDIPINE MUCOADHESIVE MICROSPHERES

A. Particle size analysis:

A sample (0.5mg) of the microspheres suspended in 5 ml of distilled water was used for the measurement. The results of measurement of mean particle size were found 272.0 nm.

B. Zeta Potential:-

The zeta potential of the drug-loaded microspheres was measured on a zeta sizer (Malvern Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25 °C in triplicate. Results of zeta potential of optimized formulation F7 was found -31.1mV.

C. Drug Entrapment:-

The drug entrapment efficacies of different formulations were in range of 48.47 - 74.19 % w/w. Drug entrapment efficacy slightly decrease with increase HPMC content and decreased EC ratio in Microspheres. This is due to the permeation characteristics of HPMC that could facilitate the diffusion of part of entrapped drug to surrounding medium during preparation of Nifedipine microspheres.

Formulation	Drug entrapment (% w/w)
F_1	76.19
F_2	70.59
F_3	66.23
F_4	64.76
F_5	61.01
F_6	57.38
F_7	78.89
F8	72.56
F9	68.56
F10	65.56
F11	60.23
F12	58.89

Table No. 5 Drug Entrapment for Different Formulation

D. Percentage Yield:-

Percentage yield of different formulation was determined by weighing the Microspheres after drying. The percentage yield of different formulation was in range of 56.84 - 82.87%.

Formulation	Percent Yield (%)	*In-vitro* wash- off test (% mucoadhesion after 1 h)
F_1	82.87	58
F_2	78.53	56
F_3	76.47	42
F_4	71.56	82
F_5	69.31	78
F_6	66.03	70

F_7	89.84	92
F_8	78.89	80
F_9	65.56	74
F_{10}	60.56	76
F_{11}	55.56	80
F_{12}	56.65	72

Table No.6. Percentage Yield for Different Formulation

E. In-Vitro Drug release study

The In vitro drug release data of the optimized formulation was subjected to linear regression analysis according to zero order, first order kinetic equation, Higuchi's and Korsmeyer's models in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that 'r' values of Higuchi was maximum i.e 0.986 hence indicating drug release from formulations was found to follow Higuchi kinetics.

Time	**% of Drug Release**										
(hr)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**
0.5	9.78	12.00	12.00	11.36	14.21	12.63	14.21	13.25	13.25	12.25	11.25
1.0	10.16	11.43	11.43	12.06	21.86	14.28	19.65	18.56	15.56	13.25	12.25
1.5	13.68	13.39	13.39	18.44	25.14	28.25	28.23	25.56	20.25	18.56	15.56
2.0	15.34	15.36	15.36	24.86	27.49	29.04	36.66	35.59	30.56	25.56	22.12
3.0	20.16	23.97	23.97	30.36	32.70	32.99	40.33	38.89	35.56	32.25	30.54
4.0	27.85	31.68	31.68	24.21	35.08	36.01	50.03	48.89	45.56	40.56	35.56
6.0	32.42	35.96	35.96	30.66	37.17	39.68	60.72	55.56	50.53	45.56	40.23
8.0	52.50	46.59	46.57	40.30	46.85	53.16	64.84	58.89	53.26	50.26	48.89

Table No. 7. Comparative Release Study data of formulation F1-F12

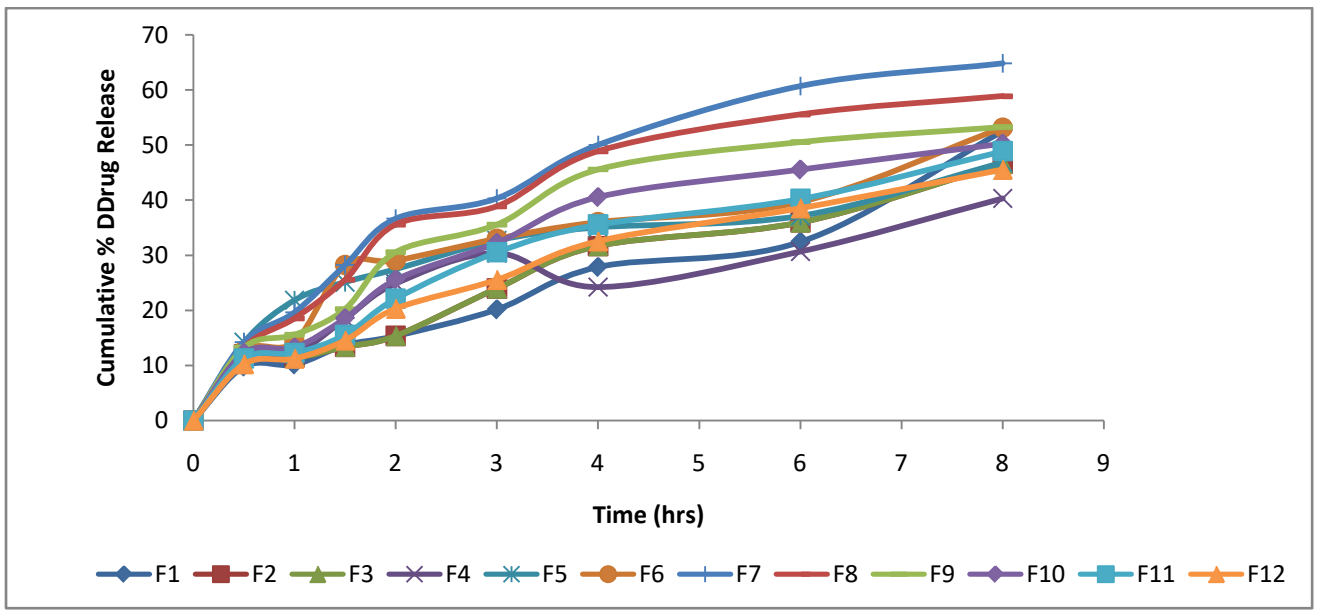

Fig No. 5 Graph of release study of formulation F1-F12

G. Release Kinetics Studies

Sr. No.	ZERO ORDER		FIRST ORDER		HIGUCHI EQUATION		KORSEMAYER -PAPAS
	Time (Hrs.)	CUM%DRS	Time (Hrs.)	LOG CUM%CDREM	CUM%CDR	Sq ROOT T	log time
1	0	0	0	2	100	0	
2	0.5	14.21	0.5	1.933	85.78	0.7071068	-0.301
3	1	19.65	1	1.904	80.34	1.000	0
4	1.5	28.29	1.5	1.855	71.70	1.224	0.176
5	2	36.66	2	1.801	63.34	1.414	0.301
6	3	40.33	3	1.775	59.66	1.732	0.477
7	4	50.03	4	1.698	49.97	2	0.602
8	6	60.72	6	1.594	39.27	2.449	0.778
9	8	64.84	8	1.545	35.15	2.828	0.903

Table No. 8. Release Kinetics of Optimized Formulation F-7

CONCLUSION:

Mucoadhesive microspheres showed good controlled release properties. Mucoadhesive drug delivery System in the form of microsphere dosage form of Nifedipine by using Carbopol and RLPO formulate. From the study it is observed that formulation act as prolonged dosage form. As the stirring speed increased the size of microsphere decreases and increases the released rate drug. The prepared microsphere of Nifedipine also gave good Micrometrics result, percent yield, drug entrapment and in-vitro release. In dissolution study of all formulations it was observed that change in process variables during the formulation of microspheres like stirring speed (RPM) and stirring time significantly affect the release rate of drug. The microspheres of F7 batch were found to be satisfactory in terms of percent yield, percent drug entrapment and in-vitro release; Surface morphology by stereomicroscope gives smooth surface of all batches.

BIBLIOGRAPHY

1. Rouge N, Buri P, Doelker E. Drug absorption sites in the gastrointestinal tract and dosage forms for site specific delivery. Int J Pharm. 1996; 136: 117-139.
2. Singh BN and Kim KH. Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. J. Control. Release. 2000; 63: 235-239.
3. Streubel A, Siepmann J, Bodmeier R. Gastroretentive drug delivery system. Expert Opin Drug Deliv. 2006; 3 (2): 217-233.
4. Ali J, Arora S, Khar RK. Floating drug delivery System: A Review. AAPS Pharm Sci Tech. 2005; 06(03): E372-E390.
5. Deshpande AA, Shah NH, Rhodes CT, Malick W. Development of a novel controlled-release system for gastric retention. Pharm Res. 1997; 14: 815-819.
6. Davis SS, Stockwell AF, Taylor MJ. The effect of density on the gastric emptying of single and multiple unit dosage forms. Pharm Res. 1986; 3: 208-213.
7. Lehr CM. Bioadhesion technologies for the delivery of peptide and protein drugs to the gastrointestinal tract. Crit. Rev. Ther. Drug Carrier Syst. 1994; 11: 119-160.Groning R, Heun G. Oral dosage forms with controlled gastrointestinal transit. Drug Dev Ind Pharm. 1984; 10: 527-539.
8. Groning R, Heun G. Dosage forms with controlled gastrointestinal passage studies on the absorption of nitrofurantion. Int J Pharm. 1989; 56: 111-116.

9. Klausner EA, Lavy E, Friedman M, Hoffman A. Expandable gastroretentive dosage forms. J Control Release. 2003; 90: 143-162.

10. Timmermans J, Moes AJ. How well do floating dosage forms float. Int J Pharm. 1990; 62: 207- 16.

11. El-Kamel AH, Sokar MS, Al Gamal SS, Naggar VF. Preparation and evaluation of ketoprofen floating oral delivery system. Int J Pharm. 2001; 220: 13-21.Oth M, Franz M, Timmermans J, Moes AJ. The bilayer floating capsule: A stomach-directed drug delivery system for misoprostol. Pharm Res. 1992; 9: 298-302.

12. Whitehead L, Fell JT, Collett JH, Sharma HL, Smith AM. Floating dosage forms: An in vivo study demonstrating prolonged gastric retention. J Control Release. 1998; 55: 3-12

13. Mojaverian P, Vlasses PH, Kellner PE, Rocci Jr ML. Effects of gender, posture, and age on gastric residence time of an indigestible solid: Pharmaceutical considerations. Pharm. Res. 1988; 10: 639-44.

14. Gansbeke BV, Timmermans J, Schoutens A, Moes AJ. Intragastric positioning of two concurrently ingested pharmaceutical matrix dosage forms. Nucl Med Biol. 1991; 18: 711-18. Timmermans J, Moes AJ. Factors controlling the buoyancy and gastric retention capabilities of floating matrix capsules: New data for reconsidering the controversy. J Pharm Sci. 1994; 83: 18-24.

15. Hilton AK, Deasy PB. In vitro and in vivo evaluation of an oral sustained release floating dosage form of amoxicillin trihydrate. Int. J. Pham. 1992; 86: 79-88.

16. Seth PR, Tossounian J. The hydrodynamically balanced system, a novel drug delivery system for oral use. Drug Dev. Ind Pharm. 1984; 10: 313-339.

17. Harrigan RM. Drug delivery device for preventing contact of undissolved drug with the stomach lining, US Patent 4, 055, 178, October 25, 1977Whitehead L, Fell JT, Collett JH. Development of a gastroretentive dosage form. Eur. J. Pharm. Sci. 1996; 4 (Suppl.): S 182.

18. Kawashima Y, Niwa T, Takeuchi H, Hino T, Itoh Y. Hollow microspheres for use as a floating controlled drug delivery system in the stomach. J. Pharm. Sci. 1992; 81: 135-140.