IJPSR (2012), Vol. 3, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 July, 2012; received in revised form 14 September, 2012; accepted 24 October, 2012

PREPARATION AND *IN-VITRO* CHARACTERIZATION OF FLOATING MICROSPHERES OF NATEGLINIDE

B.R. Rane*[1], N.A. Gujarathi [2] and J.K. Patel [3]

Department of Pharmaceutical Sciences, Jodhpur National University [1], Jodhpur, Rajasthan, India
P.S.G.V.P. Mandal's, College of Pharmacy [2], North Maharashtra University, Shahada, Maharashtra, India
Nootan College of Pharmacy [3], Visnagar, Gujarat, India

Keywords:
Nateglinide, Eudragit S100, HPMC, O/W Emulsion-Solvent Diffusion Technique, Dichloromethane, Poly Vinyl Alcohol

Correspondence to Author:

B. R. Rane

Department of Pharmaceutical Sciences, Jodhpur National University, Jodhpur, Rajasthan, India

E-mail: rane7dec@gmail.com

QUICK RESPONSE CODE

IJPSR:
ICV (2011)- 5.07

Website:
www.ijpsr.com

ABSTRACT

Gastric residence time of oral controlled release system such as microspheres is increase by different techniques such as floating and mucoadhesive system. The objective of present investigation was to formulate floating microspheres of Nateglinide in order to increase gastric residence time, increased bioavailability and to reduce dose frequency of drug molecule. Novel o/w emulsion solvent diffusion technique was used to prepare microspheres of Nateglinide by using various polymers such as HPMC, Ethyl cellulose and Eudragit S100. Entrapment efficiency of drug was upto 69%. Eudragit S100 based microspheres which were found to be hollow cavity, spherical and porous nature from the results of scanning electron microscopy. Micromeritic profile of prepared microballoons was found satisfactory. From the results of FTIR spectroscopy it was reveal that there is no drug- polymer interaction. Eudragit S100 based microspheres shows good in vitro buoyancy and sustained release profile for longer period of time > 14 hours. The formulation had followed first order kinetics as its release mechanism was diffusion controlled.

INTRODUCTION: Oral controlled release dosage forms have been developed over the past few decades due to their considerable therapeutic advantages such as ease of administration, patient compliance and flexibility in formulation. Microspheres carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery [1-3].

Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems [4-6]. They have varied applications and are prepared using assorted polymers [7].

However, the success of these microspheres is limited owing to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes. The floating microspheres have been developed in order to overcome frequent dosing to release the drug slowly into the GIT.

To control over the GRT the prolonged gastric retention is important because this helps to retain the controlled release system in the stomach for a longer period of time in a predictable manner [4]. Moreover, release the active ingredients at a sustained release rate in a larger area in the stomach is to reduce high regional concentration and risk of drug burst release compared to the single unit dosage forms [8].

At present, microspheres are considered to be one of the most promising floating systems, because they combine the advantages of multiple unit systems and good floating properties [9]. The floating microspheres were prepared by the emulsion solvent diffusion–evaporation technique. These systems are also called "microballoons" due to their low-density core [10, 11, 12].

Nateglinide acts as an agonist at peroxisome proliferator-activated receptors (PPARs) in target tissues for insulin action, such as adipose tissue, skeletal muscle, and liver. Nateglinide has all the required characteristics suitable for developing an FDDS. Hence, floating microspheres were prepared to improve the bioavailability and to achieve steady-state plasma concentration of the drug. Biodegradable anionic acrylic resin Eudragit S100, Ethyl cellulose and HPMC has been used for microencapsulation of therapeutic drugs to release of drug in controlled manner [13, 14].

The objective of present study was to fabricate microsphere by using o/w emulsion–solvent diffusion technique in order to prolong the gastric residence time, in addition to enhance bioavailability and decrease the dose frequency of Nateglinide. The influence of several factors such as the particle size, drug entrapment efficiency, floating properties and dissolution of the resulting microspheres were investigated.

MATERIALS AND METHODS:

Materials: Nateglinide was obtained as a gift sample from Dr. Reddy's Pharmaceutical Ltd. Hydrabad; Eudragit S100 was supplied by Degussa Pharmaceutical Ltd. Mumbai. Ethyl cellulose and HPMC was supplied by Lupin Pharmaceutical Ltd. Mumbai, All ingredients and solvents used were of analytical grade Supplied by Loba chem. Mumbai.

Method of preparation for Floating Microsphere: Floating microsphere with a central hollow cavity was prepared by using Emulsion solvent diffusion–evaporation technique [15]. Accurately weighed quantities of drug, Eudragit S100 were dissolved in a mixture of ethanol and dichloromethane (1:1 solvent ratio). Above prepared solution was poured into 150 ml distilled water containing 0.75% w/v Polyvinyl alcohol (PVA) and maintained at a temperature of 30–40°C. The resultant emulsion was stirred with a propeller type agitator at 400 rpm for 1 hour to allow the volatile solvent to evaporate.

% Entrapment Efficiency: Microspheres (50 mg) were crushed by using mortar and pestle, and then the crushed powder was transferred into 100ml volumetric flask. Add some quantity of 0.1 N HCl to the volumetric flask and sonicate the resulting solution for 30 min. on ultrasonicator. Further make up volume with 0.1 N HCl. And make up the suitable dilutions of resulting solution so that to obtained the solution of desired drug concentration. The absorbance was measured spectrophotometrically at 210 nm for Nateglinide.

Drug Entrapment Efficiency (%)

= (Practical drug content/ Theoretical drug content) X 100 ----- (1)

Where, Practical drug content- is the actual drug content in weighed quantity of microspheres,

Theoretical drug content- is the theoretical amount of drug in microspheres

% Yield: The Percentage yield of microsphere were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microsphere. The percentage yields were calculated as per the formula mentioned below [16].

Percentage yield = [Weight of microspheres obtained / (Weight of drug + polymer)] X 100 ----- (2)

***In-vitro* Buoyancy time:** Buoyancy of microsphere was studied using a USP dissolution test apparatus II. The microsphere (50 mg) was spread on 900 ml of 0.1M HCl containing 0.02% Tween 80 as surfactant. The medium was agitated with a paddle rotating at 100 rpm and maintained at 37°C. After 12 hours, both the floating and the settled portions of microsphere were collected separately. The microsphere were dried and weighed. The percentage of floating microsphere was calculated by using following expression [17]:

% Buoyancy = (Weight of Floating Microspheres/Initial Weight of Microspheres) X 100 --- (3)

Micromeritic Study:

1. **Particle size:** The particle size of the microsphere was measured by using an optical microscope and the mean particle size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer [18].

2. **Angle of Repose (θ):** The angle of repose was determined by the fixed funnel method. The accurately weighed powders were taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of the powder. The powder was allowed to flow through the funnel freely onto the surface. The diameter of the powder cone was measured. The angle of repose (It is the maximum angle possible between the surface of pile of powder and the horizontal plane) was calculated using the following equation.

$$\tan(\theta) = h/r$$

$$\therefore \theta = \tan^{-1}(h/r) \text{ ---------- (4)}$$

Where, h- Height of the powder cone and r- Radius of powder cone.

3. **Density:** The Bulk Density (BD) and Tapped Density (TD) of microsphere were determined. Two grams of microspheres was introduced into a 10 ml calibrated measuring cylinder. After noting down the initial volume, the cylinder was allowed to fall under its own weight onto a hard surface from the height of 2.5 inch at 2 seconds intervals. The tapping was continued until no further change in volume was noted. BD and TD were calculated using following equations.

BD = (Weight of Powder/Volume of the packaging) ----- (5)

TD = (Weight of powder/Volume of Packaging after tapping) ------- (6)

4. **Hausner's Ratio:** Hausner's ratio of the microsphere was calculated by using following formula,

Hausner's ratio = (Tapped Density/Bulk Density)---- (7)

5. **Carr's Index:** The Carr's index of microsphere was determined by following equation

Carr's Index = [(Tapped density-Bulk density)/Tapped density] X 100 ------ (8)

6. ***In-vitro* % Drug Release:** The % drug release was studied using a USP dissolution apparatus type II at 100 rpm in 0.1N HCl solution as dissolution medium (900 ml) maintained at 37±5°C. A sample (10 ml) of the solution was withdrawn up to 12 hour from the dissolution apparatus hourly and the samples were replaced with fresh dissolution medium. The samples were filtered and diluted to a suitable concentration with 0.1N HCl solution. Absorbance of these solutions was measured at 210 nm using UV spectrophotometer. Percentage drug release was calculated using an equation obtained from a standard calibration curve.

7. **Stability of Capsules:** The accelerated and long term test was carried out as per ICH guidelines for stability testing. When packaged in hard gelatin capsule shell, the microsphere capsules were stored at 40±2^{0}C, RH 75% ±5% for 6 months in case of the accelerate stability examination, and sampled at months 1, 2, 3, and 6. In long term test the microballoon capsules were preserved at 25±2^{0}C, RH 60% ±5% for 12 months, and sampled at month 0, 3, 6, 9, and 12. All physical properties, buoyancy ratio after 12 hour and drug loading amount of all samples in both studies were determined according to the above method.

RESULT AND DISCUSSION:

Preparation of Microspheres: Microspheres were prepared as per formulation **Table 1**. By emulsion solvent diffusion–evaporation technique by using organic solvents [19] such as dichloromethane and ethanol. Excellent buoyancy was shown by prepared microspheres because of their hollow nature, which can be retained for a longer period of time in the upper part of gastrointestinal tract (GIT) in order to increase

gastric residence time of the drug. The emulsion of Nateglinide and Eudragit S100 in dichloromethane and ethanol was poured into aqueous poly-vinyl alcohol solution. The ethanol rapidly partitioned into external aqueous phase and the polymer precipitated around the dichloromethane droplets, subsequently the evaporation of the dichloromethane led to formation of hollow cavities in the microballoons.

Entrapment Efficiency: The drug entrapment efficiency of formulation F_1 to F_{12} was shown in **Table 2**.

TABLE 1: FORMULATION TABLE FOR FLOATING MICROSPHERES OF NATEGLINIDE

Batch Code	Nateglinide	Eudragit R100 (mg)	HPMC (mg)	Ethyl cellulose (mg)	Glyceryl Monostearate (mg)	EtOH:DCM
F_1	400	200	-	-	0.2	1:1
F_2	400	400	-	-	0.3	1:1
F_3	400	600	-	-	0.4	1:1
F_4	400	800	-	-	0.5	1:1
F_5	400	-	200	-	0.2	1:1
F_6	400	-	400	-	0.3	1:1
F_7	400	-	600	-	0.4	1:1
F_8	400	-	800	-	0.5	1:1
F_9	400	-	-	200	0.2	1:1
F_{10}	400	-	-	400	0.3	1:1
F_{11}	400	-	-	600	0.4	1:1
F_{12}	400	-	-	800	0.5	1:1

TABLE 2: % YIELD, % ENTRAPMENT EFFICIENCY, % BUOYANCY AND % DRUG RELEASE AFTER 12 HOURS OF FORMULATIONS F_1TOF_{12}.

Batch Code	% yield	% Entrapment efficiency	% Buoyancy	% Drug release after 12 hours
F_1	87.17%	52.20(±0.23)	91.72(±0.19)	91.3
F_2	92.00%	60.24(±0.17)	95.98(±1.30)	93.56
F_3	86.15%	47.89(±0.40)	94.71(±0.44)	92.34
F_4	80.15%	69.41(±0.64)	95.72(±0.94)	92.78
F_5	80.25%	54.98(±0.30)	65.05(±0.29)	93.69
F_6	84.55%	43.89(±0.39)	61.24(±0.32)	91.05
F_7	77.26%	56.64(±0.26)	61.69(±0.55)	94.81
F_8	74.12%	54.51(±0.44)	60.14(±0.46)	95.91
F_9	76.37%	47.22(±1.62)	61.68(±0.29)	96.73
F_{10}	70.12%	53.61(±0.61)	65.78(±0.45)	94.68
F_{11}	72.45%	56.12(±0.28)	65.56(±0.27)	95.94
F_{12}	71.46%	41.78(±0.37)	64.21(±0.38)	93.75

Percentage Yield: Percentage yield of floating microspheres was depend on concentration of polymer. As the polymer concentration increases the percentage yield of floating microsphere decreases, for all formulations (F_1 to F_{12}) are shown in Table 2.

% Buoyancy test: Buoyancy of prepared microspheres were investigated by *in-vitro* buoyancy test, results of buoyancy test for all formulations (F_1 to F_{12}) are shown in Table 2.

***In-vitro* Drug Release:** % Drug release for all formulations (F_1 to F_{12}) was determined as mentioned in **Figures 1-3**. Out of all the formulations the F_1 to F_4 (Eudragit S100) formulation was found to be best formulation, as it released Nateglinide in sustained manner.

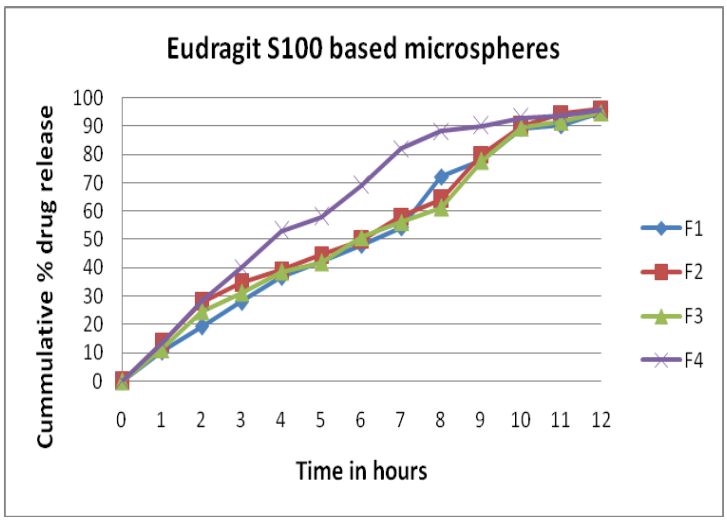


FIGURE 1: DRUG RELEASE PROFILE OF EUDRAGIT S100 BASED FORMULATION

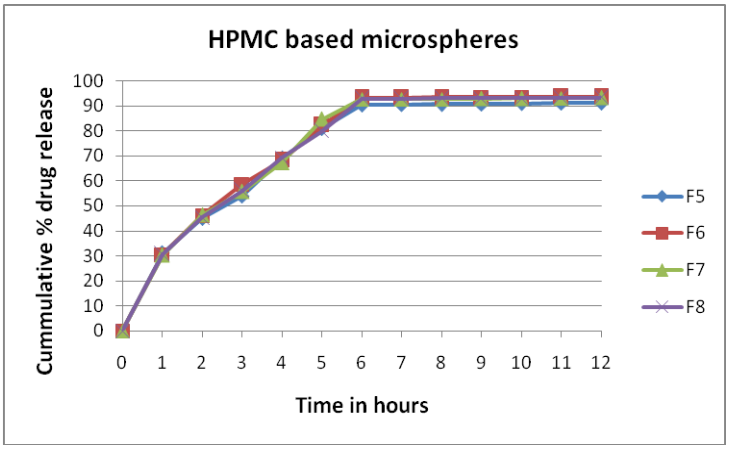


FIGURE 2: DRUG RELEASE PROFILE OF HPMC BASED FORMULATION

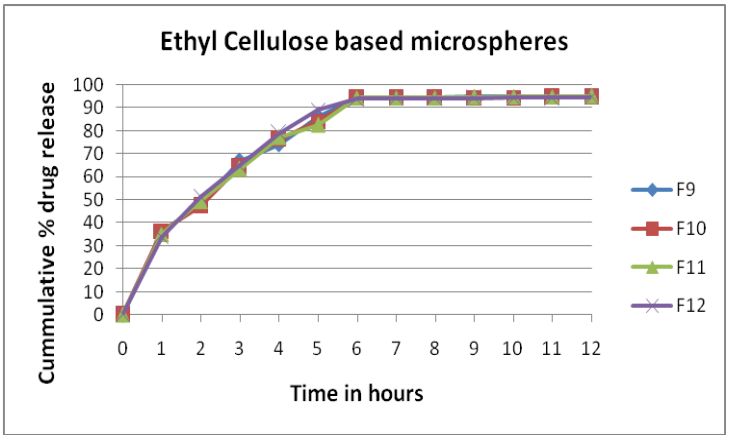


FIGURE 3: DRUG RELEASE PROFILE OF ETHYL CELLULOSE BASED FORMULATION

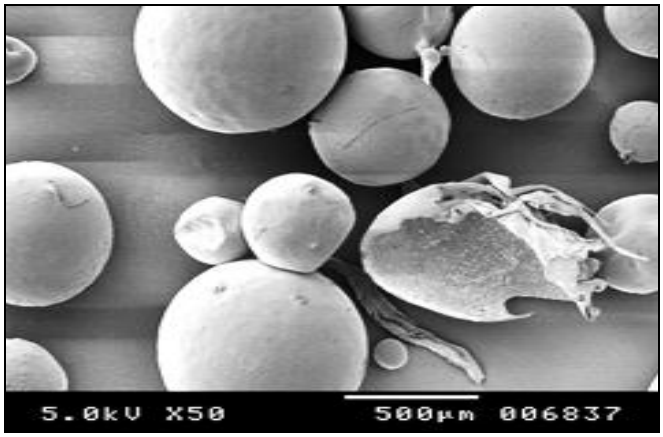
Micromeritic Properties: The average particle size of the microsphere formulations ($F_1$to F_{12}) was found to be in range of 142(±0.69) to 310 (±0.54) (as shown in **Table 3**).

The Bulk Density, Tapped Density and Hausner's ratio of formulation ($F_2M_1$to F_7M_{12}) was in range of 0.108 (±0.62) to 0.172(±0.67), 0.123(±0.47) to 0.192(±0.34) and 1.06 to 1.21 respectively. The Carr's index was in range of 6.32 to 17.73% and Angle of Repose was between 10.45(±1.32) to 25.45(±1.23) (as shown in **Table 3**).

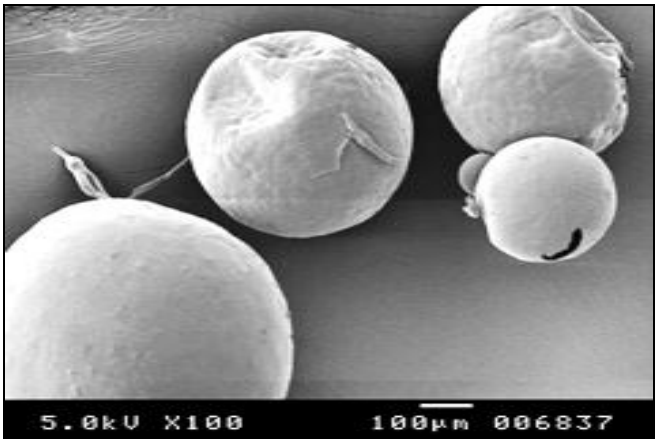
Scanning Electron Microscopy: To examine morphology of floating microspheres Scanning electron microscopic studies was performed. As shown in **Figure 4A and 4B**; it was observed that Microspheres were observed as a hollow structure with the outer surface of microsphere was smooth, while the internal surface was porous. Some pores were seen at the surface of microsphere may be due to evaporation of dichloromethane entrapped within the matrix of microsphere after forming smooth and dense layer as presented in Figure 4A and 4B.

TABLE 3: MEAN PARTICLE SIZE, ANGLE OF REPOSE, BULK DENSITY, TAPPED DENSITY, HAUSNER'S RATIO AND CARR'S INDEX FOR ALL FORMULATIONS (F_1TO F_{12})

Batch Code	Mean Particle Size	Angle of Repose	Bulk Density	Tapped Density	Hausner's Ratio	Carr's Index
F_1	286(±0.26)	11.37(±0.65)	0.108(±0.62)	0.123(±0.47)	1.13	12.19
F_2	302(±0.34)	12.94(±0.14)	0.116(±0.41)	0.141(±0.19)	1.21	17.73
F_3	310(±0.54)	10.45(±1.32)	0.132(±0.29)	0.155(±0.22)	1.17	14.83
F_4	292 (±0.13)	16.19(±0.36)	0.118(±1.25)	0.129(±0.69)	1.09	8.52
F_5	142(±0.69)	25.45(±1.23)	0.138(±0.84)	0.159(±0.85)	1.15	13.20
F_6	156(±0.72)	15.33(±1.02)	0.143(±0.52)	0.161(±0.63)	1.12	11.18
F_7	145(±0.96)	20.39(±1.46)	0.165(±0.58)	0.181(±0.94)	1.09	8.83
F_8	168(±0.17)	19.54(±0.75)	0.127(±0.87)	0.143(±0.84)	1.12	11.18
F_9	172(±0.24)	16.80(±2.44)	0.156(±0.32)	0.169(±0.68)	1.08	7.69
F_{10}	192(±0.18)	21.41(±1.02)	0.148(±0.41)	0.158(±0.54)	1.06	6.32
F_{11}	181 (±0.51)	17.21(±0.34)	0.172(±0.67)	0.192(±0.34)	1.11	10.41
F_{12}	184(±0.96)	19.15(±0.13)	0.159(±0.83)	0.172(±0.43)	1.08	7.55



A



B

FIGURE 4A AND 4B: SEM PHOTOGRAPHS OF OPTIMIZED FORMULATION

Infrared Spectroscopic Study: FT-IR spectra of pure Nateglinide, Eudragit S100 and drug-loaded microsphere were obtained to verify the chemical interaction between drug and polymer. The therapeutic activity of drug may be changed, due to chemical interaction between drug and polymer. It is reported that the peaks for functional group of active ingredient were remain same in both spectra of the drug as well as the formulation. Hence, it indicates that there will be no interaction takes place between drug and polymer. In FT-IR spectra of Nateglinide loaded microsphere, it was found that there was no significant spectral shift, as shown in **Figure 5 and 6**.

Reproducibility and Stability study: To obtain the optimal solvent formation, composed of ethanol: DCM (1:1 v/v), Nateglinide: Eudragit S100 (1:2 w/w), the yield, drug entrapment, buoyancy property and % drug release behavior was determined for Nateglinide microsphere. Three batches of Nateglinide loaded microsphere using optimal formulation were prepared to study the reproducibility of formulation. It can be reported that prepared microsphere had good reproducibility as observed from **Table 4 and Figure 7**.

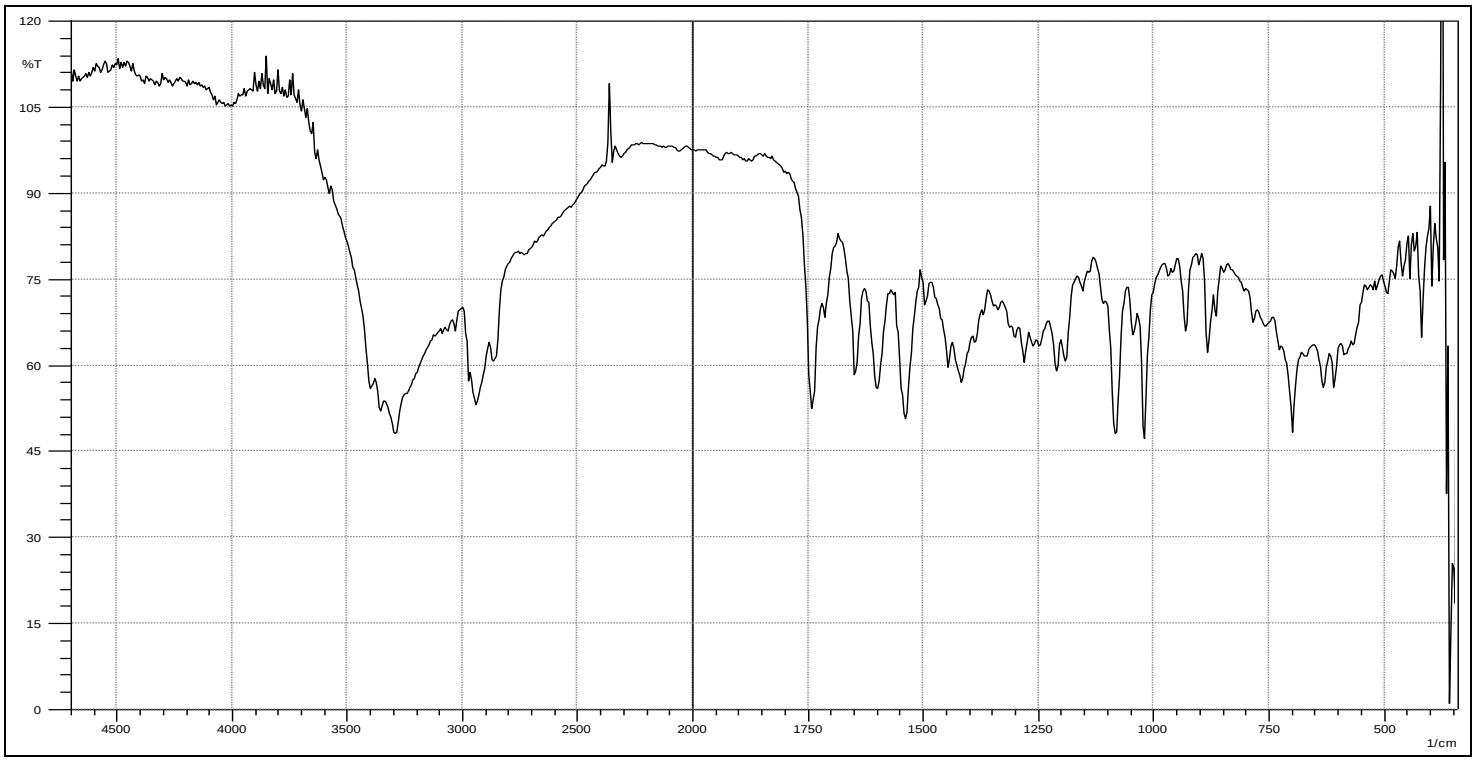


FIGURE 5: FTIR SPECTRA OF NATEGLINIDE

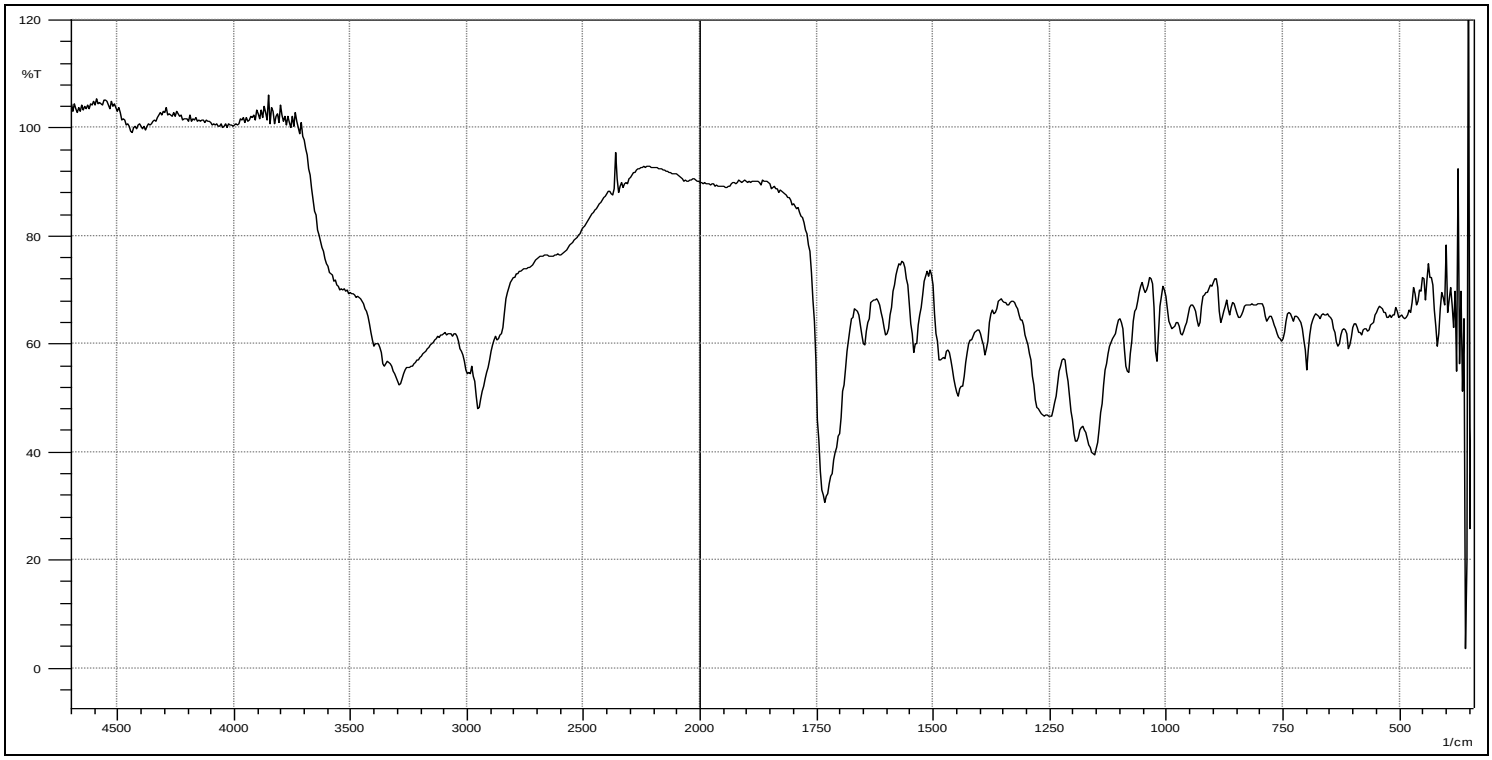


FIGURE 6. FTIR SPECTRA OF OPTIMIZED FORMULATION

TABLE 4. % YIELD, DRUG LOADING AND % BUOYANCY OF DRUG LOADED MICROSPHERES OF OPTIMIZED FORMULATION (MEAN±SD, n=3) [d]

Formulation Batches	Parameters		
	%Yield	% Drug Loading	% Buoyancy
Batch 1	91.00 (±0.72)	30.36(±0.34)	91.13(±0.61)
Batch 2	90.91(±0.46)	30.39 (±0.36)	95.78(±0.38)
Batch3	95.86(±0.30)	30.45(±0.32)	95.81(±1.37)
Average value	91.01(±0.69)	30.40(±0.32)	95.81(±0.68)

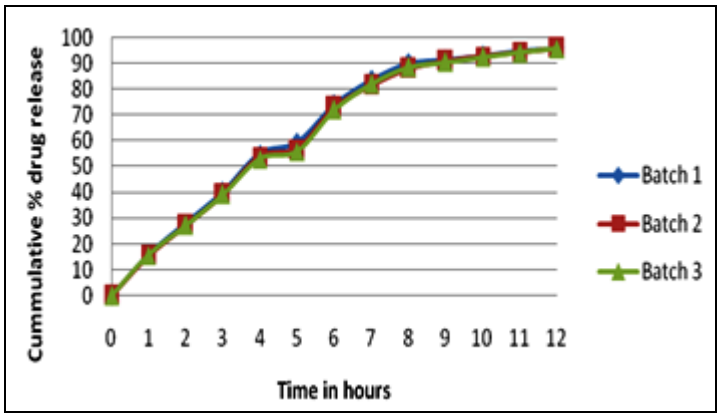


FIGURE 7: DRUG RELEASE PROFILE FOR OPTIMIZED BATCH

Stability of formulation is an important factor to determine the quality of the dosage forms. The stability of Nateglinide loaded microsphere (**Table 5 & Table 6**) were studied by performing both the accelerated testing and long term testing of optimized formulation.. The results showed no significant difference in physical properties (color, surface morphology and particle flow) and no marked change in floating ratio of test samples for microsphere. The drug loading was found in contrast with the Nateglinide loaded microsphere capsules before storage in stability chamber.

TABLE 5: THE RESULTS OF THE ACCELERATED STABILITY TESTING (MEAN±SD, n=3) [d]

Items	Time (months)				
	0	1	2	3	6
% Buoyancy	95.86(±0.30)	96.82(±0.72)	96.48(±1.41)	96.56(±0.69)	95.78(±1.39)
% Drug loading	30.36(±0.34)	30.47(±0.25)	30.51(±0.47)	30.45(±0.41)	30.48(±0.29)

TABLE 6: THE RESULTS OF THE LONG TERM STABILITY TESTING (MEAN±SD, n=3) [d]

Items	Time (months)				
	0	3	6	9	12
% Buoyancy	95.86(±0.30)	95.72(±0.68)	95.79 (±1.40)	96.79(±0.72)	95.85(±0.33)
% Drug Loading	30.36(±0.34)	30.46(±0.25)	30.52(±0.47)	30.47(±0.41)	30.48(±0.22)

CONCLUSION: Floating microsphere of Nateglinide were prepared by novel o/w emulsion solvent diffusion technique, using various biodegradable polymers such as Eudragit S100, HPMC and Ethyl Cellulose in order retain drug in body for longer period of time to increased bioavailability. Eudragit S100 based Microspheres show there buoyancy for more than 16 hours, required for sustained therapeutic activity, in comparison to HPMC and Ethyl Cellulose based microsphere due to their more hollow structure and porous nature. F_4 formulation showed good result among all other formulations. The property of polymer and its quantity in the formulation played crucial role on particle size of microspheres, their floating time and Release profile of drug molecule. From the present work it was concluded that nateglinide microspheres based on Eudragit S100 offer a most suitable floating dosage form to improve bioavailability of Nateglinide.

REFERENCES:

1. Woo BH, Jiang G, Jo YW, DeLuca PP: Preparation and characterization of a composite PLGA and poly (acryloyl hydroxymethyl starch) microsphere system for protein delivery. Pharm Res 2001;18:1600-1606.
2. Capan Y, Jiang G, Giovagnoli S, DeLuca PP: Preparation and characterization of poly (D, L-Lactide-co-Glycolide) microsphere for controlled release of human growth hormone. AAPS Pharm Sci Tech 2003;4:E28.
3. Gohel MC, Amin AF: Formulation and optimization of controlled release diclofenac sodium microspheres using factorial design. J Control Release 1998;51:115-122.
4. Soppimath KS, Kulkarni AR, Aminabhavi TM:Development of hollow microspheres as floating controlled-release systems for cardiovascular drugs: preparation and release characteristics. Drug Dev Ind Pharm 2001; 27: 507.
5. Singh BM, Kim KH. Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. J Control Release 2000; 63: 235– 259.
6. Akiyama Y, Nagahara N: Novel formulation approaches to oral mucoadhesive drug delivery systems, in: E. Mathiowitz, D.E. Chickering III, C.M. Lehr (Eds.), Bioadhesive Drug Delivery Systems: Fundamentals, Novel Approaches and Development, Marcel Dekker, New York 1999; 477–505.
7. Curatolo WJ, Lo J: Gastric retention system for controlled drug release. US Patent 1995; 5:443,843.
8. Stithit, S, Chen W, Price JC: Development and characterization of buoyant theophylline microspheres with near zero order release kinetics. J Microencapsul 1998; 15, 725-737.
9. Mitra SB: Sustained-release oral medicinal delivery device. US patent 1984; 4451260 29.
10. Kawashima Y, Niwa T, Takeuchi H, Hino T, Ito Y: Hollow microspheres for use as a floating controlled drug delivery system in the stomach. J Pharm Sci 1992; 81: 135-40.
11. Sato Y, Kawashima Y, Takeuchi H, Yamamoto H: *In vitro* and *in vivo* evaluation of riboflavin-containing microballoons for a floating controlled drug delivery system in healthy humans. Int J Pharm, 2004;275: 97-107.
12. Jayanthi G, Jayaswal SB, Srivastava AK: Formulation and evaluation of terfenadine microballoons for oral controlled release. Pharmazie, 1995; 50: 769-70.
13. Ruiz JM, Tissior B, Benoit JP: Microencapsulation of peptide: a study of the phase separation of poly (D, L-lactic acid-co-glycolic acid) copolymer 50/50 by silicon oil. Int J Pharm 1989; 49:69-77.
14. Lewis DH.: Biodegradable polymer as drug delivery system. In Chasin M, Langer R.(Eds). Drugs and pharmaceutical sciences, Marcel Dekker, New York 1990; 45:1-42.
15. Ichikawa M, Watanabe S, Miyake Y: A new multiple– unit oral floating dosage system, I: preparation and in vitro evaluation of floating and sustained–release characteristics. J Pharm Sci, 1991; 80: 1062-6.
16. Hardman JG, Limbird LE.(Eds): Goodman &. Gilman's The Pharmacological Basis of Therapeutics. 9th edition, New York: McGraw-Hill Companies 1996; 1487–1517.
17. Abd El-Hameed MD, Kellaway IW: Preparation and In Vitro Characterization of Mucoadhesive Polymeric Microspheres as Intra-Nasal Delivery System. Eur J Pharm Biopharm, 1997; 44: 53-60.
18. Yasunori S, Yoshiaki K, Hirofumi T, Hiromitsu Y.: In vitro evaluation of floating and drug releasing behaviors of hollow microspheres (microballoons) prepared by the emulsion solvent diffusion method. European Journal of Pharmaceutics and Biopharmaceutics, 2004; 57:235–243.
19. Lee JH, Park TG, Choi HK.: Development of oral drug delivery system using floating microspheres. J Microencapsul, 1999; 16, 715-729.

How to cite this article:
Singh S, Mann R and Sharma SK: Phytochemical Analysis and Pharmacognostical Standardization of stem of *Cayratia trifolia* (Linn.) Domin. *Int J Pharm Sci Res*. 3(11); 4306-4313.