ASIAN JOURNAL OF BIOMEDICAL & PHARMACEUTICAL SCIENCES

e-ISSN: 2249-622X

RESEARCH ARTICLE

Design and *In-vitro* Evaluation of Ethyl Cellulose Based Floating Microspheres Containing Antidiabetic Drug

Tekade B. W., Jadhao U. T., Thakare V. M., Yogita A. Chaudhari., Vaishali D. Patil, Chaudhari C. S.

Department of Pharmaceutics, TVES's Honorable Loksevak Madhukarrao Chaudhari College of Pharmacy, Faizpur, (India)

Received:
10th Aug 2013
Received in revised form:
20th Aug 2013
Accepted:
30th Aug 2013
Available online:
15th Sept 2013

OPEN ACCESS

Online ISSN 2249–622X
http://www.jbiopharm.com

ABSTRACT

The present investigation was concerned with formulation and evaluation of floating microspheres for Nateglinide using Ethyl cellulose as a release retarded material by solvent evaporation technique. seven different batches of microspheres were prepared by varying the concentration of ethyl cellulose from 0.25% to1.75% The microspheres were characterized for drug content, percentage yield, particle size analysis and surface morphology.The results of all the physiochemical tests of all formulations were found to be favourable. *In-vitro* floatability studies reveled that most of the microspheres (44 to 95.45%) were floatable. The in-vitro % drug release was found to be in range of 89.18 to 98.78 %. at the end of 12 hrs. optimized formulation F4 was evaluated for FTIR, XRD and SEM. XRD and FTIR studies showed that the nature of pure drug Nateglinide remains unaffected till the completion of process of microspheres formation. SEM photographs showed that the Floating microspheres were spherical in nature with smooth surface and uniform distribution of the drug within the microsphere.

Keywords: Nateglinide, Floating microspheres, Solvent evaporation technique, Ethyl cellulose.

1. INTRODUCTION:

Floating microspheres are gastro-retentive drug delivery systems based on non-effervescent approach. Hollow microspheres are in strict sense, spherical empty particles without core[1]. These microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, ideally having a size less than 200 μm. Solid biodegradable microspheres incorporating a drug dispersed or dissolved throughout particle matrix have the potential for controlled release of drugs[2].

Gastro-retentive floating microspheres are low-density systems that have sufficient buoyancy to float over gastric contents and remain in stomach for prolonged period. As the system floats over gastric contents, the drug is released slowly at desired rate resulting in increased gastric retention with reduced fluctuations in plasma drug concentration .When microspheres come in contact with gastric fluid, the gel formers like polysaccharides and polymers hydrate to form a colloidal gel barrier that controls the rate of fluid penetration into the device and consequent drug release. As the exterior surface of the dosage form dissolves, the gel layer is maintained by the hydration of the adjacent hydrocolloid layer. The air trapped by the swollen polymer lowers the density and confers buoyancy to the microspheres. However, a minimal gastric content needed to allow proper achievement of buoyancy[3,4].

In the present study Nateglinide was selected as model drug as it is the prototype antidibetic agent used to treat type 2 diabetes mellitus having short half life (1.5 hours) and low bioavailability (20-30%) in the upper part of GIT hence, it is suitable for gastro-retentive system. Ethyl cellulose was used to achieve the controlled delivery of drug from polymer matrix and emulsion solvent diffusion technique is selected for formulation.

2. MATERIALS AND METHODS

Nateglinide was obtained as kind gift sample from Cadila Healthcare Ltd, Ahmadabad, ethyl cellulose (EC) and Tween 20 were obtained from Sigma (India). All other

*Corresponding author: Tekade B.W. | Department of Pharmaceutics, TVES's Honorable Loksevak Madhukarrao Chaudhari College of Pharmacy, Faizpur, (India) | Email: bharattekade@yahoo.co.in

chemicals/reagents used were of analytical grade, available commercially and used as such without further processing.

Method of Preparation:

Microsphere containing nateglinide as a core material was prepared by O/W emulsion solvent diffusion method. The polymer ethylcellulose dissolve in the mixture of acetonitrile and dichloromethane (1:1). The core material nateglinide was dispersed in polymer solution with constant stirring to get uniform mixture. This mixture poured dropwise into stirring 100 ml, 0.25 % w/v aqueous solution of PVA. The emulsion was stirred at 1500 rpm for 1 Hr. Then the prepared microsphere was collected by filtration and wash with N-hexane, microsphere dried at room temperature and store in desiccators[5,6].

Batches	Drug: Polymer Ratio	Internal Organic Phase
F1	1:0.25	Acetonitrile:Dichloromethane
F2	1:0.5	
F3	1:0.75	
F4	1:1	
F5	1:1.25	
F6	1:1.50	
F7	1:1.75	

Table 1: Formulation of floating Microspheres.

EVALUATION OF MICROSPHERE:

Micromeritic Properties of Floating Microspheres

Angle of repose:

Angle of repose is defined as the maximum angle possible between the surface of pile of powder and horizontal plane. The angle of repose for the granules of each formulation was determined by the funnel method. The microspheres were allowed to flow out of the funnel orifice on a plane paper kept on the horizontal surface. It forms a pile of microsphere on the paper. The angle of repose was calculated by substituting the values of the base radius 'R' and pile height 'H' in the following equation[7].

$$\tan\theta = H/R$$

Where, H = Pile Height. R = Radius of Pile.

Bulk Density:

Bulk density of all batches of microsphere was determined by pouring gently 0.5 g of sample through a glass funnel into a 10 ml graduated cylinder. The volume occupied by the sample was recorded. Bulk density was calculated as per given formula.

$$\text{Bulk density} = \frac{\text{Weight of sample}}{\text{Volume occupied by the sample}}$$

Tapped Density:

The tapped density was determined by pouring 0.5 g of microsphere through a glass funnel into a 10 ml graduated cylinder. The cylinder was tapped from height of 2 inches until a constant volume was obtained. Volume occupied by the sample after tapping was recorded .The values for tapped density was calculated as per given formula[7].

$$\text{Tapped density (g/ml)} = \frac{\text{Weight of sample}}{\text{Volume occupied by the sample}}$$

Compressibility index:

The compressibility indices of the formulation blends were determined using Carr's compressibility index formula.

$$\text{Carr's Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Determination of particle size:

The particle size was determined using stage micrometer. The diameters of about 300 microspheres were measured and the average particle size determined[8].

Percentage yield:

The percentage yield of different formulations was determined by weighing the floating microspheres after drying[9]. The percentage yield was calculated as follows.

$$\% \text{ Yield} = \frac{\text{Total weight of floating microspheres}}{\text{Total weight of drug and polymer}} \times 100$$

Drug entrapment:

The various batches of the floating microspheres were subjected to estimation of drug content[10,11]. The floating microspheres equivalent to 50 mg of nateglinide from all batches were accurately weighed and crushed. The powdered of microspheres were dissolved in ethanol (10 ml) in volumetric flask (100ml) and made the volume with 0.1 N HCl. This solution is then filtered through Whatmann filter paper No. 44. After filtration, from this solution accurate quantity (10 ml) was taken and diluted up to 100 ml with 0.1 N HCl. From this solution, accurate volume (2 ml) was pipette out and diluted up to 10 m1 with 0.1 N HCI and the absorbance was measured at 254 nm against 0.1 N HCI as a blank. The percentage drug entrapment was calculated as follows.

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

Floating ability of microspheres:

Floating microspheres (50 mg) were placed in 0.1 N HCI (100 ml) containing 0.02% Tween 20. The mixture was stirred at 100 rpm in a magnetic stirrer. The layer of buoyant microspheres was pipette and separated by filtration at 1, 2, 4 and 8 hours. The collected microspheres were dried in a desiccator over night. The

percentages of microspheres were calculated by the following equation.

$$\% \text{ floating microsphere} = \frac{\text{Weight of floating microspheres}}{\text{Initial weight of floating microspheres}} \times 100$$

***IN-VITRO* DISSOLUTION STUDY:**

Accurately weighed microsphere equivalent to 200 mg of Nateglinide were taken in muslin cloth and it was kept in baskets. Dissolution study was carried out in 0.1 N Hydrochloric acid (pH 1.2) at 100 rpm at temp 37 ºC ± 0.5^0C. During dissolution study 10 ml aliquot was withdrawn at a time intervals of 1 to 12 hrs and same was replaced with equal volume of fresh medium. The withdrawn samples were filtered through Whatmann filter paper and absorbance's were measured at 237 nm. Drug concentration in the samples was determined from the standard calibration curve. Cumulative percent of drug dissolved was found out at each time point[12].

Characterization of Microspheres:

Surface Morphology:

This study was performed by Scanning Electron Microscopy (SEM) using JSM 6380 A(JOEL, Japan).The microspheres were coated with Platinum by ion sputtering using Autofine coater JFC-1600 (JOEL, Japan) The microspheres were kept on the sample holder and the scanning electron micrographs were taken[13].

IR Analysis:

The spectral analysis was done using FT-IR (Schimadzu 8400 SCCE).The dry sample of Nateglinide, Ethyl cellulose, physical mixture, optimized formulation (F4) was mixed by triturating with dry potassium bromide (A.R. Grade) and placed in sample cell[14,15].

X-Ray Diffraction:

The X-Ray powder diffraction patterns were obtained by using Philips PW 1700 with Cu Ķ α (λ=1.54056A^O) radiation and a crystal monochrometer, voltage: 45 kV and current 20 mA. The diffraction patterns run at 5-10^0 / min in terms of 2θ angle. The graph was plotted in 2 theta angle Vs intensity count[16,17].

3. RESULT AND DISCUSSION

All the formulations show angle of repose value in the range of 25.74± 0.732 -22.78±0.534 . These values for angle of repose (< 30) indicated good flow properties. The values for bulk density were found to range from 0.354±0.035 to 0.475±0.073. The values for tapped density were found to range from 0.395±0.064 to 0.548±0.064. Compressibility index values were found in the range of 10.37±0.075to 13.32±0.024 respectively. These values for compressibility index (5-15) indicated excellent flow properties of Microsphere. Hausner Ratio was ranging from 1.15±0.014 to 1.15±0.0675, i.e., all the preparation showed that microspheres had good flow Properties. The average particle size of Microsphere was found to be within 112.546± 1.42 to 171.342± 1.36 µm.

Batches	Angle of Repose (θ)	Bulk Density(g/ml)	Tapped Density(g/ml)	Carr's Index (%)	Hausner's Ratio
F1	23.32±0.645	0.475±0.073	0.548±0.064	13.32±0.024	1.15±0.0675
F2	22.78±0.534	0.452±0.024	0.521±0.036	11.32±0.032	1.15±0.064
F3	23.95±0.633	0.358±0.024	0.403±0.014	11.16±0.075	1.12±0.023
F4	25.74±0.732	0.354±0.035	0.395±0.064	10.37±0.075	1.15±0.046
F5	24.68±0.59	0.471±0.025	0.712±0.012	13.30±0.18	1.52±0.019
F6	21.32±0.80	0.574±0.022	0.658±0.051	18.31±0.98	1.15±0.014
F7	24.69±0.55	0.552±0.016	0.634±0.026	13.22±0.23	1.10±0.003

Table 2:Physicochemical properties of floating Microspheres.

The drug % encapsulation efficiency of ethyl cellulose Microsphere is shown in Table No.3. The drug: polymer ratio showed significant effect on the encapsulation efficiency of Microsphere. The increase in concentration of drug showed the increase in drug encapsulation efficiency. The Microsphere formulated using acetonitrile and dichloromethane as internal organic phase or solvent showed better encapsulation efficiency than other Formulations. The % encapsulation efficiency is found to be in the range of 65.36 to 75.58 %. The percentage yield of formulations varies with drug: polymer ratio. The percentage yield of different formulation was in range of 73.5% - 88.5% .The drug entrapment efficiency of different formulation was in the range of 65 % - 75 % w/w.

Batches	Drug : Polymer	Theoretical loading (%)	Actual Drug Loading (%)	Encapsulation Efficiency (%)	Yield (%)
F1	1:0.25	35	25.21 ± 0.905	65.36 ± 1.81	73.95
F2	1:0.5	40.12	27.02 ± 0.455	70.55 ± 1.13	79.2
F3	1:0.75	46.53	30.81 ± 0.355	69.43 ± 1.06	83.96
F4	1:1	50	35.29 ± 0.35	75.58 ± 0.7	88.2
F5	1:1.25	45	28.30±0.33	72±0.77	84.3
F6	1:1.50	44	32.30±0.45	73±1.3	85.6
F7	1:1.75	48	34.73±0.72	74±1.07	87.1

Table 3: Data for Percentage yield, percentage loading and encapsulation efficiency of Nateglinide Microsphere.

The floating test was carried out to investigate the floating ability of the prepared microsphere. Floating Microsphere were dispersed in 0.1 N HCl containing Tween 20 (0.02% w/v). Tween 20 was added to counteract the downward

pulling at the liquid surface by lowering surface tension. Floating ability of different formulations was found to be differed according to polymer ratio. F4 formulations showed best floating ability (94.25 – 49.66 %) in 8 hours.Given in table no.4

Batches	Time (Hours)			
	1 hr.	2 hrs.	4 hrs.	8 hrs.
F1	95.62	91	80	62
F2	94.33	89	74	56.33
F3	90.66	81.03	64	44
F4	94.25	86	68.27	49.66
F5	92.6	87.2	67.52	48.14
F6	91.35	85.14	67	45.2
F7	93.8	88	68.34	49.65

Table 4: The percentage floating ability of different batches of floating microspheres.

Fig. No.1, 2 shows IR spectra for Nateglinide, and formulation F4. Major functional groups of Nateglinide (di-substituted aromatic ring, ketone bond.) can be seen in spectra of individual drugs as well as in spectra of formulation. So there is no interaction between Nateglinide and ethyl cellulose.

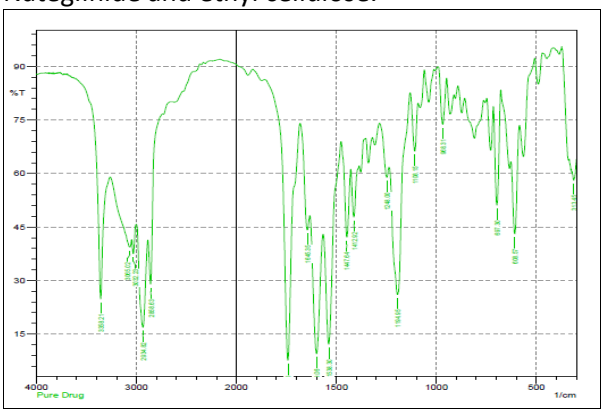

Fig. No. 1: IR spectrum of Nateglinide.

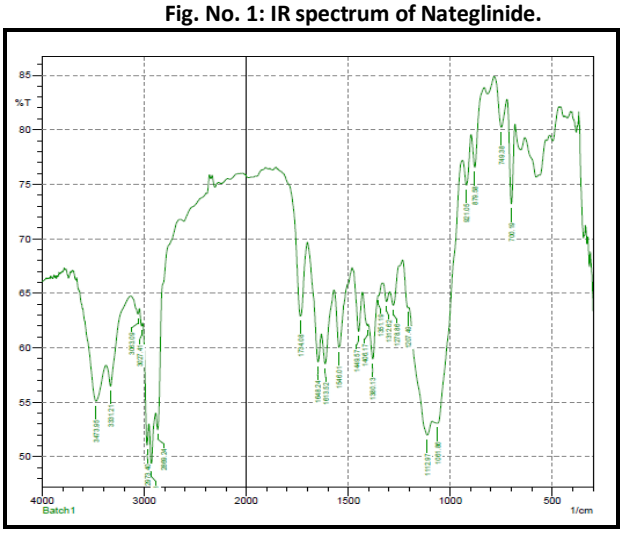

Fig. No. 2: IR spectrum of Nateglinide & physical mixture.

Fig.No:3 show the SEM photographs of prepared formulations. SEM photographs showed that the Microsphere were spherical in nature and had a smooth surface. SEM photographs revealed the absence of crystals of drug on the surface of Microsphere and uniform distribution of the drug within the Microsphere.

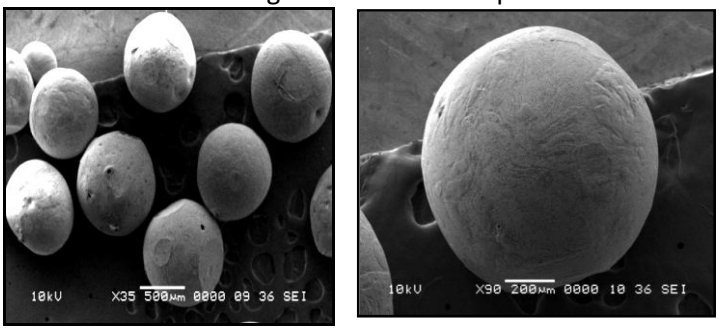

Figure 3: SEM Photographs of Microsphere

The XRD scan of plain Nateglinide showed intense peaks of crystallinity. Diffractogram of Nateglinide showed high intensity peaks between 2θ of 20-30^0 values demonstrating the crystalline nature of drug. No intense peaks were observed in diffractogram of ethyl cellulose which indicates amorphous nature. The XRD pattern of formulation exhibited halo pattern with less intense and denser peaks compared to plain Nateglinide. This indicates that Nateglinide is dispersed at the molecular level in the blend polymeric matrix. Given in Fig.No.4, 5.

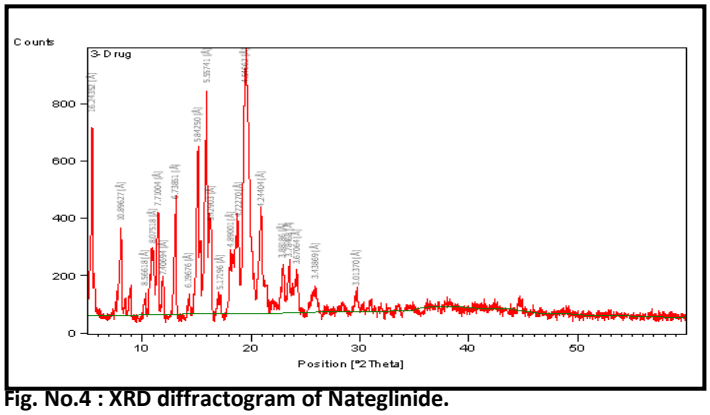

Fig. No.4 : XRD diffractogram of Nateglinide.

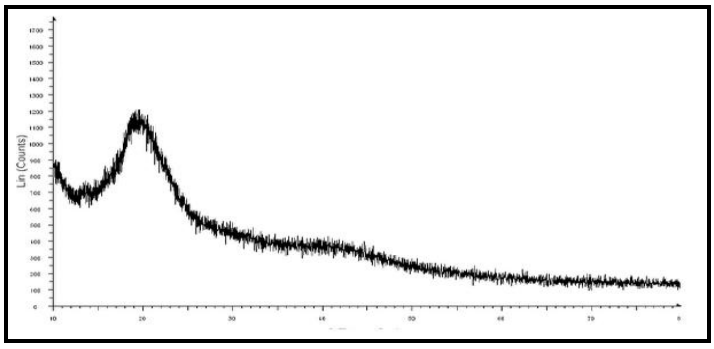

Figure 5: XRD diffractogram of formulation F4

The drug release study was carried out up to 12 hrs. The percentage drug release from batch F1 to F7 vary from 89.18 to 98.78 %. Large concentration of high viscosity polymer induces the formation of strong viscous gel layer that slowed down the rate of water diffusion into the tablet matrix, which may result in the retardation or decreases the drug release. Dissolution profiles for all batches were shown in (Fig. 6).

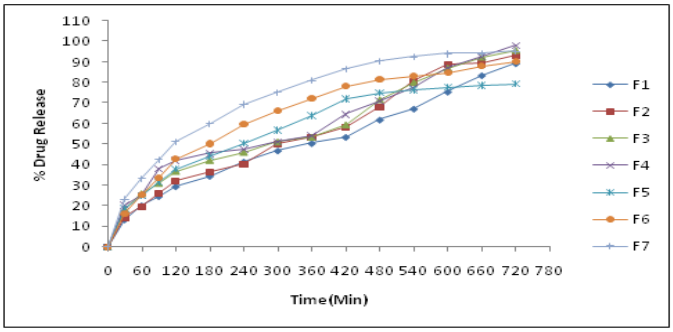

Fig.No-6- % Drug release of microspheres Nateglinide.

5. CONCLUSION

The Nateglinide floating microspheres were successfully prepared by solvent evaporation method for prolonged as well as controlled action of drug. Due to their low density, these multi particulate drug delivery systems showed good floating ability (> 12 h). From *in-vitro* drug release studies, it may be concluded that by changing the ratio of polymers Nateglinide release can be controlled. This system could be useful for narrow absorption window and/or gastric site specific delivery. Therefore, it may be concluded that drug loaded floating microspheres are suitable delivery system for Nateglinide.

7. REFERENCES

1. Bankar, G.S., Rhodes, C.T., Modern Pharmaceutics, 3rd edn. vol. 40. Marcel Dekker, Inc. New York, 1996. pp. 668-669.

2. Baumgartner, S., Kristel, J.,Vreer, F., Vodopivec, P., Zorko, B.,(2000). Optimisation of floating matrix tablets and evaluation of their gastric residence time. Int. J. Pharm. 195, 125-135.

3. Choi, B.Y., Park, H.J., Hwang, S. J.,(2002). Preparation of alginate beads for floating drug delivery system effects of CO_2 gas forming agents. Int. J. Pharm. 239, 81-91.

4. Indian Pharmacopoeia. 2007. vol. 1. The Indian Pharmacopoeia Commission, Ghaziabad, pp. 107.

5. Samal, H., Dey, S., Kumar D., Sreenewas, S., Rahul, V., 2011. Formulation ,characterization and in vitro evaluation of floating microsphers of nateglinide. Int. J. Pharma. Bio Sci. 147-155

6. Yoo, H. S., 2006. Preparation of biodegradable polymeric hollow microspheres using O/O/W emulsion stabilized by Labrafil. Colloids and Surfaces B: Biointerfaces. 52, 47-51.

7. Lachman, L., Libermann, H. and Kanig, J., 1991. The Theory and Practice of Industrial Pharmacy, 3rd edn. Varghese Publication, Bombay, pp. 183-84.

8. Khandelwal, K.R., 2004. Practical Pharmacognosy Techniques and Experiments. 11th edn. Nirali Prakashan, Pune, pp. 42.

9. Mishara, S. K., Pathak, K., 2008. Formulation and evaluation of oil entrapped gastroretentive floating gel beads of loratadine. Acta Pharm. 58, 187-197.

10. Park, K., Yeo, Y., 2007. Microencapsulation technology. In: Swarbrick, J., Boylon, J. C. (Eds.), Encyclopedia of Pharmaceutical Technology. 3rd edn. vol. 4. Marcel Dekker Inc. New York, pp. 2315-2327.

11. Schimdt, P., 2007. Secondary electron microscopy in pharmaceutical technology. In: Swarbrick J., Boylon J.C. (Eds.), Encyclopedia of Pharmaceutical Technology. 3rd Edn. vol. 5. Marcel Dekker Inc. New York, pp. 3217- 325.

12. Dyas, A., Shah, U., 2007. Dissolution and dissolution testing. In: Swarbrick J., Boylon J.C. (Eds.), Encyclopedia of Pharmaceutical Technology. 3rd Edn. vol. 2. Marcel Dekker Inc. New York, pp.908- 928.

13. Mali S, Nighute A, Deshmukh V, Gonjari I, Bhise S, Microcrystals: For Improvement Of Solubility And Dissolution Rate Of Lamotrigine, **Int.J.Ph.Sci**., May-Aug 2010, 2(2), 515-521.

14. Duerst, M., 2007. Spectroscopic methods of analysis: infrared spectroscopy. In: Swarbrick J., Boylon J.C. (Eds.), Encyclopedia of Pharmaceutical Technology. 3rdEdn. vol. 5. Marcel Dekker Inc. New York, pp. 3405- 3418

15. Skoog, D.A., Holler, F.J., Nieman, T.A., 2004. Principles of Instrumental Analysis. 5 th edn. Sounder's College Publishing, pp. 798-808.

16. Achutha, N.U., Mutalik, S., Reddy, M.S., 2008. Preparation and in-vitro, preclinical, clinical studies of aceclofenac spherical agglomerates. **Eur. J. Pharm. Biopharm.** 70. 674–683.

17. Radke R.S., S. B. Deshmukh, P. S. Jagtap, P. S. Gangane, D. R. Godwani, 2010. Design and Evaluation of Gastro-Retentive Floating Tablets of Captopril, International Journal of Pharma and Bio Sciences , Vol. 1 (2), 1-10.

Conflict of Interest: None Declared

Cite this article as:
Tekade B. W., Jadhao U. T., Thakare V. M., Yogita A. Chaudhari., Vaishali D. Patil, Chaudhari C. S. Design and *In-vitro* Evaluation of Ethyl Cellulose Based Floating Microspheres Containing Antidibetic Drug Asian Journal of Biomedical and Pharmaceutical Sciences, 2013, 3: (23), 33-37.

