FORMULATION AND EVALUATION OF FLOATING MICROSPHERES OF NATEGLINIDE

Noopur Pandey*, Dr. Archana Negi Sah, Kamal Mahara
*Research Scholar
Department of Pharmaceutical Sciences, Bhimtal Campus,
Kumaun University, Nainital Uttarakhand, India
E-mail: noopurpandey56@gmail.com
Tel: +91-8859442013

ABSTRACT - Gastroretentive dosage forms have potential for use as controlled- release drug delivery systems. Gastro retentive floating drug delivery systems have a bulk density lower than that of gastric fluids and thus increase residence time of drug in stomach and provide controlled delivery of many drugs. The aim of the present study is formulation and characterization of floating microspheres using nateglinide as a model drug for the management of type-2 diabetes mellitus. Floating microspheres were prepared by oil-in-water emulsion solvent evaporation technique using ethyl cellulose and eudragit S-100 as release retarding polymers. The floating microspheres were evaluated for percentage yield (%), particle size, drug content, drug entrapment efficiency, *in-vitro* floating ability and *in-vitro* drug release studies. The surface morphology of prepared microspheres was characterized by scanning electron microscopy. The microspheres were found to be spherical in shape and porous in nature. Compatibility studies were performed by fourier transform infrared (FTIR) technique. The prepared microspheres showed prolonged drug release of 12 h and remain buoyant for more than 12 h. *In-vitro* release kinetics were studied in different release kinetics models like zero order, first order, higuchi and korsmeyer peppas model and the best fit model was found to be higuchi plot with release exponent n value less than 0.89. It was concluded that developed floating microspheres of nateglinide offers a suitable and practical approach for prolonged release of drug over an extended period of time and thus oral bioavailability, efficacy and patient compliance is improved.

Keywords: Antidiabetic, Ethyl cellulose, Eudragit S-100, Gastro retentive drug delivery, Floating drug delivery system, Emulsion solvent evaporation method.

1. INTRODUCTION

Oral route is considered to be highly suitable route and frequently used for delivery of drug due to ease of administration, patient compliance and flexibility of formulation. The success of oral controlled delivery system depends on the fact that the drug can be better absorbed from GI tract. But the main problem with conventional delivery is to maintain the drug concentration within the therapeutic effective concentration level, which can be achieved only when taken several times a day. Although attempts have been made to develop controlled release delivery systems for oral route but various limitations like variable drug absorption, uncontrolled gastric transit time have established the need of more intelligent drug delivery systems, which can prolong the transit time of drug, or provide effective concentration locally. The gastro retentive drug delivery system can be retained in the stomach and help in improving the oral sustained delivery of drugs that have an absorption window in a particular region of the GI tract. Various methods have been designed to increase the gastric residence time (GRT) which include: floating drug dosage systems (FDDS), swelling or expanding systems, mucoadhesive systems and high-density systems. These system give advantage in improving bioavailability of drugs that have a narrow absorption window, reduces drug waste, and improves solubility for drugs that are less soluble in a high pH environment. It also has applications for local drug delivery to the stomach and proximal small intestines. [1,2]

Floating microspheres are gastroretentive drug delivery systems based on non-effervescent approach. They are spherical empty particles without core. These microspheres are characteristically free flowing powders consisting of protein or synthetic polymers with diameters 1 μm to 1000 μm. Hydro dynamically controlled drug delivery systems (Floating drug delivery system) are low density systems that have sufficient buoyancy to float over the gastric contents and remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. The sustained release of drug from floating systems improves the gastric retention of drugs and reduces the fluctuations in plasma drug concentration. Commonly used polymers to prepare floating microspheres include polycarbonate, HPMC, cellulose acetate, calcium alginate, Eudragit S, chitosan etc. Thus floating microspheres are considered as one of most promising buoyant systems. They possess the unique

advantages of multiple unit systems and in addition better floating properties. The general techniques involved in their preparation include emulsion solvent evaporation and emulsion solvent diffusion. The drug release and better floating properties mainly depend on the type of polymer, plasticizer and the solvent employed for the preparation. [3,4]

Diabetes is one of the major causes of death and disability in the world. Nateglinide is used to control hyperglycemia in type II diabetes. It is commercially available as conventional tablets.

Nateglinide belongs to the meglitinide category, used in the treatment of type-2 diabetes mellitus. It helps to lower blood glucose levels by blocking ATP- sensitive potassium channels in pancreatic beta cells, which stimulates insulin secretion. The drug has a rapid onset and short duration of action. It has been used alone or in combination with other medications to treat typr-2 diabetes. The half life of the drug is small i.e. 1.5 h. The absolute bioavailability of drug is 73%. The general dosage regimen is 60-120 mg three times a day. It requires frequent dosing to maintain therapeutic effect. Therefore, it would be beneficial to develop a drug delivery system which remains at the gastro intestinal tract for an extended period of time. [5,6,7]

Microspheres encapsulated with anti-diabetic drug, increase the effectiveness and release of drug in control manner from polymer membrane and thereby maintain its concentration for longer duration. Due to short acting action, fast clearance, enzymatic stability and absorption throughout GIT make nateglinide a suitable target for developing floating dosage form. The aim of the study was to increase the bioavailability and reduce the side effects of drug. Various polymers like ethyl cellulose and biodegradable acrylic polymers eudragit S-100 was used to achieve the controlled delivery of drug from polymer matrix and oil in water emulsion solvent evaporation technique was selected for formulation. The influence of various factors such as percentage yield, particle size, drug entrapment efficiency, floating properties and *in-vitro* release of the resulting microspheres were investigated. [3]

2. MATERIALS AND METHODS

2.1 MATERIALS

Nateglinide was purchased from Yarrow chem products, Mumbai. Ethylcellulose and Eudragit S-100 were purchased from CDH laboratory reagents, New Delhi. Analytical grade ethanol, dichloromethane, tween-80 were purchased from Molychem, India. All other chemicals used were of analytical grade.

2.2 METHODS

2.2.1 Preformulation studies

Preformulation studies are the first step in the rational development of dosage forms of a drug. It can be defined as the determination of physical, chemical and mechanical properties of a new drug substance alone and when combined with excipients. The overall objective of preformulation studies is to generate information useful in developing stable and bioavailable and sustained release dosage forms which can be mass produced.

2.2.2 Solubility studies

Solubility analysis was done to select a suitable solvent system to dissolve the drug and also to test its solubility in the dissolution medium which was to be used. Drug solubility study was performed by taking an excess quantity of drug in different solvents like water, ethanol, methanol, acetone and buffers. [8,9]

2.2.3 Melting point

Melting point determination of the drug sample was done by open capillary method using melting point apparatus. Drug was taken in glass capillary tube whose one end was sealed by means of flame. The capillary tube was placed in a melting point apparatus attached to a thermometer to measure the melting point. The sample holder was heated gradually and the temperature at which drug melts was recorded. Melting point of a drug sample is a first indication of purity of sample.

2.2.4 Preparation of standard calibration curve by using UV spectroscopy

Preparation of 0.1 N HCl: 8.5 ml of HCl was dissolved in distilled water and volume was made up to 1000 ml to make 0.1 N HCl.

Preparation of stock solution- 10 mg of drug was dissolved in 10 ml of ethanol and volume was made upto 100 ml with 0.1 N HCl in a clean and dry volumetric flask. Finally volume was made up to the mark. This gives a stock solution of 100 µg/ml. The aliquots of stock solution of nateglinide were subsequently diluted to obtain a series of dilutions containing 2, 4, 6, 8, 10, 12, 14 µg/ml of drug. The absorbance of these solutions was measured by using UV visible spectroscopy at 210 nm using stock solution as a blank. The absorbance was plotted against concentration. The method obeys Beer's law in the concentration range of 5-25 µg/ml.

2.2.5 FTIR Spectrophotometric analysis

A spectra of FTIR helps in identification of different functional groups of a molecule. It helps in identification of a compound based on the functional groups present in it. The FTIR spectrum of nateglinide was recorded on Perkin Elmer spectrophotometer using KBr pellet technique and reported in wave number (cm^{-1}). The scanning range was 450-4000 cm^{-1} and the resolution was 2 cm^{-1}. The FTIR spectra of drug with polymer were compared with standard FTIR spectrum of the pure drug. [10]

2.3 Preparation of microspheres

Oil-in-water emulsion solvent evaporation technique

Microspheres containing nateglinide as a core material were prepared by oil-in-water emulsion solvent evaporation technique. In this process, both the drug and the polymer should be insoluble in water while a water immiscible solvent is required for the polymer. The polymer was dissolved in an organic solvent such as dichloromethane and ethanol (1:1). The drug was dissolved into polymer solution and this solution containing the drug was emulsified into an aqueous phase containing tween 80, 0.2% v/v to make an oil-in water emulsion by using as emulsifying agent. After the formation of a stable emulsion, the organic solvent was evaporated either by increasing the temperature under pressure or by continuous stirring. Solvent removal from embryonic microspheres determines the size and morphology of the microspheres. Oil-in water emulsion is widely used due to simplicity of the process and easy cleans up requirement for the final product. [11,12]

Table 1: Various formulation of floating microspheres of nateglinide

Formulation code	Drug (mg)	Ethyl cellulose (mg)	Eudragit S-100 (mg)	Ethanol: Dichloromethane	Stirring speed (r/min)
F1	100	100	-	1:2	800
F2	100	200	-	1:2	800
F3	100	300	-	1:2	800
F4	100	400	-	1:2	800
F5	100	-	100	1:2	1000
F6	100	-	200	1:2	1000
F7	100	-	300	1:2	1000
F8	100	-	400	1:2	1000

Figure 1: Pictures of microspheres

2.4 Evaluation of prepared floating microspheres

2.4.1 Percentage yield (%)

Percentage yield of floating microspheres was calculated by dividing actual weight of product to total amount of all non-volatile components that are used in the preparation of floating microspheres and is represented by following formula.

$$\% \text{ yield} = (\text{weight of floating microspheres} / \text{ weight of drug and polymers}) \times 100 \quad \text{Eq. (1)}$$

2.4.2 Particle size analysis

Particle size and shape of the microspheres was determined by optical microscopy. The freshly prepared microspheres were examined on an optical microscope and the size of microspheres was measured by pre-calibrated ocular micrometer and stage micrometer. About 100 particles of each formulation were observed and measured.

2.4.3 Micromeritic properties

The prepared microspheres are characterized by their micrometric properties, such as microsphere size (mean particle size), Bulk density, Tapped density, Carr's compressibility index, Hausner's ratio and angle of repose.

a) Bulk and Tapped density

Bulk and tapped densities were measured by using 50 ml of graduated cylinder. Accurately weighed amount of 5g of sample passed through a glass funnel. The sample poured in cylinder was tapped mechanically for 3 times and 100 times for calculating bulk volume (V_b) and tapped volume (V_t) respectively. Then tapped volume was noted down and bulk density and tapped density were calculated. It was expressed in g/cm^3.

$$\text{Bulk density } (\rho_b) = \frac{\text{Mass of microspheres (M)}}{\text{Volume of microspheres after tapping } (V_b)} \quad \text{Eq. (2)}$$

$$\text{Tapped density } (\rho_t) = \frac{\text{Mass of microspheres (M)}}{\text{Volume of microspheres after tapping } (V_t)} \quad \text{Eq. (3)}$$

b) Carr's Compressibility Index

Compressibility index (C.I.) or Carr's index value of microspheres was calculated according to the following equation

$$\%\ \text{Compressibility index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \quad \text{Eq. (4)}$$

The value given below 15% indicates a powder with usually give rise to good flow characteristics, whereas above 25% indicate poor flow ability.

c) Hausner ratio

Hausner's ratio of microspheres was determined by comparing the tapped density to the bulk density using the equation.

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \times 100 \quad \text{Eq. (5)}$$

d) Angle of repose

The maximum angle which is formed between the surface of a pile of powder and horizontal surface is called the angle of repose.

$$\text{Tan } \theta = h/r \quad \text{Eq. (6)}$$

Where θ= angle of repose

h = height of the circle formed by the powder heap

r = radius of heap

2.4.4 Percentage floating

Floating microspheres of about (200 mg) was weighed and placed in simulated gastric fluid (pH 1.2, 100 ml) containing 0.02 w/v % Tween 80. The mixture was stirred at 100 r/min in a magnetic stirrer. After 12 h, the layer of buoyant microparticles was pipetted 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.

$$\%\ \text{Buoyancy of microspheres} = [W_f / (W_f + W_s)] \times 100 \quad \text{Eq. (7)}$$

Where W_f and W_S are the weight of floating and settled particles, respectively

2.4.5 Floating time

It is defined as the time taken by floating microspheres to remain buoyant in the medium. The floating microspheres were placed in the beaker containing 200 ml of 0.1N HCl and examined for the duration of time till they float.

2.4.6 Drug content

The floating microspheres equivalent to 50 mg of nateglinide were weighed accurately and crushed. The powdered microspheres were placed in 100 ml volumetric flask and the volume was made up using pH 6.8 phosphate buffer and kept for 24 h. The solution was then filtered through whatman filter paper No. 44. The solution was diluted with fresh solvent and absorbance was measured at 210 nm using UV spectrophotometer (Shimadzu-1601) and the percent drug content was calculated. [13]

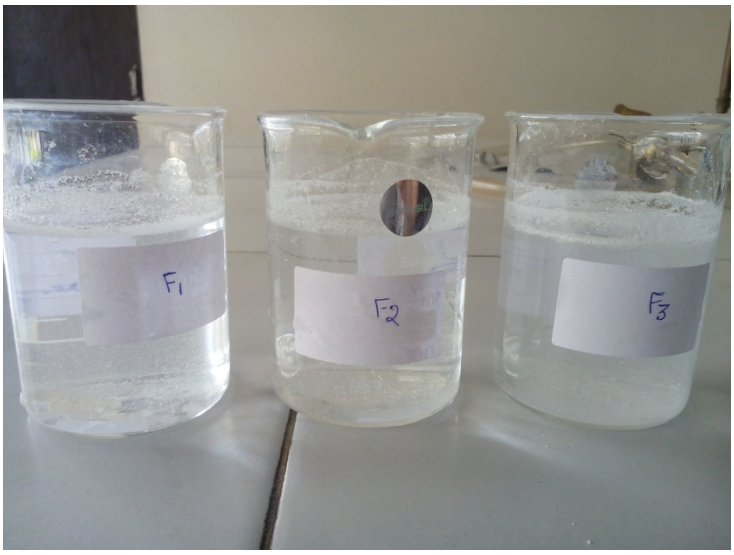


Figure 2: *In-vitro* evaluation of floating ability of formulations

2.4.7 Drug entrapment efficiency

The percent drug entrapped was calculated as follows

$$\% \text{ Entrapment efficiency } = \frac{\text{Calculated drug content}}{\text{Theoretical drug content}} \times 100 \qquad \text{Eq. (8)}$$

2.4.8 Surface morphology

The surface morphology was measured by using scanning electron microscope (SEM).

2.4.9 *In-vitro* drug release study

The *in-vitro* drug release studies of formulations were carried out in 0.1 N HCl (pH 1.2) for 2 h and in pH 6.8 buffer for 10 h. The drug release rate from floating microspheres was determined using paddle type eight-station dissolution test apparatus (Electrolab). A weighed amount of floating microspheres equivalent to 100 mg drug was kept in 0.1 N HCl (1.2 pH) maintained at 37± 0.5 ºC at a rotation speed of 100 r/min. Sink condition was maintained during the study. 5 ml sample was withdrawn at 60 min time interval, the initial volume of the dissolution fluid was maintained by adding 5 ml of fresh dissolution fluid after each withdrawal, passed through 5 μm membrane filter and analyzed spectrophotometrically at 210 nm. The same process was repeated using pH 6.8 as dissolution medium. [14]

2.4.10 Release kinetics study

The drug release kinetics was studied by various kinetic models such as Korsmeyer-peppas, Higuchi plot, First order plot and Zero order plot. To study the release kinetics, data obtained from *In-vitro* drug release studies were plotted in various kinetic models. Zero order as cumulative amount of drug released Vs time, First order as log cumulative percentage of drug remaining Vs time, and Higuchi's model as cumulative percentage of drug released Vs square root of time. The best fit model was confirmed by the value of correlation coefficient near to 1. The data was presented for the most appropriate model. If n value is 0.45 or less, the release mechanism follows "Fickian diffusion" and higher values of 0.45 to 0.89 for mass transfer follow a non-fickian model (anomalous transport). The drug release follows Higuchi model of drug release and case II transport if the n value is 0.89. For the values of n higher than 0.89, the mechanism of drug release is regarded as super case II transport. [15,16]

3. RESULTS AND DISCUSSION

3.1 Preformulation studies

Solubility analysis: Sample of Nateglinide was found to be soluble in ethanol, methanol, dichloromethane and chloroform and insoluble in water.

Melting point determination: The melting point of drug sample was found to be 137º C.

Preparation of calibration curve by using UV spectroscopy

Table 2: Absorbance value of nateglinide in 0.1 N HCl

Concentration (µg/ml)	Absorbance
0	0
2	0.068
4	0.1251
6	0.178
8	0.245
10	0.302

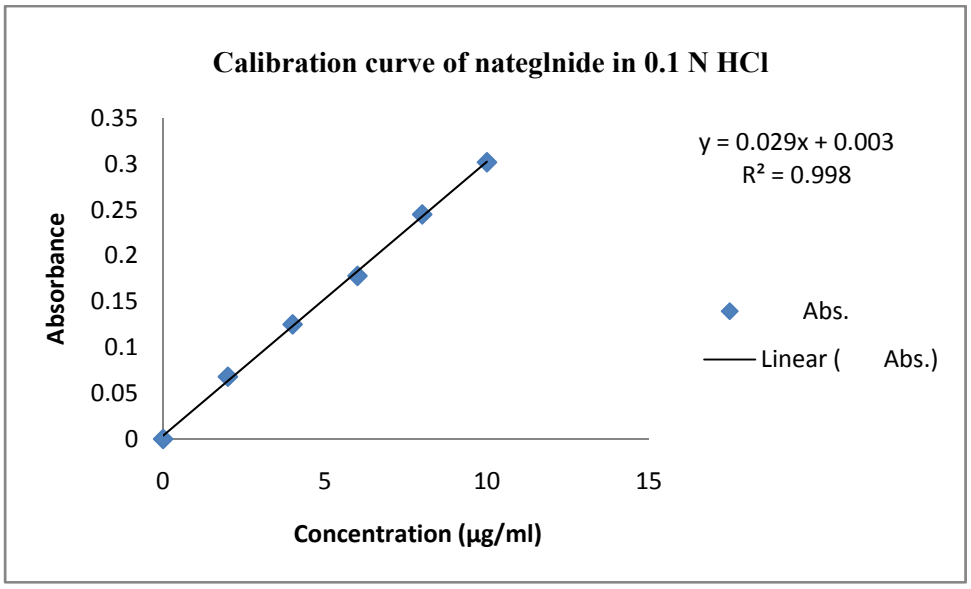


Figure 3: Calibration curve of nateglinide in 0.1 N HCl

FTIR Spectrophotometric analysis

Drug identification and compatibility study of drug and excipients was done by using fourier transform infrared spectrum (FTIR). Characterstic bonds of nateglinide (fig 4) were observed at 1647 cm^{-1} (-C=O), 1715 cm^{-1} (-COOH), 2859-3064 cm^{-1} (-CH_2- cycloalkane) and 3308 cm^{-1} (-NH). This FTIR of Nateglinide shows its correct identification as it matches with reference values.

Table 3: Characterstic peaks of Nateglinide

S.No.	Reference peak (cm^{-1})	Obtained peaks (cm^{-1})	Functional group	Stretching/Bending
1	1680-1630	1647	-C=O (carbonyl)	Stretching
2	1725-1700	1715	-C=O (COOH)	Stretching
3	1320-1210	1287	-C-O (COOH)	Stretching
4	2859-3064	2859-3064	-CH_2 - (cycloalkane)	Stretching
5	3296-3310	3308	-NH	Stretching

However, FTIR spctrum of Nateglinide with Ethyl cellulose (fig. 5) showed the respective characterstics bonds of nateglinide at 1647 cm^{-1} (-C=O), 1717 cm^{-1}(-COOH), 2858-3072 cm^{-1} (-CH_2- cycloalkane) and 3304 cm^{-1} (-NH). The results confirmed that there was no chemical interaction between Nateglinide and Ethyl cellulose.

Similarly FTIR spectrum of nateglinide with Eudragit S-100 (fig. 6) showed the characteristic peaks of nateglinide at 1645 cm^{-1}, 1715 cm^{-1}, 2860-3063 cm^{-1} and 3308 cm^{-1}, so indicating no interaction between nateglinide and Eudragit S-100.

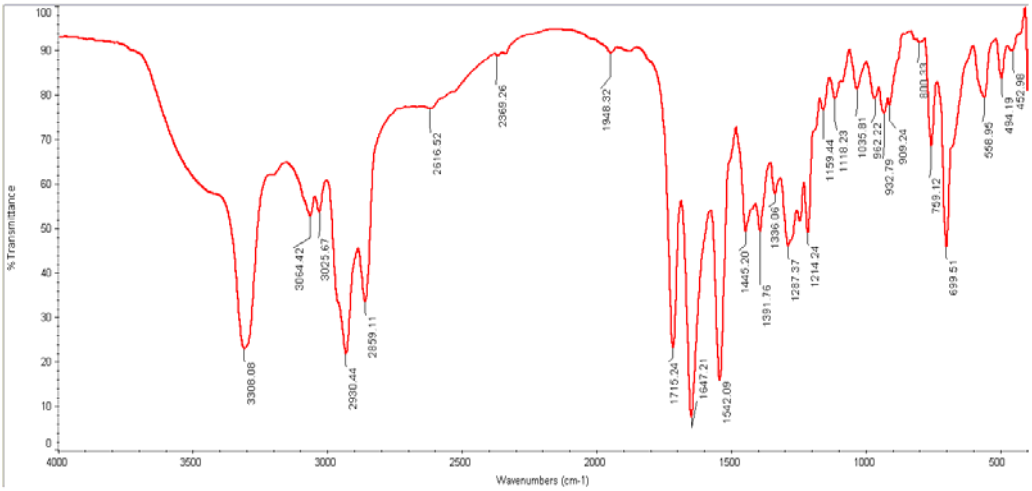


Figure 4: FTIR spectrum of Nateglinide

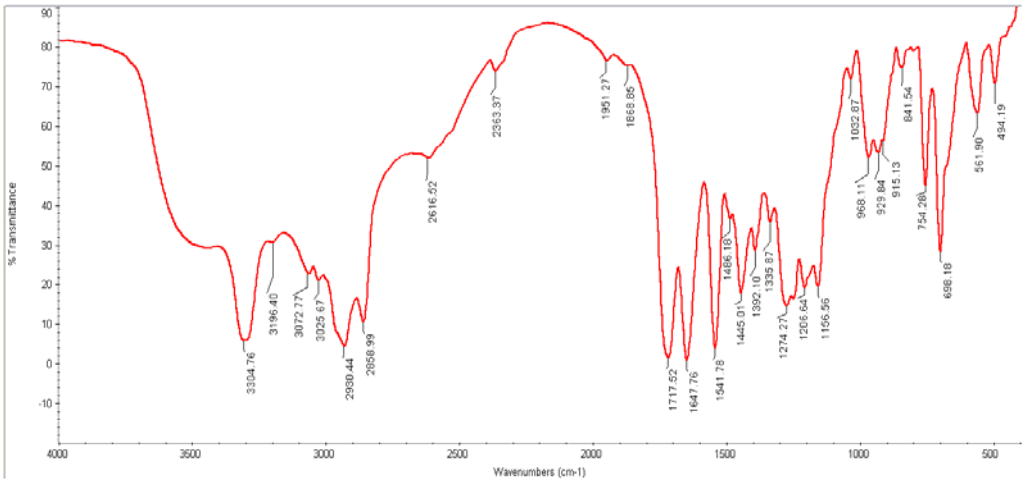


Figure 5: FTIR spectrum of Drug and Ethyl cellulose

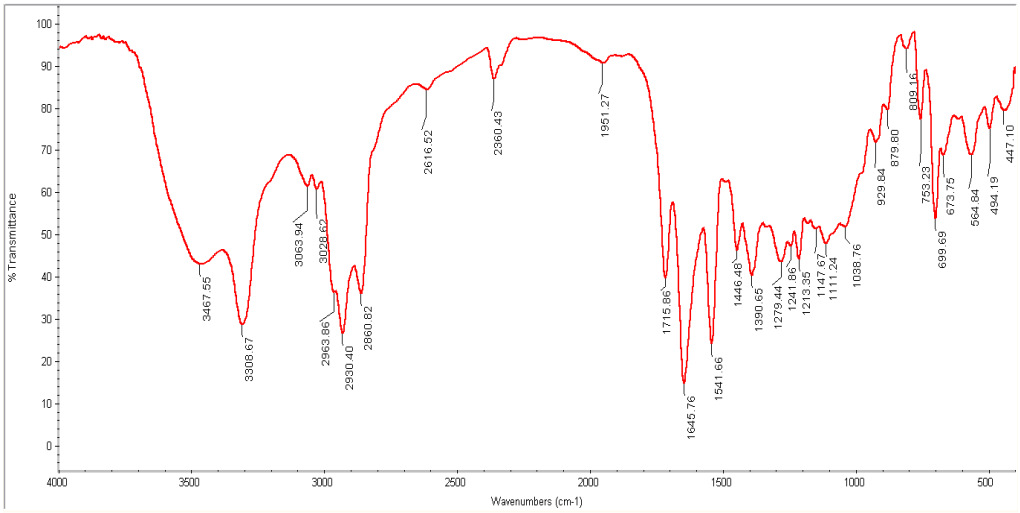


Figure 6: FTIR spectrum of Drug and Eudragit S-100

3.2 Formulation of floating microspheres- It is well known that the success of formulation depends upon, its percentage yield as well as percentage floating of microspheres, drug content and drug release study. Floating microspheres were prepared by emulsion solvent diffusion technique and oil-in-water emulsion solvent evaporation method by using organic solvents such as dichloromethane and ethanol. From the preformulation studies, it was concluded that oil-in-water emulsion solvent evaporation method was suitable to produce free flowing, smooth, spherical and uniform size microspheres.

Various polymers are used for the preparation of floating microspheres in different ratio (1:1, 1:2, 1:3, 1:4) using ethyl cellulose and eudragit S-100. It was concluded that microspheres prepared by eudragit S-100 produce appropriate microspheres.

A major factor in determination of formation of microspheres was agitation speed. When agitation speed was increased, the mean particle size also decreases. The reason for this could be due to frothing and adhesion of microspheres to the wall of the beaker and when agitation speed was decreased, the stable emulsion droplets were not able to formed, hence larger particles were formed. The optimum speed was found to be 1000 r/min for formation of microspheres. For the formation of microspheres the optimum temperature is 35-40ºC at room temperature. At low temperature, the yield was low and at higher temperature, the buoyancy of microspheres decreases.

The type and concentration of emulsifier play a key role in the preparation of microspheres. This might be due to low emulsifier concentration was not sufficient to reduce the surface charge and particles were aggregated resulting in an increase in particle size. In case of 0.2% v/v emulsifier concentration, the particle size was found to be optimum size.

3.3 Characterization of microspheres

3.3.1 Percentage yield- The Percentage yields of floating microspheres were found in the range of 75–88.2 %. It was observed that with the increase in the polymer concentration (i.e. decrease in drug to polymer ratio) in the formulation, the product yield increased. It was found that average percentage yield was greater than 50 % for all the batches which shows the suitability of this method for preparation of microspheres. The results were showed in table 4.

Table 4: Percentage yield, average particle size of floating microspheres

S.No.	Formulation	Percentage yield (%)	Average particle size (μm)
1	F1	75	44.598
2	F2	80	66
3	F3	79	80
4	F4	85	85
5	F5	87	70
6	F6	79.5	60
7	F7	88.2	120

3.3.2 Particle size- The average particle size range for formulations F1, F2, F3 and F4 was found to be 44.598 µm, 66 µm, 80 µm, 85 µm respectively and for formulations F5, F6, F7 and F8 was found to be 70 µm, 60 µm, 120 µm, 80 µm respectively. The results were showed in table 4. The particle size of the microspheres increases with increase in polymer concentration respectively. This is because the viscosity of polymer solution increases with increasing polymer concentration resulting in enhanced interfacial tension, which in turn decreases the stirring efficiency, which results in increased particle size.

3.3.3 Micromeritic properties- The results of micromeritic properties were showed in table 5. The tapped density values obtained in the range from 0.116-0.168 gm/cm.^{3} and bulk density values obtained in the range from 0.106-0.154 gm/cm^3. for all the formulations. For the prepared formulations angle of repose ranged between (25º- 30º), the compressibility index ranged between 6.89 %-13.81 % and Hausner's ratio ranged between (1.07-1.16), confirmed good flow properties of the microspheres. Thus the floating microspheres showed better flow property and were non-aggregated.

Table 5: Micromeritic properties of formulations

Formulation code	Angle of repose	Bulk density g/cm^3	Tapped density g/cm^3	Carr's index (%)	Hausner ratio
F1	26	0.106	0.116	8.62	1.09
F2	25	0.108	0.116	6.89	1.07
F3	24	0.108	0.121	10.74	1.12
F4	29	0.119	0.129	7.75	1.08
F5	27	0.154	0.168	8.33	1.09
F6	26	0.127	0.143	11.18	1.12
F7	28	0.131	0.152	13.81	1.16
F8	30	0.106	0.121	12.39	1.14

3.3.4 Percentage floating- 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.

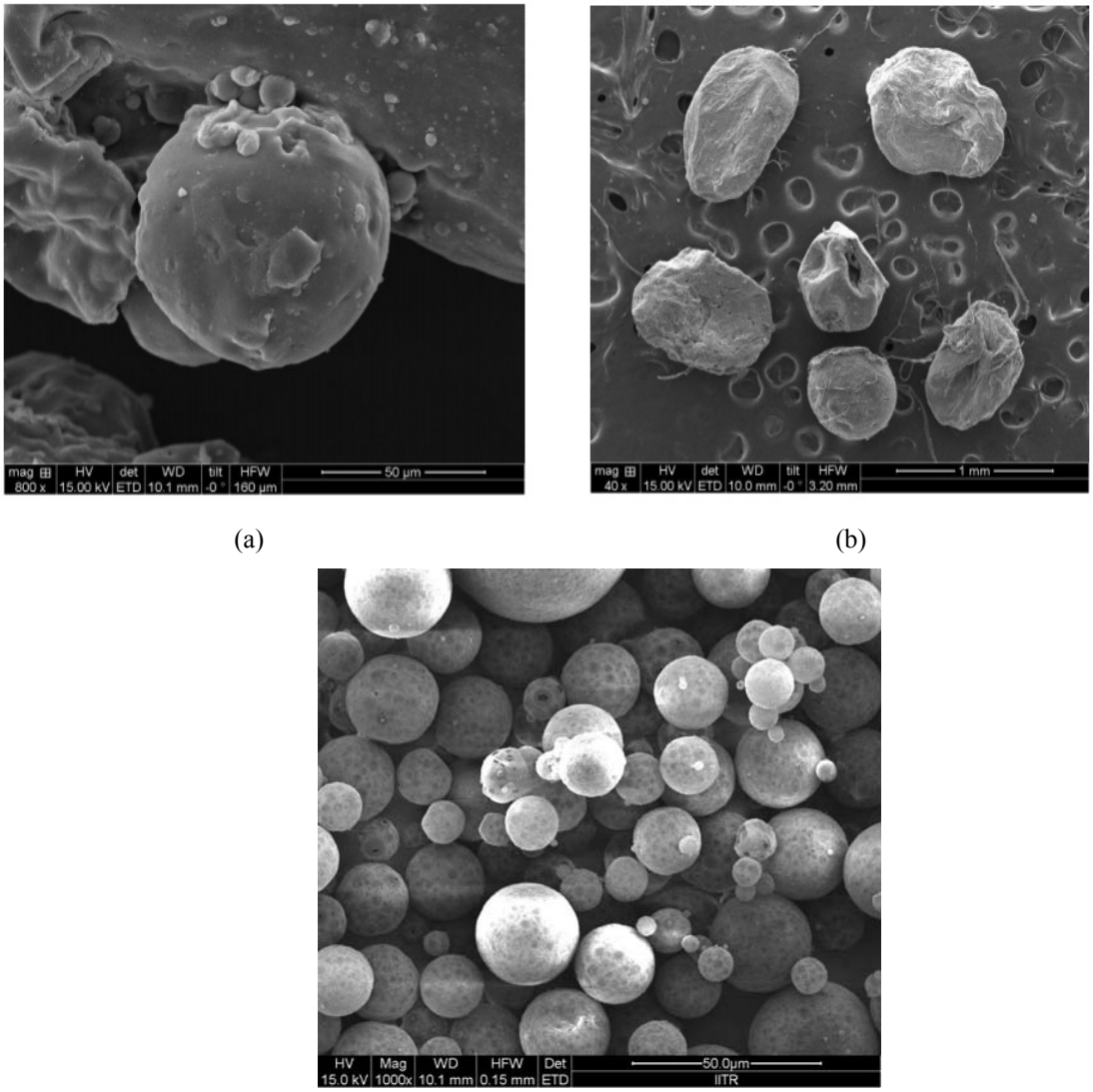
Buoyancy of prepared microspheres was investigated by *in-vitro* buoyancy test and the buoyancy of all the formulations were found to be in the range of 66 - 88%, the results were showed in table 6. Formulation F5 showed least percentage buoyancy of 66%, while F7 showed highest buoyancy of 88%. The formulations prepared with the various drug and polymer ratios were evaluated for floating time. In the test of floating time, more than 80% microspheres remained floating for more than 12 hour. The good buoyancy behavior of the microspheres may be attributed to the hollow nature of the microspheres. As the concentration of polymers increases, buoyancy also increases. Formulation F7 gave the best floating ability (88%) in SGF. Smaller the microspheres lesser was the floating ability, while larger the size, floating ability was found to be more and sustained was the release of drug.

Table 6: Evaluation of floating microparticulated drug delivery systems

Formulation code	Percentage floating (%)	Floating time (hours)	Drug content (%)	Drug entrapment efficiency (%)
F1	84	12	66.38	70.48
F2	78	15	71.6	85.95
F3	70	8	76.53	88.64
F4	76	14	80.79	90.17
F5	66	13	64.35	75.76
F6	72	11	73.80	85.12
F7	88	15	85.2	91.2
F8	86	10	74.52	82.8

3.3.5 Drug content and entrapment efficiency- The drug entrapment efficiency of all formulations was found to be in the range between 70.48 to 91.2% and the drug content was found to be in the range of 64.35 to 88.2%, the results were showed in table 4. With the increase in polymer concentration, increased entrapment efficiency was seen because with increasing polymer content, more particles of drug would be coated leading to higher encapsulation efficiency as can be seen from Table 6. An increase in polymer concentration in the internal phase shows increase in drug loading. This may be due to increase in viscosity of internal phase which reduces the migration of drug in aqueous phase, thus entrapping greater amount of drug.

3.3.6 Surface morphology using SEM- Morphology of floating microspheres was examined by scanning electron microscopy. The SEM images of prepared formulations were showed by figure 7. SEM analysis showed that the prepared floating microspheres were having size in micrometers and the particles were nearly spherical.



(a) (b)

(c)

Figure 7: (a), (b) and (c) SEM of microspheres of formulation F7

3.3.7 *In vitro* drug release- For comparison of the release rate of formulations prepared by using different ratio of polymers *in vitro* release study was done. *In-vitro* drug release studies were performed in 0.1 N HCl for 2 h and in pH 6.8 buffer for 10 h. The results of cumulative drug release were showed in table no. 7. The graph was plotted between cumulative drug release and time and showed in figure no. 8. The cumulative release of drug significantly decreased with increase in polymer concentration. The increased density of polymer matrix at higher concentration resulted in an increased diffusion path length. This may decrease the overall drug release from the polymer matrix. And it was found that as the polymer concentration was increased the release rate decreases. The selected formulation percentage of drug released was found to be initially 5.39% at 1 h and 80.67% up to 12 h.

Table 7: *In-vitro* dissolution studies of floating microspheres of Nateglinide Cumulative % drug release for different batches of microspheres formulations

S. No.	Time (h)	Percent cumulative drug release for different batches of microspheres formulations							
		F1	F2	F3	F4	F5	F6	F7	F8
1	0	0	0	0	0	0	0	0	0
2	1	2.63	2.68	2.85	2.9	3.86	4.46	5.39	5.77
3	2	11.07	14.72	16.81	20.93	13.79	16.91	17.94	18.25
4	3	14.16	27.77	26.80	24.31	21.15	21.50	21.37	21.72
5	4	27.39	31.05	27.52	27.74	27.86	23.46	24.52	23.68
6	5	30.54	35.85	30.58	29.74	30.70	27.89	30.42	27.71
7	6	30.83	41.87	38.85	38.47	39.50	46.03	46.34	45.93
8	7	38.40	48.02	45.12	46.18	45.40	54.70	55.08	54.76
9	8	41.63	55.57	49.05	49.46	53.33	58.76	59.13	57.92
10	9	49.48	57.76	55.73	55.42	57.17	58.94	64.94	60.26
11	10	56.78	62.07	56.92	57.82	60.60	61.91	69.81	61.57
12	11	67.15	64.59	60.60	62.07	61.75	62.19	75.49	67.62
13	12	77.54	66.93	70.18	73.49	75.16	71.18	80.67	69.49

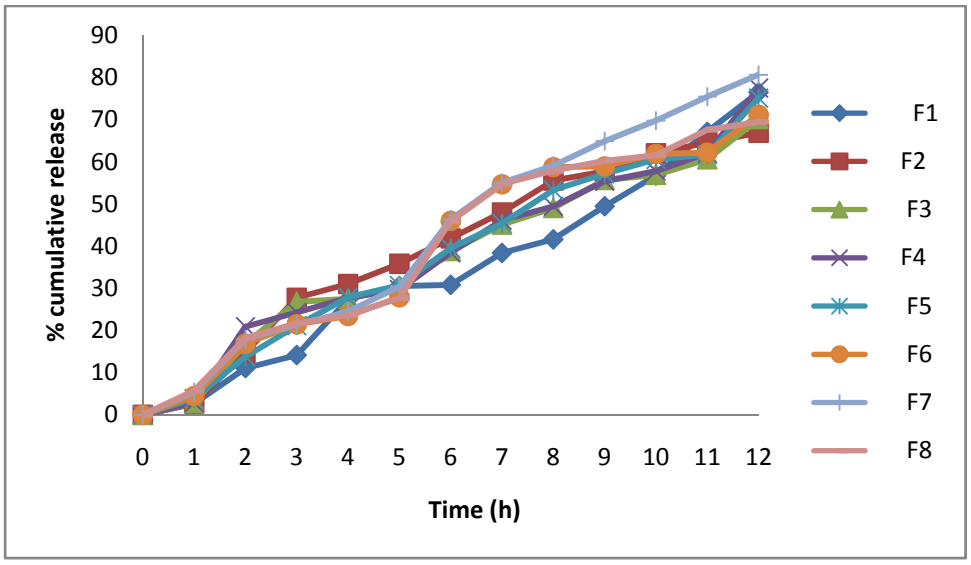


Figure 8: Graph showing % cumulative drug release vs time of the prepared formulations

3.3.8 Release kinetic study- In order to determine the release model which best describes the pattern of drug release, the *in-vitro* release data were substituted in various models such as zero order, first order, higuchi plot and korsmeyer peppas kinetics models. Model fitting release profiles of formulation were showed in table 8. The highest regression (0.947) was obtained for higuchi equation. To explain the mechanism of drug release, korsmeyer-peppas equation was used. Value of slope (n) was calculated and found to be (0.713) which is less than 0.89 which indicates anomalous non-fickian diffusion i.e. coupling of diffusion and erosion, which indicates that the drug release is sustained by more than one process.

From the above parameters the best selected formulation was found to be F7 having, 88.2% yield, 88% buoyancy, 85.2% drug content, 91.2% entrapment efficiency and 80.67% drug release.

Table 8: Model fitting release profile of formulations F1 to F8

Formulation	Zero order (r^2)	First order (r^2)	Higuchi (r^2)	Korsmeyer-Peppas	
				(r^2)	n value
F1	0.918	0.976	0.974	0.885	0.836
F2	0.746	0.857	0.916	0.773	0.752
F3	0.812	0.926	0.949	0.791	0.727
F4	0.834	0.946	0.958	0.789	0.715
F5	0.837	0.939	0.958	0.874	0.735
F6	0.747	0.830	0.897	0.856	0.703
F7	0.827	0.944	0.947	0.904	0.713
F8	0.754	0.837	0.905	0.88	0.659

4. CONCLUSION

Floating microspheres of nateglinide were prepared by novel oil-in-water emulsion solvent evaporation technique, using various biodegradable polymers such as ethyl cellulose and eudragit S-100 in order to retain drug in body for longer period of time. Nateglinide is insoluble in water and has short half life of 1.5 h. It requires frequent dosing before meals due to short half life and thereby imposing side effects. The drug requires a novel gastroretentive drug delivery system which can provide an extended period of time in stomach and improve oral bioavailability. Floating microspheres were characterized for floating ability, compatibility study, particle size and shape, drug content, *in vitro* drug release, entrapment efficiency. Due to their low density, these multi particulate drug delivery systems showed good floating ability and remained in gastric environment for more than 12 h. Eudragit S-100 based microspheres showed its buoyancy for more than 15 h, required for sustained therapeutic activity in comparison to Ethyl cellulose based microspheres.

Major advantages of the system include ease of preparation, good floating ability, high encapsulation efficiency and sustained drug release over several hours. From this study it was concluded that formulation of floating microspheres of nateglinide offers prolonged gastric residence time and continuous release of the medication over an extended period of time thus oral bioavailability of the drug and subsequent efficacy is improved.

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