FORMULATION AND EVALUATION OF FLOATING MICROSPHERES NATEGLINIDE

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ABSTRACT : The objective of the present work was to formulate floating microspheres of Nateglinide which is soluble and shows better absorption in gastric pH. Microspheres were prepared by emulsion solvent diffusion technique. Using various such as carbopol 934, ethyl cellulose and Eudragit polymers. The formulations were evaluated for micromeritic properties, in vitro buoyancy, % yield, entrapment efficiency and in vitro studies. They were characterized by FT-IR. FT-IR and studies indicated that there was no interaction between the drug and polymers. SEM photographs showed the outer surface of microspheres was smooth and dense where as internal surface was porous which helped to prolong floating to increase residence time in stomach. The results showed that floating microspheres could be successfully prepared with better yield. Results showed larger the particle size, longer was the floating time. In vitro drug release studies showed controlled release of Nateglinide for over 8 h. From the results it can be concluded that gastric floating microspheres can be successfully used for the delivery of Nateglinide to control blood glucose level.
Keywords: Nateglinide, Polymers, FTIR studies, emulsion solvent diffusion technique, floating time, in vitro drug release studies.

I.INTRODUCTION

The primary aim of oral controlled drug delivery is the most preferable route of drug delivery system is to achieve better bioavailability and release of drug from the system which should be predictable and reproducible, easy for administration, patient compliances and flexibility in formulation for effective therapy or to improve therapeutic efficiency of the drug through improved bioavailability [1,2]. Gastro retentive dosage forms significantly extend for the period of time, over which drug may be released and thus prolong dosing intervals and increase patient compliance[3]. Gastric retention can be achieved by the mechanism of mucoadhesive or bio adhesion systems, expansion system, high density systems, magnetic systems, super porous hydrogels, raft forming systems, low density system and floating ion exchange resins. Floating drug delivery systems or hydro dynamically balance systems have a bulk density lower than gastric fluids and thus remain buoyant in the stomach for a prolonged period of time without affecting the gastric emptying rate[4]. The drug is released slowly at a desired rate from the system and drug residual systems are emptied from the stomach. This results in increase in the gastric residence time and a better control of qualification in plasma drug concentration[5]. Microspheres are small spherical particles, with diameters in the micrometer range (typically 1µm to 1000µm). Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Glass microspheres, polymer microspheres and ceramic microspheres are commercially available.[6] Solid and hollow microspheres vary widely in density and, therefore, are used for different applications. Hollow microspheres are typically used as additives to lower the density of a material.[7] The aim of present study was to develop a Floating microsphere of Nateglinide in order to achieve an extended retention in upper GIT, which may result in enhanced absorption and there by improves bioavailability. Nateglinide is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). It belongs to the meglitinide class of short-acting insulin secretagogues, which act by binding to β cells of the pancreas to stimulate insulin release[8]. Nateglinide is an amino acid derivative that induces an early insulin response to meals decreasing postprandial blood glucose levels. Controlling high blood sugar helps prevent kidney damage, blindness, nerve problems, loss of limbs, and sexual function problems[9].

II. MATERIALS AND METHOD

2.1 MATERIALS

Nateglinide was collected as a gift sample from Hetero labs, Hyd polymers and other excipients were purchased from Vijaya Chemicals,Hyd.

2.2 METHODODOLOGY

Compatibility studies:

The drug-polymer compatibility was ascertained by subjecting the drug and homogenates of drug and polymer to Infrared spectrophotometric study.

Fourier Transform Infrared Spectroscopy (FTIR)[10,11]

Assessment of possible incompatibilities between an active drug substance and different excipients forms an important part of the preformulation stage during the development of a dosage form. The use of FTIR technique allows pointing out the implication of the different functional groups of drug and excipients by analyzing the significant changes in the shape and position of the absorbance bands. In this method individual samples as well as the mixture of drug and excipients were ground mixed thoroughly with potassium bromide (1:100) for 3-5 mins in a mortar and compressed into disc by applying pressure of 5 tons for 5 mins in hydraulic press. The pellet was kept in the sample holder and scanned from 4000 to 400 cm-1 in FTIR spectrophotometer. Then the characteristics peaks were obtained of all sample as well as mixtures.

Preparation and evaluation of Nateglinide Floating microspheres

Formulation table:

Table-: 1 Formulation development of Nateglinide Floating microspheres

F. no	Nateglinide	Ethycellulose	Hpmc	Eudragit	stirring speed
F1	100	100		-	1000
F2	100	200		-	1000
F3	100	300		-	1000
F4	100	400		-	1000
F5	100	-	100	-	1000
F6	100	-	200	-	1000
F7	100	-	300	-	1000
F8	100	-	400	-	1000
F9	100	-		100	1000
F10	100	-		200	1000
F11	100	-		300	1000
F12	100	-		400	1000

Method:

Emulsion-solvent diffusion technique with some modifications was used to prepare carbopol 934 and ethyl cellulose microspheres containing nateglinide. Briefly Nateglinide was dissolved in 5 ml distilled water. Polymers was dissolved in Dichloromethane at various drug - polymer ratios (1:1, 1:2). Then these drug and polymer solutions were mixed and emulsified using a Remi Lab Magnetic stirrer at 500 rpm for about 10 min to form stable w/o emulsion. This stable w/o emulsion was slowly added to 200 ml aqueous solution containing 1 % PVA and stirred at 1000 rpm by a mechanical stirrer equipped with a three bladed propeller (Remi motors, India) at room temperature for 2 h to allow the solvent to evaporate completely. Microspheres were isolated by filtration and washed with distilled water several time to remove PVA. The produced microspheres were dried at ambient temperature for 24 h and dried in vacuum chamber at 25 ^{0}C for 2 h to remove any residual solvent.[12]

Evaluation of Floating microspheres

Particle size analysis:

Particle size analysis plays an important role in determining the release characteristics and floating property. The sizes of Floating microspheres were measured by using a set of standard sieves ranging from 14, 16, 18, 22, 30 and pan. The sieves were arranged in increasing

order from top to bottom. The Floating microspheres were passed through the set of sieves and amount retained on each sieve was weighed and calculate the % weight of Floating microspheres retained by each sieve. Mean particle size for all formulation was determined by dividing the total weight size of formulation to % total weight of Floating microspheres.[13]

Floating Property of Floating microsphere:

100 mg of the Floating microsphere were placed in 0.1 N HCI (300 ml) containing 0.02% Tween 20. The mixture was stirred with paddle at 100rpm. The layer of buoyant micro balloons was pipetted and separated by filtration at 1, 2, 4 and 6 hours. The collected micro balloons were dried in a desiccator over night.[14]

The percentage of micro balloons was calculated by the following equation:

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

Drug Entrapment:

The various formulations of the Floating microspheres were subjected for drug content. 50 mg of Floating 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 diluted up to 10 m1 with 0.1 N HCI and the absorbance was measured against 0.1 N HCI as a blank.[15]

The percentage drug entrapment was calculated as follows.

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

Percentage Yield:

The percentage yield of different formulations was determined by weighing the Floating microspheres after drying. The percentage yield was calculated as follows.[16]

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

Shape and Surface Characterization by Scanning Electron Microscopy:

From the formulated batches of Floating microspheres, formulation which showed an appropriate balance between the buoyancy and the percentage release were examined for surface morphology and shape using scanning electron microscope Hitachi, Japan. Sample was fixed on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was set at 20KV during scanning. Microphotographs were taken on different magnification and higher magnification (200X) was used for surface morphology[17].

In vitro drug release study

In vitro drug release studies were carried out for all formulations in Franz diffusion cell. Microspheres equivalent to 10 mg of Nateglinide were poured into 1 ml aliquots were withdrawn at a predetermined intervals and equal volume of dissolution medium was replaced to maintain sink conditions. The necessary dilutions were made with 1.2 pH buffer and the solution was analysed for the drug content spectrophotometrically using UV-Visible spectrophotometer.[18]

Drug release kinetics

In order to describe the Drug release kinetics from individual tablet formulations, the corresponding dissolution data were fitted in various kinetic dissolution models:

Zero order, first order, and Higuchi respectively.

$$Qt = Q0 + K0\ t \ldots\ldots\ldots..$$

where, Qt is the amount of drug released at time t; Q0 the amount of drug in the solution at t = 0, (usually, Q0 = 0) and K0 the zero order release constant.

$$\log Qt = \log Q\alpha + (K1 / 2.303)\ t \ldots\ldots..$$

Qα being the total amount of drug in the matrix and K1 the first order kinetic constant.

$$Qt = KH.\ t\ ½ \ldots\ldots\ldots$$

where,

KH is the Higuchi rate constant.

Further, to better characterise the mechanism of drug release from matrices, dissolution data were analyzed using

the equation proposed by Korsmeyer and Peppas.

$$Q\ (t\text{-}l)\ /Q\alpha = KK\ (t\text{-}l)n \ldots\ldots$$

where, Qt corresponds to the amount of drug released in time t, *l* is the lag time (*l* = 2 hours), Qα is the total amount of drug that must be released at infinite time, KK a constant comprising the structural and geometric characteristics of the tablet, and n is the release exponent indicating the type of drug release mechanism. To the determination of the exponent n, the points in the release curves where Q (t-*l*)/Qα>0.6, were only used. If n approaches to 0.5, the release mechanism can be Fickian. If n approaches to 1, the release mechanism can be zero order and on the other hand if 0.5<n<1, non-Fickian (anomalous) transport could be obtained. Anomalous (non-Fickian) transport generally refers to the drug release by the summation of both diffusion and erosion of the polymeric matrix. The criteria employed to select the ‘‘best model’’ was the one with the highest coefficient of determination (r^2)[18].

Stability Study

From the prepared Floating microspheres which showed appropriate balance between the buoyancy and the percentage release was selected for stability studies. The prepared formulation were placed in borosilicate screw capped glass containers and stored at room temperature (27 ± 2° C), oven temperature (42±2° C) and in refrigerator (5-8° C) for a period of 90 days[19].

III.RESULTS AND DISCUSSION

FT-IR Spectrum of Nateglinide

FT-IR Spectra of Nateglinide and F4 formulation were recorded. All these peaks have appeared in formulation and physical mixture, indicating no chemical interaction between Nateglinide and polymer. It also confirmed that the stability of drug during microencapsulation process.

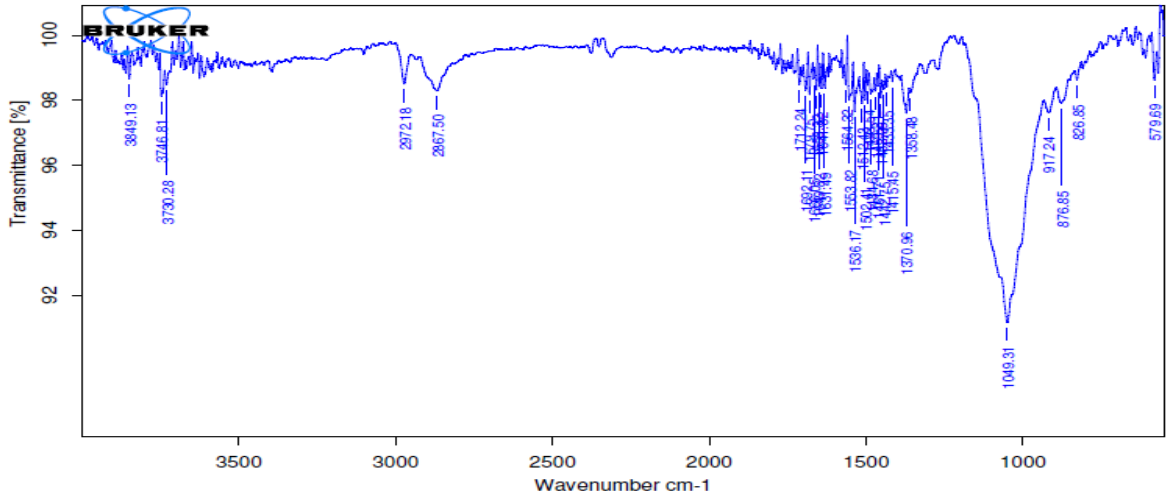

Fig.1. FTIR Studies of Nateglinide

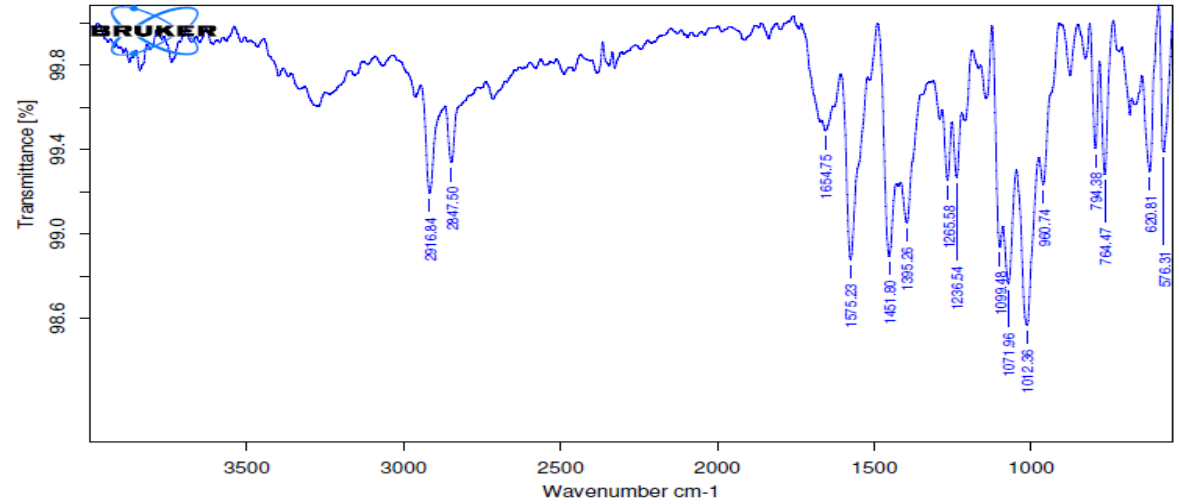

Fig.2. FTIR Studies of optimized formulation

Evaluations of Floating microspheres

Particle size analysis

Particle size was determined by sieving method it plays important role in floating ability and release corrected of drug from microspheres. If size of microspheres less than 500 mm so release rate of drug will be high and floating ability will reduce, while microspheres range between 500mm - 1000mm, floating ability will be more and release rate will be in sustained manner. The mean particle size of Floating microsphere was in range 799 - 864. mm

Table-:2 Mean particle size of Different Batches of Floating microsphere

S. No	Formulation code	Mean particle size* (mm)
1	F1	758
2	F2	762
3	F3	788
4	F4	842
5	F5	786
6	F6	764
7	F7	812
8	F8	806
9	F9	798
10	F10	824
11	F11	816
12	F12	789

Floating Property of microsphere

Microsphere were dispersed in 0.1 N HCl to simulate gastric fluid. Floating ability of different formulation were found to be differed according to polymer ratio.

Table-: 3 Percentage buoyancy for different formulation

Formulation	1 hour	2 hours	4 hours	6 hours
F1	97.2	96.15	92.49	95.25
F2	96.15	96.46	95.23	93.25
F3	97.55	95.51	90.96	91.49
F4	96.62	92.42	92.68	96.16
F5	96.21	97.23	91.43	94.56
F6	95.18	93.16	94.14	9152
F7	94.48	92.15	91.69	89.29
F8	95.76	90.24	93.67	92.61
F9	96.27	91.59	94.38	92.52
F10	93.48	92.52	90.09	94.82
F11	91.48	88.63	91.34	89.79
F12	89.81	91.82	92.52	92.1

Drug Entrapment

The drug entrapment efficacy of different formulations were in range of 68.18% - 92.20 % w/w. Drug entrapment efficacy slightly decrease with increases Hpmc and eudragit content and drug entrapment increases with increasing ethyl cellulose ratio in microspheres.

Table-: 4 Drug entrapment for different formulation

Formulation	Drug Entrapment (% w/w)
F1	73.52
F2	72.73
F3	79.12
F4	92.20
F5	73.26
F6	74.82
F7	71.42
F8	72.19
F9	74.25
F10	84.38
F11	80.36
F12	79.06

Percentage Yield

Percentage yield of different formulation was determined by weighing the micro balloons after drying. The percentage yield of different formulation were in range of 65.10 - 92.67% as shown in Table.

Table –: 5 Percentage yield for different formulation

Formulation	Percent Yield*(%)
F1	82.96
F2	86.34
F3	86.28
F4	92.67
F5	68.14
F6	72.98
F7	76.26
F8	80.14
F9	82.68
F10	83.96
F11	85.16
F12	87.62

Percent yield:

The percentage (%) yield values ranged from 65.10 - 92.67% for all the formulations.

Scanning Electronic Microscopy

Shape and surface characteristic of Floating microspheres examine by Scanning Electronic Microscopy analysis as shown in Fig. Surface morphology of F4 formulation examine at different magnification 40X and 200X, which illustrate the smooth surface of floating microballoons and small Floating cavity present in microsphere which is responsible for floating property.

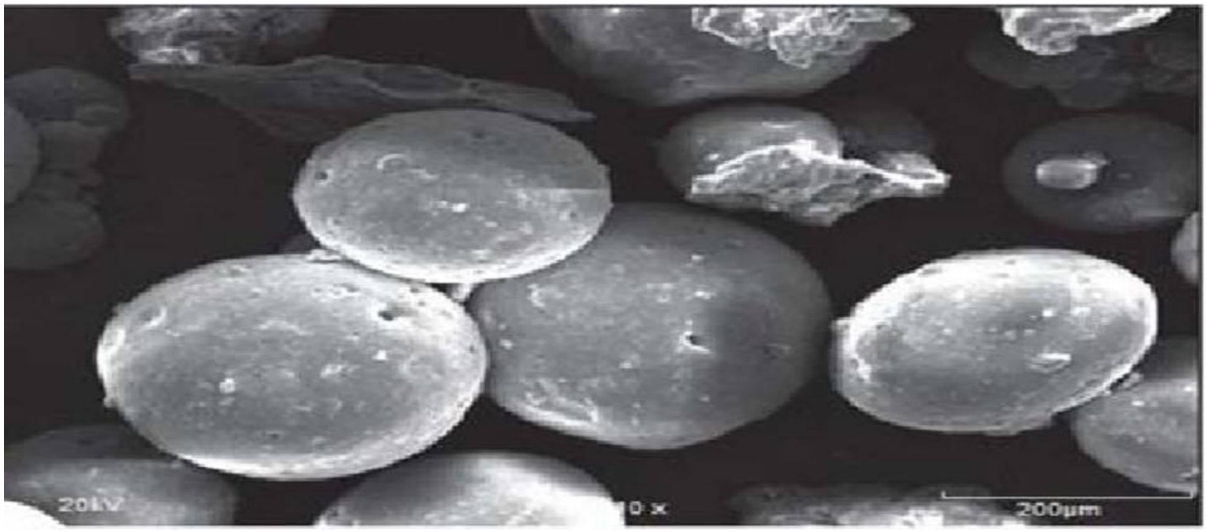

Fig.3. SEM Photographs of Formulation F4

IN-VITRO Drug release study

Table-: 6 Comparititive *In-Vitro* Drug Release Profile for Formulations(F1-F6)

TIME	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	15.18	16.5	16.2	18.62	11.06	17.62
2	28.15	26.75	28.15	26.6	26.21	25.62
3	35.11	40.14	32.88	38.29	32.19	36.24
4	43.08	56.03	44.15	51.74	49.23	52.47
5	55.75	67.25	59.18	69.92	71.69	68.29
6	69.5	72.65	66.63	77.63	75.68	76.36
7	80.15	84.92	74.16	83.33	80.42	82.36
8	85.25	89.18	85.9	95.53	87.24	93.84

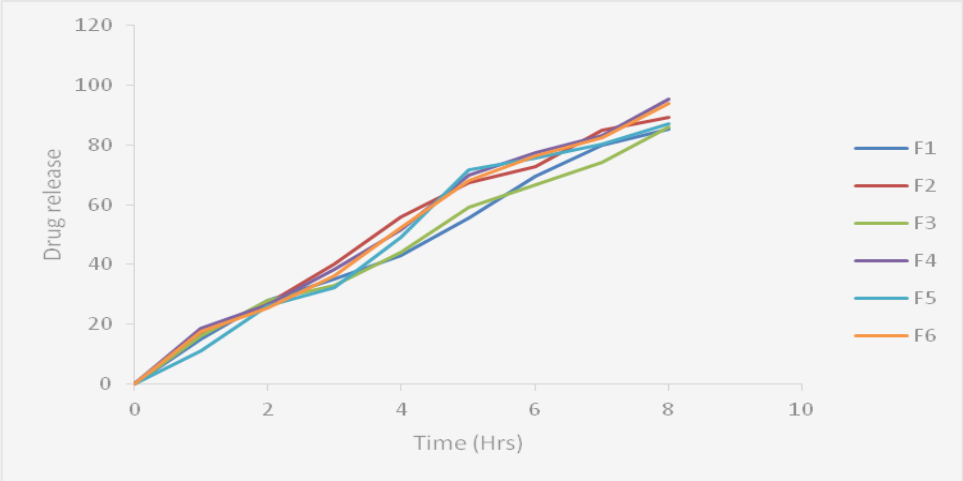

Fig.4. Comparative In-Vitro Drug Release Profile Of (F1-F6) formulations

Table-: 7 Compariitive *In-Vitro* Drug Release Profile for Formulations(F7-F12)

TIME	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0
1	19.25	17.48	14.81	15.76	15.28	14.12
2	28.14	21.88	27.26	24.72	27.52	26.52
3	31.54	34.25	34.12	44.42	33.84	37.27
4	48.28	50.14	42.81	54.13	45.56	46.25
5	55.96	69.29	53.52	65.54	56.84	59.15
6	60.32	75.18	68.26	71.26	67.39	66.92
7	76.82	82.54	81.54	83.21	75.62	85.16
8	88.52	91.21	90.54	91.34	84.16	87.81

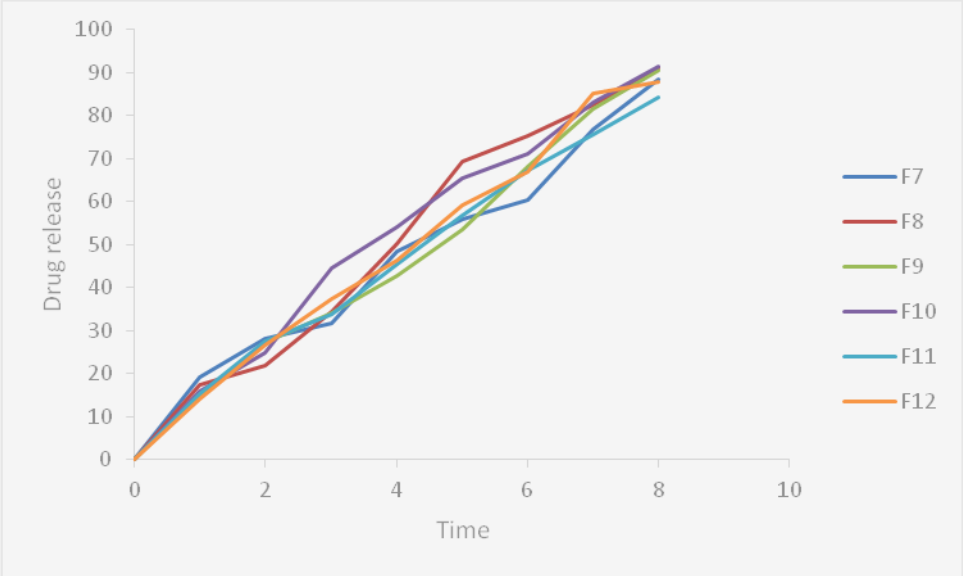

Fig.5.Comparative In-Vitro Drug Release Profile of (F7-F12) formulations

DISCUSSION:

All the 12 formulations of Floating microspheres were subjected to dissolution studies. Dissolution was carried out in franz diffusion cell apparatus at 100 rpm in the volume of 10 ml dissolution media of 0.1N HCL for 8 hours. F4 showed a release rate of 95.53 by end of 8th hour of dissolution study.

Release kinetic

Kinetics and mechanism of drug release from all formulation was evaluated on the basis of zero order, Higuchi equation and Pappas model. Correlation coefficient (r^2) and slop value for each equation was calculated from Microsoft excel. Zero order plot for all formulations were found to be linear in both dissolution medium. That indicates it may follow zero order mechanism. Higuchi plot was found to be linear, which indicates diffusion may be the mechanism of drug release for each formulation. Peppas plot was found good linear, n > 0.5 for all formulations, indicated that drug release may follow anomalous diffusion. Zero order plot for F4 formulation was found to be linear in both dissolution medium, it considered as a best fit for drug release

Zero order kinetics

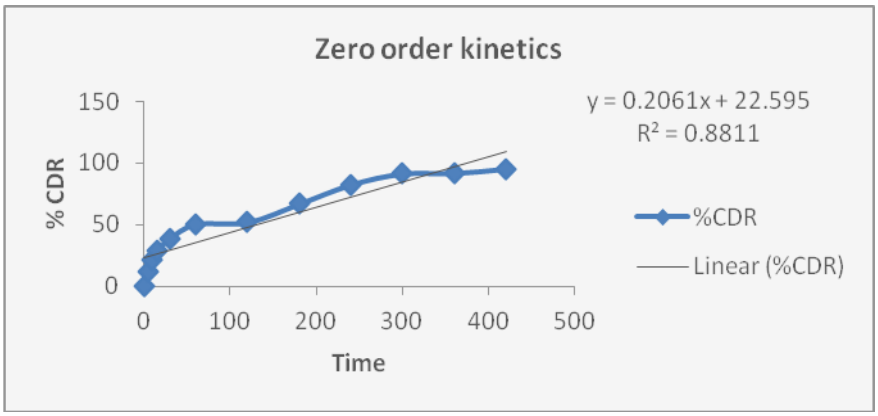

Fig.5. Zero order kinetics

First order kinetics

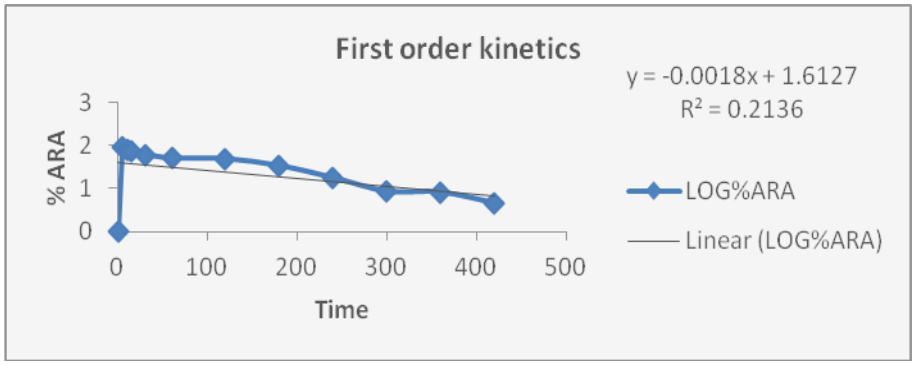

Fig.7. First order kinetics

Higuchi model

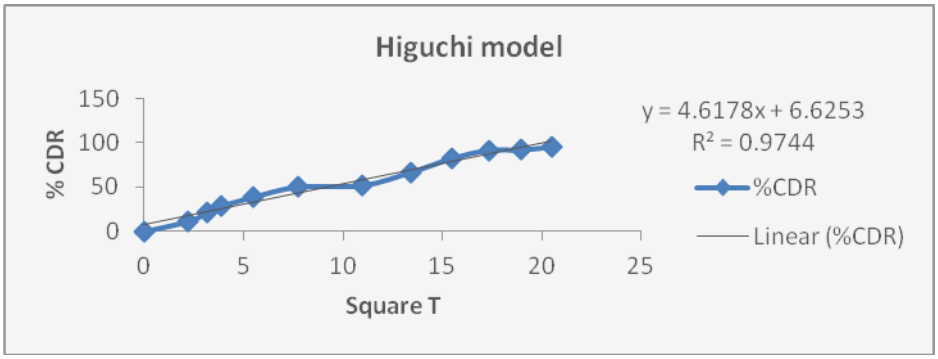

Fig.8. Higuchi model

Krosmayer peppas

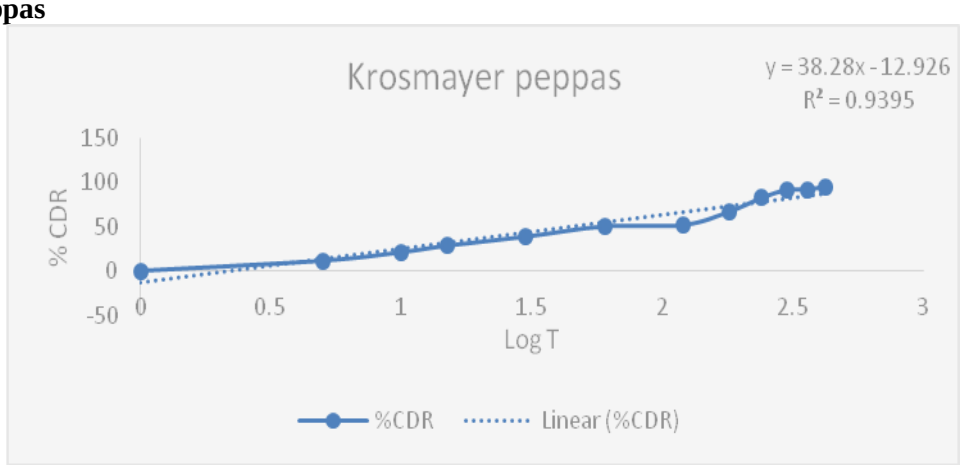

Fig.9. Krosmayer peppas

This indicates that r^2 values are higher for Higuchi's model compared for all the microspheres. Hence Nateglinide release follows diffusion rate controlled mechanism.

Stability Study

Stability study was carried out for the F4 formulation by exposing it to different temperature 25°C, 30°C and 40°C for 90 days. The sample was analysed for drug content at the regular intervals. It was found that no remarkable change in the drug content of F4 formulation. This indicates that F4 was stable for following temperature.

Table-: 8 Results of stability studies of optimized formulation F-4

S.NO	Time in days	Physical changes	Mean % drug release		
			Nateglinide		
			25^0C/60%	**30^0C/75%**	**40^0C/75%**
1.	01	No Change	95.53	95.53	95.53
2.	30	No Change	94.83	94.82	94.81
3.	60	No Change	93.79	93.69	93.75
4.	90	No Change	92.64	92.68	92.69

Discussion:

The optimized formulation was stored in different conditions to check the stability. Drug content of the optimized formulation F4 initially was 95.53%. From the above result it can be concluded that there was no significant change in physical and chemical properties of the FloatingmicrospheresofformulationF-4after3Months.

IV. CONCLUSION

Emulsion Solvent diffusion method used for preparation of Floating microspheres was suitable for poor water-soluble drugs, because the drug was soluble in the internal organic phase. FT-IR studies indicated that there was no chemical interactionϖ between the drug and the polymers used. The morphology of Floating microspheres was examined using SEM. The view of microspheres showed a Floating spherical structure with rough surface morphology. It was also evident that the Floating microspheres exhibited porous surfaces. The particle size of microspheres ranged between 735 to 850 µm. Results of drug content determination from Floating microsphere inferred that there was proper and uniform distribution of drug. The percentage encapsulation efficiency of microspheres also showed that the drug loading was optimum and increased with increasing amount of polymers. The prepared microspheres exhibited good micromeritic properties. From the results of particle size analysis, it is clear that all the process variables were within the limits and the process was reproducible. The study of micrometric properties indicated fair to good flow of microspheres. All the formulations floated for more than 8 hours. In vitro test showed that larger the particle size, longer the floating time. The microspheres of all the formulations were spherical and free flowing. The in vitro release data showed maximum drug release of more than 90 % in 8 hours. Among the formulated microspheres, those prepared from a blend of polymers showed optimum release. Drug was released in 0.1N HCl buffer.Results of the stability studies showed that there were no significant change in the drug content and physical appearance. F4 formulation is considered as optimized formulation.

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