International Journal of Research in Advent Technology, Vol.7, No.4, April 2019
E-ISSN: 2321-9637
Available online at www.ijrat.org

Formulation and Evaluation of RSM Based Floating Alginate Microspheres of Gymnemic Acid & In Vitro-In Vivo Correlation (IVIVC) for Treatment of Diabetes Mellitus

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Abstract- The purpose of this research was to formulate and Characterize floating gastro-retentive microspheres of Gymnemic acid, an anti-diabetic drug by prolonging its stay in stomach for long period of time, thereby achieved improved bioavailability. Floating microspheres were prepared by ion-tropic gelation method using Sodium Alginate and Microcrystalline cellulose in varying ratios. The formulations were optimized on the basis of Dependent variables like Particle size in µm (Y1), Cumulative % drug release (Y2), Entrapment efficiency (Y3) by using Response surface methodology. The floating microspheres were evaluated for micrometric properties, drug entrapment efficiency, as well as in-vitro buoyancy study and in-vitro drug release study. Zeta potential, DSC, FTIR, XRD, UV-study, In-vivo drug release pattern were also studied. *IVIVC* level- A by using New Zealand white rabbit species were also studied. SEM study, Particle size (601±0.85 to 718±0.63), %of drug loading (75.9to 90.11), buoyance property (64to89%) support our ultimate objective. Drug release had been achieved up to 87.58%..All other parameters like C_{max}, T_{max}, AUC_{0-t} were at desired ranges.

Index Terms- Gymnemic Acid, Floating Microspheres, Response surface methodology, In Vitro in Vivo Correlation.

1. INTRODUCTION

The Novel drug delivery system (NDDS) which provide such a dosage form which can deliver the therapeutic amount of drug to the proper site in the body to achieve and maintain the desired drug concentration. GRDDS under NDDS which is retain in stomach for longer time and improve bioavailability of drug and thereby decrease first pass metabolism. If the drugs are poorly soluble in the intestine due to alkaline pH, gastric retention may increase solubility before they are emptied, resulting in gastrointestinal absorption of drugs with narrow therapeutic absorption window, as well as controlling release of drugs having site specific –absorption limitation. Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body can't effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar. Gymnema Sylvestre: (family-Asclepiadaceae). Gymnema sylvestre has been linked with significant blood glucose lowering via different mechanisms - Regeneration of islet cells. Increase in beta-cell function, Delay the glucose absorption in the blood. Gymnema sylvestre is the most popular herb for control of diabetes and Gymnemic acid is reported to be the main active constituent responsible for hypoglycemic activity. Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications.[1]Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death.[3] Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes. In floating types the bulk density is less than the gastric fluid and so remains buoyant in stomach without affecting gastric emptying rate. The drug is released slowly at the desired rate, and the system is found to be floating on gastric content and increases gastric residence and increases fluctuation in plasma concentration. Moreover it also reduces chances of dose dumping. It produces prolonged therapeutic effect and therefore reduces dosing frequencies. Drug is given in the form of floating microspheres. **[2][3]**

2. MATERIAL AND METHODS

Gymnemic Acid was obtained as a gift sample from Bioprex Labs, Pune, Maharashtra. Sodium Alginate was obtained from Salus Pharmaceuticals, Baddi, H.P. Microcrystalline cellulose was obtained from Loba Chemic Pvt.Ltd. Calcium Chloride, Calcium Carbonate, Acetic Acid, Ethanol, and Hydrochloric acid etc. were purchased locally. All chemicals were used as analytical grade.

3. PREPARATIONS OF FLOATING ALGINATE BEADS

Sodium alginate solutions of different concentrations were prepared by dissolving required amount of alginate in 100 ml of deionized water under gentle agitation. Gymnemic acid and calcium carbonate (as gas forming agent) were dispersed in alginate solution under constant stirring for uniform mixing. The dispersion was sonicated for 30 minutes to remove any air bubbles. The resultant dispersion was dropped through a 22 gauge syringe needle into 100 ml of 1% (w/v) calcium chloride solution containing 10% (v/v) acetic acid at room temperature. Then the beads formed were allowed to remain in the stirred solution for 10 min. The beads were filtered, washed with plain water and subsequently oven-dried at 50ºC for 4 hours. [4]

4. EXPERIMENTAL DESIGN

The optimization of floating microspheres of Gymnemic acid was done by using Central composite Design (center point 1), [lack of fit 3], DESIGN EXPERT SOFTWARE BY STAT EASE **(Design Expert® Software 11.0 trial version).** Based on the preformulation study the quantity of Sodium Alginate in mg (X1) and quantity of MCC in mg (X2) was selected as the independent variables, studied at 3 levels each. The central point (0,0) was studied in triplicate. All other formulations and processing variables were kept invariant throughout the study. The following table summarizes an account of the 9 experimental runs studied, their factor combination, and the translation of the coded level to the experimental units employed during the study. Particle size (Y1), Cumulative % Drug release at 12 hours (Y2), % Drug Entrapment Efficiency (Y3) were taken as response variables.

Trial No	**Coded Factor Levels**	
	A	**B**
I.	0	1
II.	1	1
III.	0	0
IV.	-1	0
V.	1	0
VI.	-1	1
VII.	-1	-1
VIII.	1	-1
IX.	0	-1
Translation of coded levels in actual units		
Coded level	-1 0 +1	
Independent Variables		
X1 : Sodium Alginate (mg)	320 560 800	
X2 : MCC (mg)	80 240 400	
Dependent Variable		
Y1	Particle Size (µm)	
Y2	Drug Release (%)	
Y3	Entrapment Efficiency (%)	

Table 1: Formulation trial carried out for floating microspheres of Gymnemic Acid with quantity of sodium alginate used at different quantity of MCC as per experimental design

5. DATA ANALYSIS AND VALIDATION OF OPTIMIZATION MODEL

Various RSM computations for the current optimization study were performed employing Design Expert software (11.0 trial version) Stat- Ease Inc. Polynomial models including interaction and quadratic terms were generated for all the response variables using multiple linear regression analysis (MLRA) approach. The general form of the MLRA model is represented as the following equation:

$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_1X_2^2 + \beta_7 X_1^2X_2$

Here, β_0 is the intercept representing the arithmetic average of all quantitative outcomes of 9 runs; β_1 to β_7 are the coefficients computed from the observed experimental response values of Y; and X1 and X2 are the coded levels of the independent variable(s). The terms X1X2 and Xi^2 (i =1 to 2) represent the interaction and quadratic terms, respectively. Statistical validity of the polynomials was established on the basis of ANOVA provision in the design expert software. Three-Dimensional (3D) response surface plots and two dimensional (2-D) contour plots were constructed based on the model polynomial functions using design expert software .These plots are very useful to see interaction effects on the factors on the responses. Eight optimum check point were selected by intensive grid search, performed over the entire experimental domain, to validate the chosen experimental design and polynomial equations. The formulations corresponding to these checkpoints were prepared and evaluated for various response properties. Subsequently, the resultant experimental data of response

6. MATHEMATICAL MODELING FOR RSM OPTIMIZATION

Mathematical relationships in the form of polynomial equation for the measured response particle size. % drug release (at 12 hours) and % drug entrapment efficiency were taken as the response variables obtained with the stat-ease software. The polynomial equation relating the different response and independent variable is given below:

Y1 (Particle size in µm) = $665.556 + 37.8241X_1 + 20.4173X_2$

Y2 (% Cumulative Drug Release) = $79.9256 – 5.33413X_1 – 3.85063X_2$

Y3 (% Drug entrapment efficiency) = $73.5789 + 1.69537X_1 + 1.52983X_2$

The above equation represents the quantitative effect of process variables and their interaction on the response. For estimation of the significance of the model, the analysis of variance (ANOVA) was determined as per the provision of design expert software as shown below. Using 5% significance level. A model is considered significant if the p-value (significance probability value) is less than 0.05.

Std	Run	Factor 1 A:Na Alginate (mg)	Factor 2 B:MCC (mg)	Response 1 Particle Size (µm)	Response 2 %CDR (%)	Response 3 %DEE (%)
6	1	800	240	708	74.56	76.45
1	2	390.294	126.863	601	93.85	72.17
9	3	560	240	664	77.53	73.22
5	4	320	240	610	87.58	71.88
7	5	560	80	637	83.67	68.78
2	6	729.706	126.863	698	75.89	74.64
8	7	560	400	703	74.87	75.44
3	8	390.294	353.137	651	78.84	72.5
4	9	729.706	353.137	718	72.54	77.13

Table 2: Response variables (Y1, Y and Y3) obtained from various trial formulations

Table 3: Formulation Table

INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9
Gymnemic Acid (mg)	400	400	400	400	400	400	400	400	400
Sodium alginate (mg)	560	800	560	320	800	320	320	800	560
Microcrystaline	400	400	240	240	240	400	80	80	80

cellulose(mg)									
$CaCO_3$(mg)	400	400	400	400	400	400	400	400	400
$CaCl_2$ solution & Acetic acid (%)	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10	1:10

Fig 1: Contour plot showing the effect of quantity of sodium alginate in mg (X1) and quantity of MCCi n mg (X2) on Particle Size in µm (Y1) of formulation

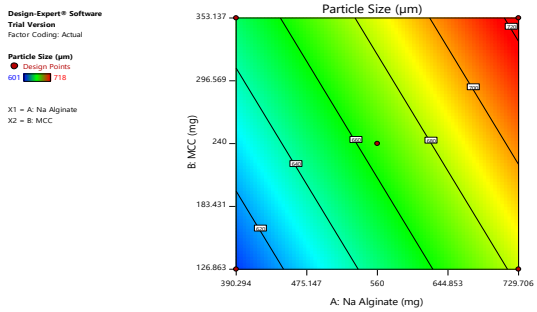


Fig 2: Response surface plot showing the effect of quantity of Sodium alginate (X1) and quantity of MCC (X2) on Particle size (Y1) of formulation

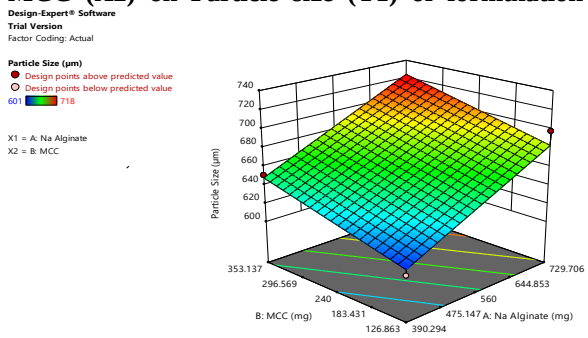


Fig 3: Contour plot showing the effect of quantity of sodium alginate in mg (X1) and quantity of MCC in mg (X2) on % Cumulative drug release (Y2) of formulation

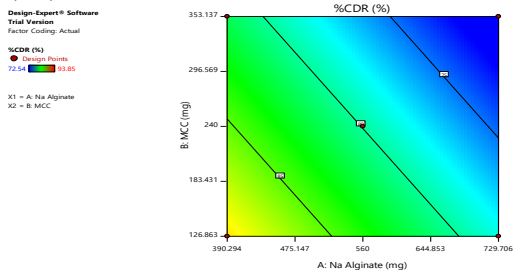


Fig 4: Response surface plot showing the effect of quantity of Sodium alginate (X1) and quantity of MCC (X2) on % Cumulative Drug Release (Y2) of formulation

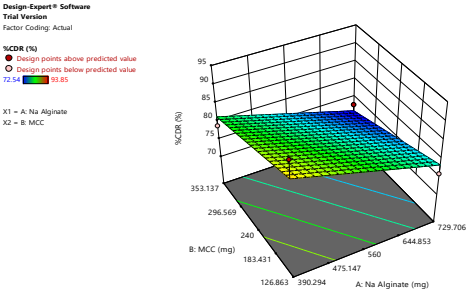


Fig 5: Contour plot showing the effect of quantity of sodium alginate in mg and quantity of MCC in mg on % Drug Entrapment Efficiency (Y3)

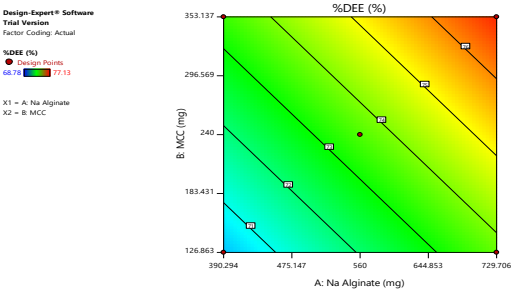
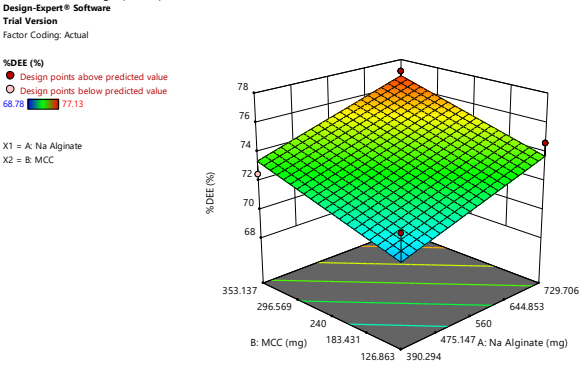


Fig 6: Response surface plot showing the effect of quantity of sodium alginate in mg and quantity of MCC in mg on % Drug Entrapment Efficiency(Y3)



Response Surface Analysis (Particle Size in µm): Figures 1 and 2 represent the contour plot and three dimensional analysis for the studied response property , i.e. particle size .The graphs show the effects of the two independent variables , i.e. quantity of sodium alginate (X1) and quantity of MCC in mg (X2) , on the response variable i.e. particle size (Y1).

From the contour plot it can be concluded that the particle size (in µm) increases with increase in quantity in mg of X1 and X2.The response changes the variables in a linear and ascending manner. From the three-dimensional graph it can be concluded that the particle size (in µm) increases with increase in quantity in mg of X1 and X2.From the contour plot

and three-dimensional analysis, it was concluded that the particle size in µm increases with augmentation of both the variables.

Response Surface Analysis (% Drug Release at 12 hours): Figures 3 and 4 represents the contour plot and three – dimensional analysis for the studied response property i.e. % cumulative drug release .The graphs show the effects of the two independent variables i.e. quantity of sodium alginate in mg (X1) and quantity of MCC in mg (X2), on the response variable i.e. % cumulative drug release (Y2). From the contour plot it can be concluded that the % cumulative drug release decreases with increase in quantity in mg of X1 and X2.The response changes the variables in a linear and descending manner. From the three-dimensional graph it can be concluded that the % cumulative drug release decreases with increase in X1 and X2. From the contour plot and three-dimensional analysis, it is concluded that the % cumulative drug release decreases with augmentation of both the variables.

Response Surface Analysis (% Drug Entrapment Efficiency): Figure 5 and 6 represent the contour plot and three-dimensional analysis for the studied response property i.e. % drug entrapment efficiency .The graphs show the effects of the two independent variables i.e. quantity of sodium alginate (X1) and quantity of MCC in mg (X2),on the response variable i.e. % drug entrapment efficiency (Y3). From the contour plot it can be concluded that the % drug entrapment efficiency increases with increase in quantity in mg of X1 and X2. The response of the variables in a linear and ascending manner. From the three-dimensional graph it can be concluded that the % drug entrapment efficiency increases with increase in X1 and X2. From the contour plot and three-dimensional analysis, it is concluded that the % drug entrapment efficiency increases with augmentation of both the variables.

7. PREFORMULATION STUDY

Solubility Study:

The drug was found to be soluble in acetone, ethanol, water and 0.1N HCl buffer pH 1.2 Solubility of Gymnemic acid was found to be more in 1.2 pH 0.1 N HCl buffer. Therefore 1.2 pH 0.1N HCl buffers were used as dissolution medium.

Micromeritic Properties

Angle of repose

Angle of repose of different formulations was measured according to fixed funnel method. Completely dried Microspheres were weighed and passed through the funnel, which was kept at a height 'h' from the horizontal surface. The passed micropsheres formed a pile of the height 'h' above the horizontal surface and the diameter of the pile was measured and the angle of repose was determined for all the formulation using the formula,

$\tan \theta = h / r$

Angle of repose (θ) = $\tan^{-1} (h / r)$

Where, h is the height of the pile and r is the radius.

Hausner's Ratio

Hausner's ratio is an indirect index of ease of powder flow. It is calculated by the following formula,

Hausner's ratio = Tapped density/Bulk density

Lower Hausner's ratio (<1.25) indicates better flow properties than higher ones (>1.25)

Particle size analysis

The particle size of microspheres was determined using an optical microscope with calibrated ocular micrometer. /sieve size analysis. The mean particle size was calculated by measuring 100 particles of each formulation.

Preparation of 0.1 N HCl buffer pH 1.2:

To prepare the 0.1 N HCl, at first take 1000ml of volumetric flask .After that measure 8.5 ml of conc. HCl by pipette properly. Then the volume is make up to 1000 ml by distilled water with 8.5 ml of conc. HCl in the volumetric flask and shake properly that the solution dissolved properly.

Bulk density and Tapped Density

The loose bulk density (LBD) and tapped bulk density (TBD) of microspheres (200mg) were determined. The prepared microspheres was poured into a calibrated measuring cylinder (10 ml) then noted initial volume. Then the cylinder was allowed to fall under its own weight onto the hard surface from the height of 2.5 cm at 2 seconds intervals. The tapping was the continued no further change in volume was noted. LBD and TBD were calculated using following equation,

LBD = weight of the powder/volume of the packing

TBD = weight of the powder/tapped volume of the packing.

Compressibility Index

The compressibility index (Carr's Index) of the all formulations were determined by using the below mentioned equation,

Carr's Index (%) =(TBD- LBD)/ TBD × 100

Preparation of standard curve of the drug in 0.1 N HCl buffer 1.2

An accurately weighed quantity of Gymnemic acid equivalent to 10 mg was taken in a 100 ml volumetric flask and it is dissolved by using 5 ml of ethanol and volume was made to mark with 1.2 pH 0.1 N HCl buffer to give a 100 µg/ml of the drug. The aliquot portion of standard stock solution of Gymnemic acid was diluted with 1.2 pH 0.1 N HCl buffer to obtain concentration 10 µg/ml.Appropriate dilutions was made for the drug from the standard stock solution and scanned in the spectrum mode from 200-600 nm .Gymnemic acid showed absorbance at 237 nm in 0.1 N HCl buffer 1.2.From the above stock solution 2,4,6,8 and 10 ml were taken and dilute up to 10 ml with 0.1 N HCl buffer pH 1.2 to get 2,4,6,8,10 µg/ml concentrated solution of Gymnemic acid. Absorbance of solution was measured at 237 nm in 1.2 pH 0.1 N HCl buffer solution. The graph was plotted for concentration vs. absorbance to get calibration curve of the drug.

Fourier-transform infrared spectroscopy (FTIR):

Drug polymer interactions were studied by FT-IR spectroscopy. The infrared spectra of sodium alginate, Gymnemic acid and drug loaded beads were recorded on FT-IR (Shimadzu FTIR 8400S). The samples were prepared on KBr press and the spectra were recorded over the wave number range of 4,000 to 400 cm^{-1}.

Scanning electron microscopy (SEM):

The surfaces and cross-section morphologies of the beads were observed using a scanning electron microscope (SEM) (JSM-6490 LA, JEOL, Tokyo, Auto fine coater and then the images were recorded at different magnifications.[9]

Swelling Index Studies

The swelling behavior of a dosage unit was measured by studying its weight gain. The swelling index of microspheres was determined by placing the microspheres in the basket of dissolution apparatus using dissolution medium as 0.1N HCl at 37±0.5°C. After 0.5, 1, 2, 3, 4, 5, and 6h, each dissolution basket containing microspheres was withdrawn, blotted with tissue paper to remove the excess water and weighed on the analytical balance. The experiment was performed in triplicate for each time point13.Swelling index was calculated by using the following formula

Determination of drug encapsulation efficiency:

50 mg of beads from each formulation were weighed and crushed in a mortar and pastel and the crushed material was dissolved in 100 ml of 0.1 N HCl buffer at pH 1.2. This solution was mechanically agitated on shaker at 200 rpm for 2 hours. The resultant dispersions were filtered and analyzed at 237 nm using UV spectrophotometer (schimadzu 1700, Japan). The encapsulation efficiency was determined by the following formula. [10][11][12]

Encapsulation efficiency = (AQ/TQ) X 100 where AQ is the actual drug content of beads and TQ is the theoretical quantity of drug present in beads.

Buoyancy test:

The obtained beads were studied for buoyancy12 and floating time using USP Apparatus II (paddle type). 300 mg beads of each batch were placed in 900 ml of 0.1 N HCl buffer (pH 1.2) containing 0.02% w/v Tween 80 and agitated at 50 rpm, temperature was maintained at 37ºC**.**

$$\% \text{ Buoyancy} = \frac{Qf}{(Qf + Qs)} \times 100$$

Where,
Qf = Weight of the floating Beads
Qs = Weight of settled Beads

Japan) operated at an acceleration voltage of 25 kV. The beads were made conductive by sputtering thin coat of platinum under vacuum using Joel JFC-1600

Swelling index = (Wet weight of microspheres – Dry weight of microspheres)/Dry weight of microspheres.

In vitro dissolution studies:

In vitro dissolution studies were performed for all the formulations using USP apparatus II (paddle type). An accurately weighed floating alginate beads were taken into 900 ml 0.1 N HCl buffer (pH 1.2). The temperature was maintained at 37ºC and stirred at a speed of 50 rpm. At 30 minutes time intervals, a 10-ml aliquot of the sample was withdrawn and the volume was replaced with an equivalent amount of plain dissolution medium kept at 37ºC. The collected samples were filtered and analyzed at 237 nm using UV- visible spectrophotometer against 0.1 N HCl buffer (pH 1.2) taken as blank. **[11]**

8. RESULTS AND DISCUSSION:

Formulation code	**Bulk density (g/cc)**	**Tapped density (g/cc)**	**Carr's Index**	**Hausner's Ratio**	**Angle of repose(θ)**
F1	0.45±0.045	0.52 ± 0.09	15.60±0.2	1.15±0.02	28.06± 0.31
F2	0.45±0.045	0.50 ± 0.07	12.23±0.6	1.11±0.04	27.58± 0.15
F3	0.44±0.044	0.50 ± 0.09	12.58±0.8	1.13±0.08	28.44± 0.11
F4	0.45±0.045	0.52 ± 0.04	15.19±0.1	1.15±0.06	28.36± 0.13
F5	0.44±0.044	0.52± 0.01	15.48±0.6	1.18±0.08	28.52± 0.19
F6	0.45±0.045	0.51 ± 0.04	13.48±0.8	1.13±0.09	29.32± 0.19
F7	0.51±0.045	0.59 ± 0.04	14.48±0.8	1.15±0.09	29.69± 0.19
F8	0.45±0.041	0.52 ± 0.10	15.60±0.21	1.15±0.04	28.06± 0.41
F9	0.44±0.041	0.52 ± 0.11	15.48±0.54	1.18±0.12	28.52± 0.15

Table 12: Pre-formulation Parameters (Mean ± SD)

SL.NO	FORMULATION CODE	MEAN PARTICLE SIZE µm (±S.D.)
1	F1	708±0.33
2	F2	601±0.85
3	F3	664±0.78
4	F4	610±0.51
5	F5	637±0.68
6	F6	698±0.43
7	F7	703±0.72
8	F8	651±0.64
9	F9	718±0.63

Table 13: Particle Size Analysis

n= 3(average of 3 batches)

SL.NO	FORMULATION CODE	% YIELD	DRUG LOADING		% BUOYANCY	% DRUG ENTRAPMENT EFFICIENCY	%SWELLING INDEX
			TDL	EDL			
1	F1	80.23	27.78	21.24	64	76.45±0.56	33.54
2	F2	84.33	77.35	55.82	79	72.17±0.53	34.11
3	F3	87	50.00	36.61	73	73.22±0.28	36.87
4	F4	85.61	71.42	51.33	83	71.88±0.89	38.33
5	F5	83.34	62.5	42.98	81	68.78±0.65	31.74
6	F6	89.27	46.69	34.85	72	74.64±0.45	32.65
7	F7	75.9	41.67	31.43	65	75.44±0.27	30.25
8	F8	86	53.81	39.01	84	72.50±0.32	37.29
9	F9	90.11	36.93	28.48	89	77.13±0.78	36.14

Table 14: Drug Loading, Drug Content, Yield of Microsphere, Buoyancy, Swelling Index, Drug Entrapment Efficiency

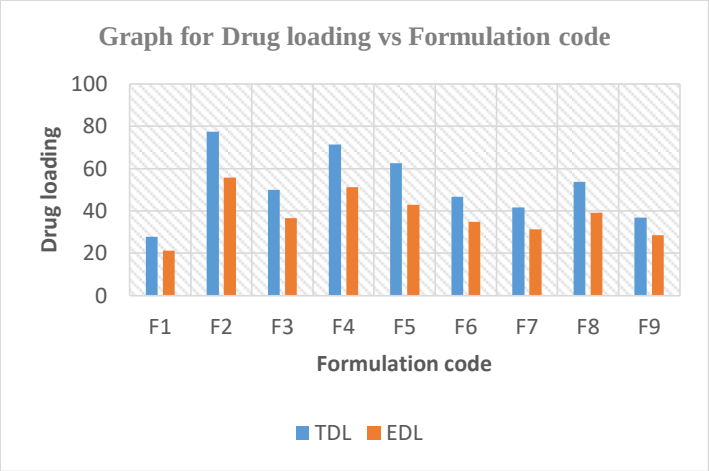


Fig 7: Graph for TDL (Theoretical drug loading) and EDL (Experimental drug loading) vs. formulation code

UV ANALYSIS:

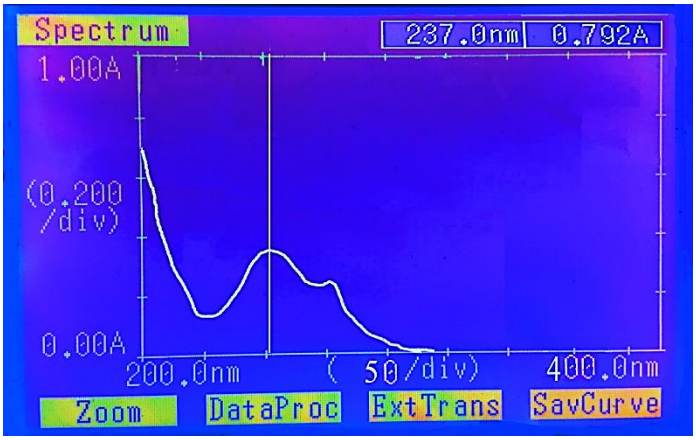


Fig 8: UV-Spectroscopy

CALIBRATION CURVE OF GYMNEMIC ACID

Serial No	Conc (mcg/ml)	Absorbance
1	2	0.233
2	4	0.461
3	6	0.627
4	8	0.859
5	10	0.964

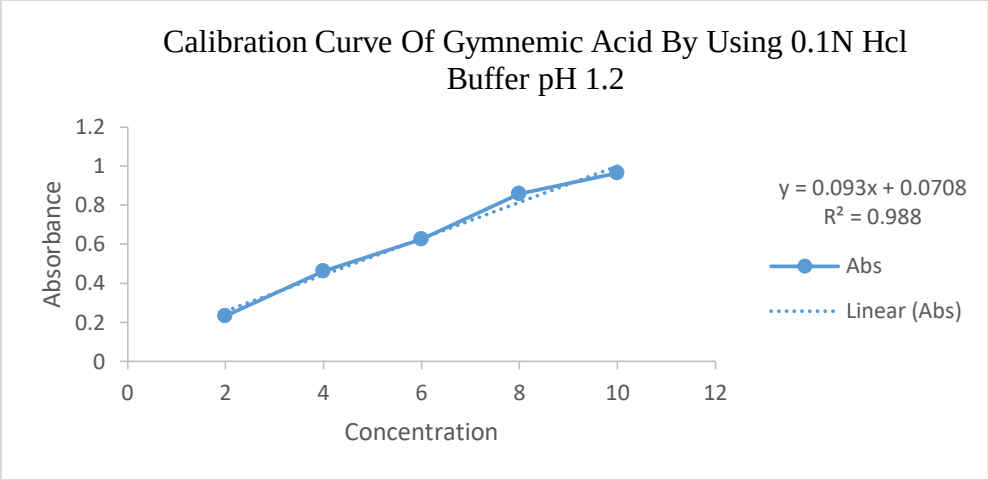


Fig 9: calibration curve of Gymnemic acid

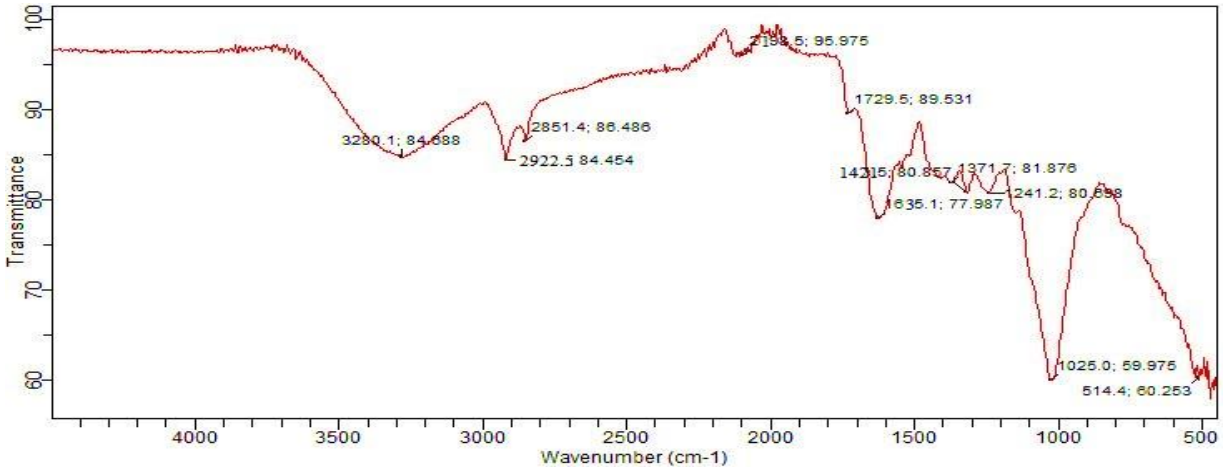


Fig 10: FTIR spectra of Gymnemic acid with peak

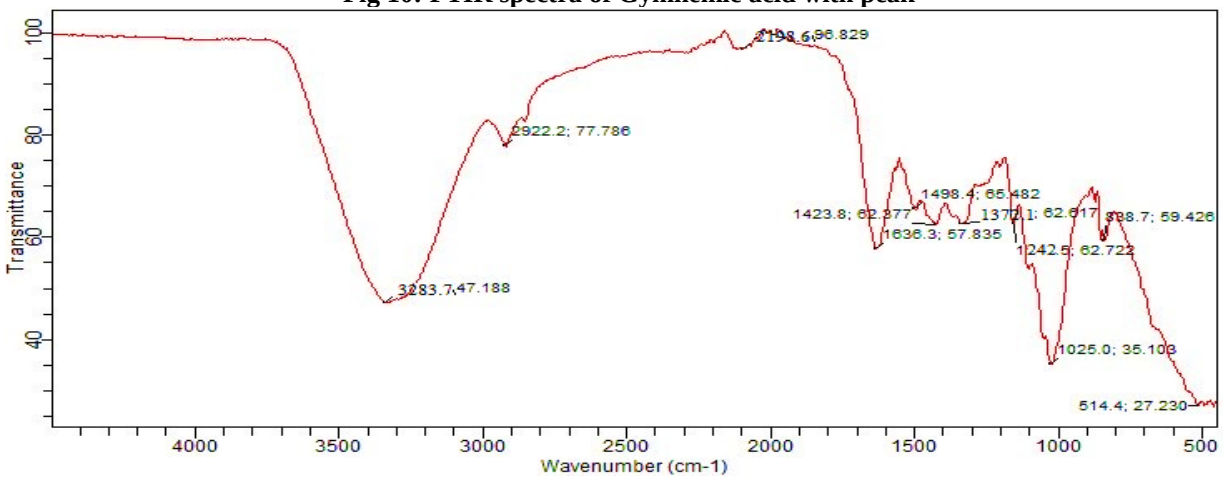


Fig 11: FTIR spectra of Drug + excipients

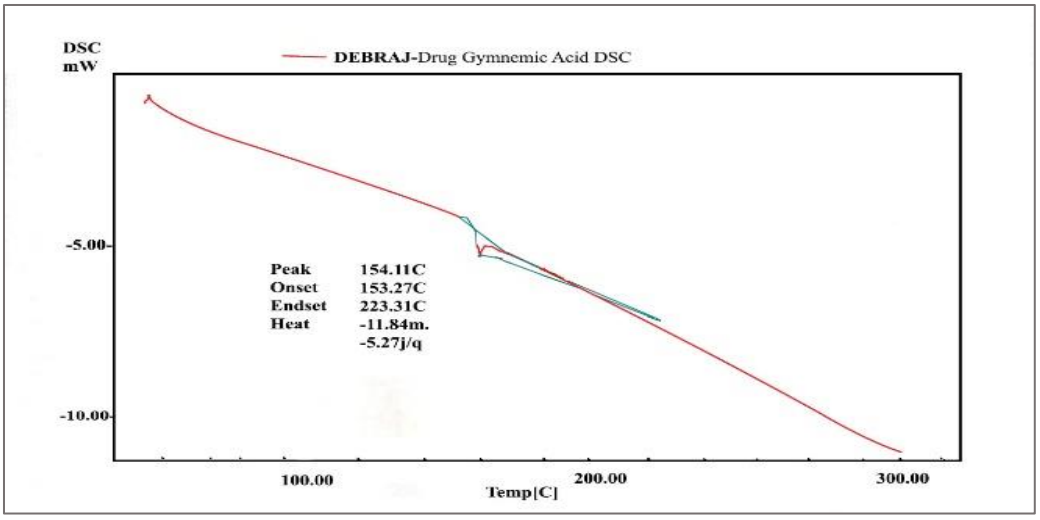


Fig 12: DSC of Gymnemic Acid

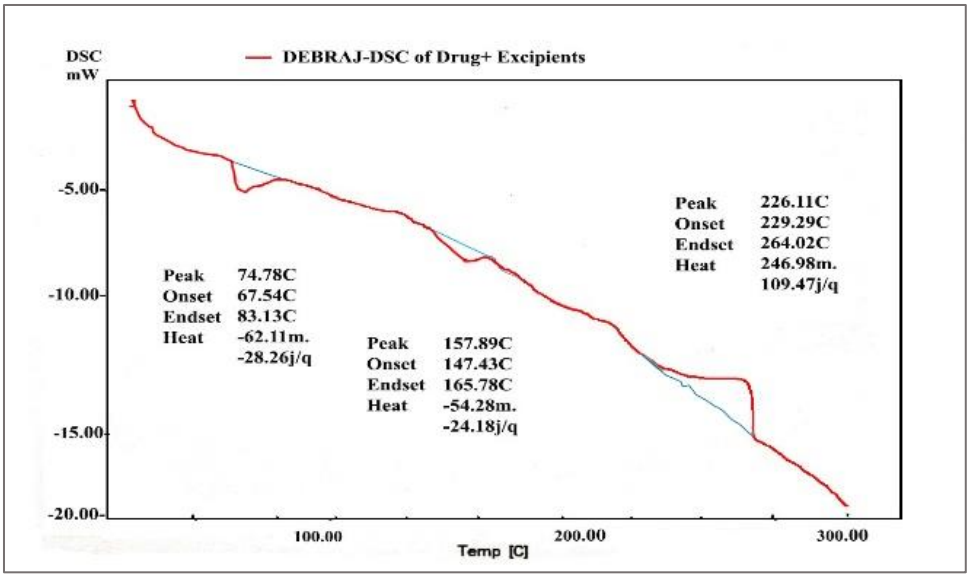


Fig 13: DSC of Drug + Polymer

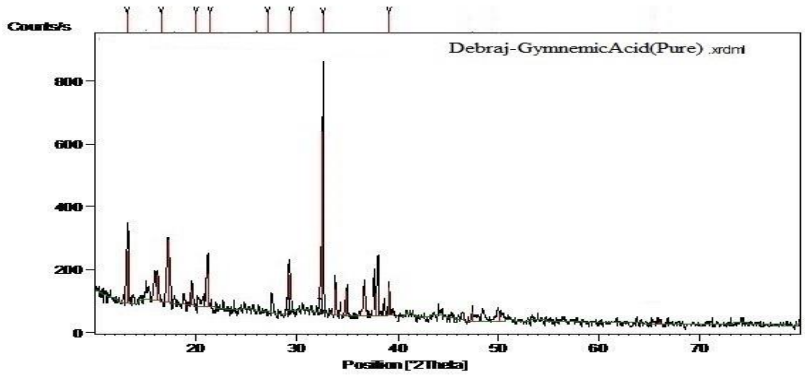


Fig 14: XRD of GymnemicAcid (Pure)

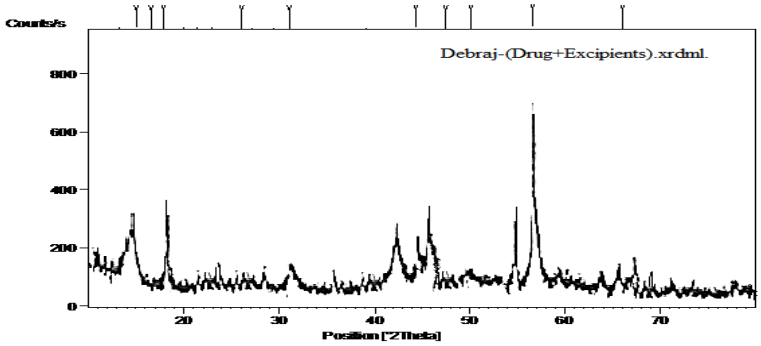
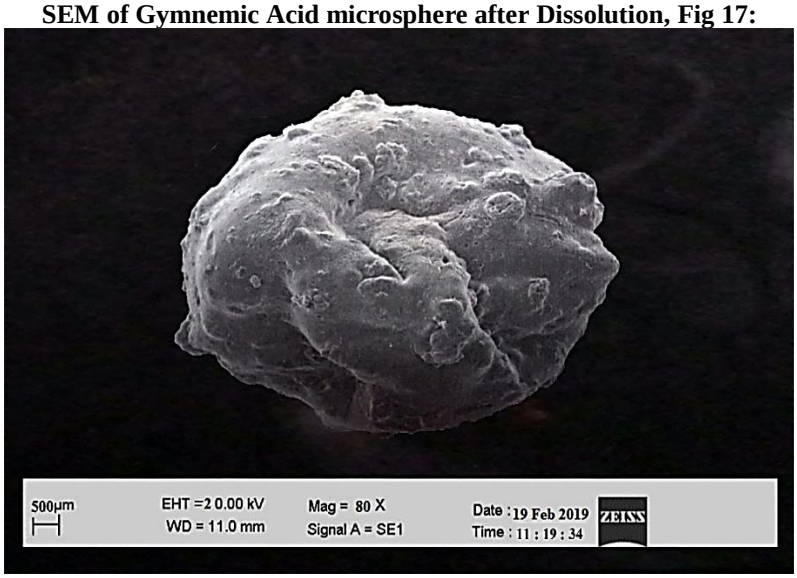


Fig 15: XRD of GymnemicAcid + Excipients

SHAPE AND SURFACE CHARACTERISTICS BY SCANNING ELECTRON MICROSCOPY (SEM)

Fig 16: SEM of GymnemicAcid microsphere before dissolution

SEM of Gymnemic Acid microsphere after Dissolution, Fig 17:



Zeta Potential:

Formulation Code	Zeta Potential (mV)
F1	13.43
F2	14.91
F3	10.31
F4	17.6
F5	15.23
F6	11.78
F7	14.64
F8	9.43
F9	8.64

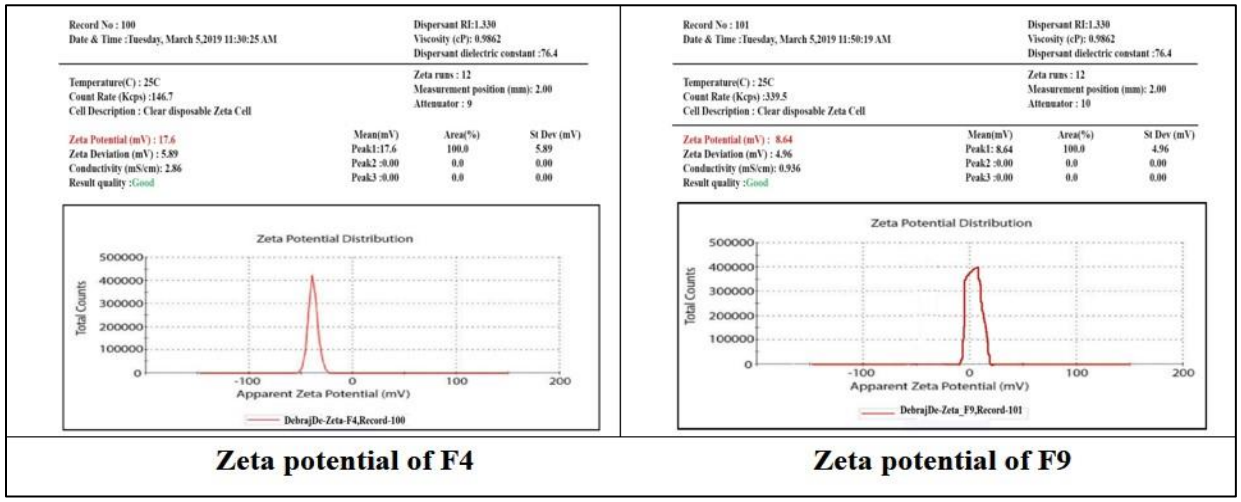


Fig 18: Zeta Potential

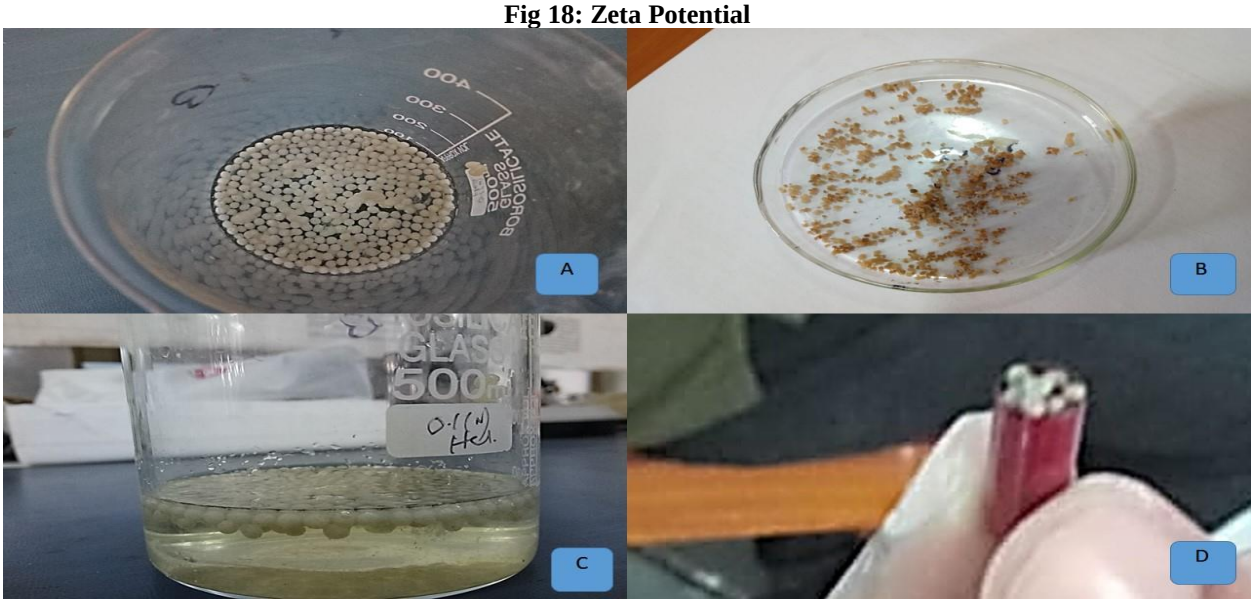


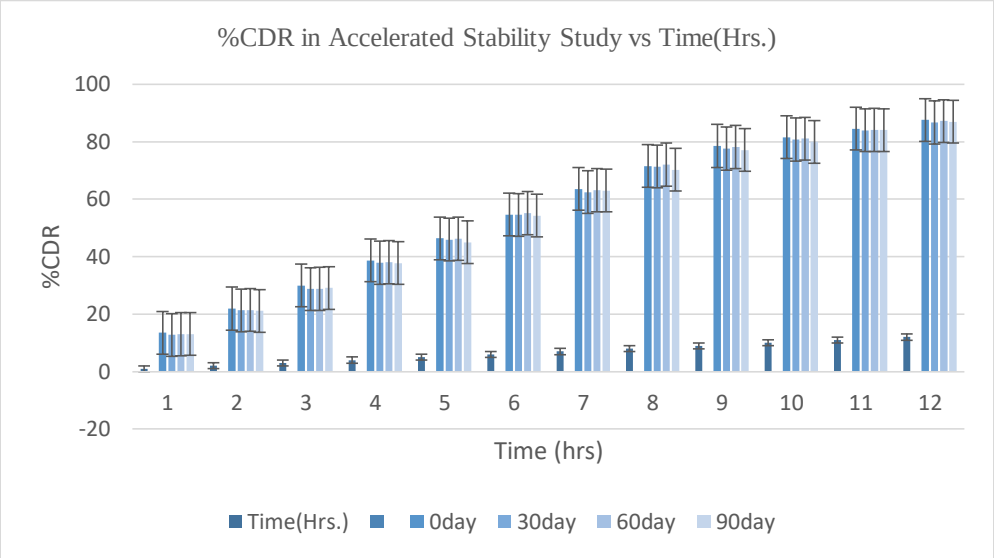
Fig 19: Structure of formulated floating microspheres (A, B, C) and filling of the formulated microspheres in empty capsule shell (D)

ACCELERATED STABILITY STUDY OF PREPARED FLOATING GYMNEMIC ACID MICROSPHERES: Accelerated stability studies were performed on Optimized formulation (F4) as per international conference on harmonization (ICH) guidelines. Stability studies were performed by keeping the sample at accelerated condition. These studies were performed for the period of 3 months.

The formulations were evaluated for parameter i.e. physical appearance ,Drug release, Buoyancy at storage condition at 40ºC ±5ºC / 75% RH ±5ºC at the end of the stability study period.[5]

Time (Hrs.)	0 Day	30Day	60Day	90Day
1	13.56	12.76	12.97	13.05
2	21.92	21.32	21.45	21.11
3	29.97	28.77	28.85	29.10
4	38.67	37.89	38.12	37.73
5	46.4	45.94	46.21	45.02
6	54.69	54.55	55.11	54.26
7	63.56	62.49	63.17	62.98
8	71.55	71.34	72.09	70.23
9	78.56	77.63	78.14	77.09
10	81.55	80.78	81.07	80.01
11	84.55	83.97	84.11	84.06
12	87.58	86.73	87.19	86.99

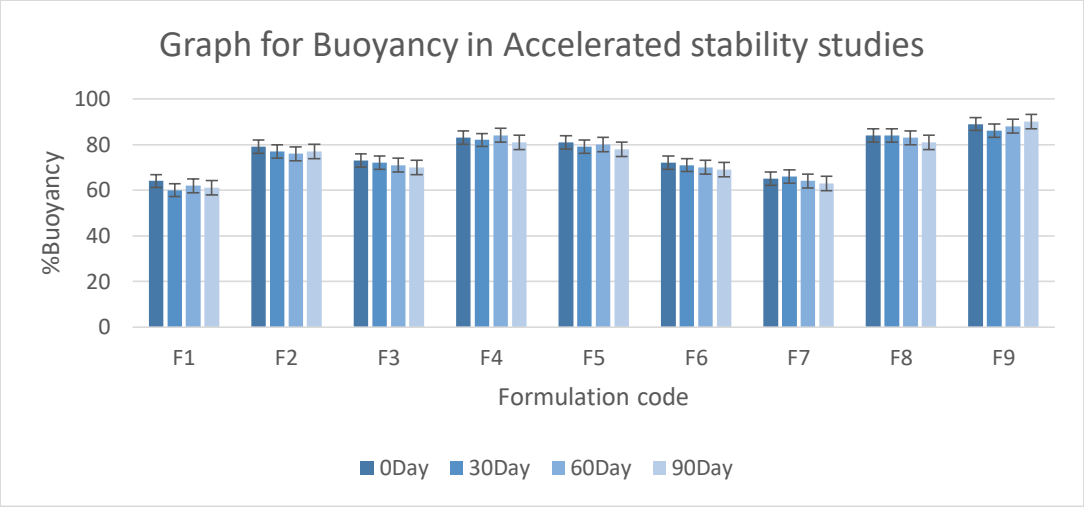
For optimized formulation F4 (%CDR)



Optimized formulation (Fig. 20)

Buoyancy test in Accelerated Stability Testing (Fig 21)

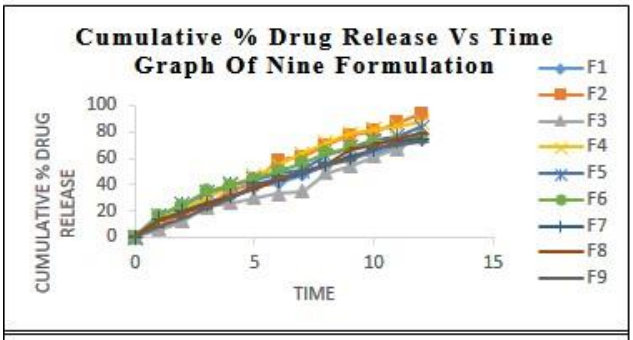
Formulation Code	0Day	30Day	60Day	90Day
F1	64	60	62	61
F2	79	77	76	77
F3	73	72	71	70
F4	83	82	84	81
F5	81	79	80	78
F6	72	71	70	69
F7	65	66	64	63
F8	84	84	83	81
F9	89	86	88	90



IN-VITRO DRUG RELEASE STUDY:

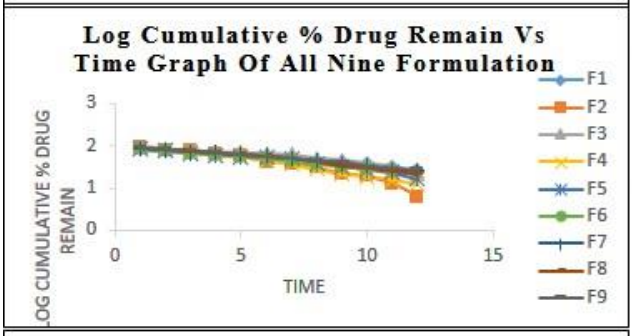
(Cumulative % Drug Release Vs. Time)

Time (Hrs.)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	11.97± 1.71	7.6± 1.5	6.21± 7	13.56± 1.3	16.67± 1.44	16.39± 1.1	13.7± 1.23	12.56± 1.2	7.8± 0.68
2	21.99± 1.33	19.6± 1.5	12.51± 1.6	21.92± 1.8	24.59± 1.3	24.29± 1.1	19.67± 1.8	18.7± 1.7	14.9± 1.5
3	26.4± 1.6	22.7± 1.45	23.07± 1.2	29.97± 1.79	33.9± 1.2	34.7± 1.4	25.7± 1.44	24.89± 1.5	21.98± 1.89
4	31.45± 1.2	35.89± 1.1	26.09± 1.6	38.67± 1.4	40± 1.75	39.67± 1.75	31.45± 1.2	31.5± 1.8	29.78± 1.75
5	38.16± 1.2	41.22± 1.3	29.81± 1.6	46.4± 1.3	43.8± 1.1	44.31± 1.5	37.43± 1.6	37.12± 1.5	39.78± 1.8
6	41.45± 1.4	58.68± 1.8	32.91± 1.8	54.69± 1.3	48.29± 1.8	50.1± 1.8	43.23± 1.23	43.19± 1.1	45.17± 1.3
7	47.6± 1.8	61.26± 1.1	35.19± 1.7	63.56± 1.12	50.78± 1.91	56.73± 1.4	49.17± 1.6	49.27± 1.7	49.79± 1.78
8	54.9± 1.4	69.67± 1.87	49.13± 1.04	71.55± 1.8	62.34± 1.3	63.87± 1.2	55.09± 1.3	55.37± 1.5	55.27± 1.2
9	59.87± 1.23	77.29± 1.3	54.06± 1.9	78.56± 1.6	67.75± 1.5	67.89± 1.9	61.2± 1.98	66.89± 1.44	58.67± 1.66
10	63.41± 1.08	80.37± 1.1	61.32± 1.6	81.55± 1.2	73.67± 1.1	71.78± 1.09	66.89± 1.5	70.6± 1.8	67.75± 1.5
11	68.67± 1.3	87.06± 1.8	67.27± 1.59	84.55± 1.23	76.67± 1.4	73.29± 1.45	72.7± 1.89	74.87± 1.36	70.32± 1.46
12	74.56± 1.1	93.85± 1.12	77.53± 1.2	87.58± 1.5	83.67± 1.6	75.89± 1.41	74.87± 1.26	78.84± 1.36	72.54± 1.4



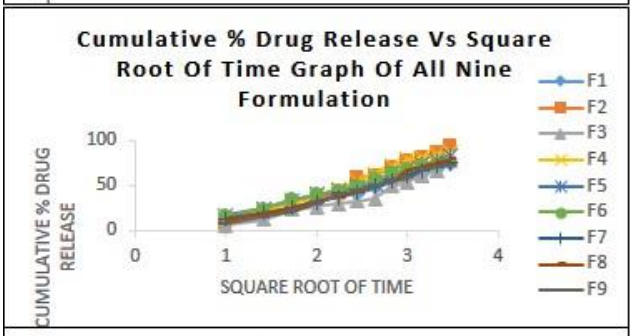
ZERO ORDER DRUG RELEASE KINETICS GRAPHS OF ALL NINE FORMULATION

Fig 22 :Cumulative % drug release vs time graph of all 9 Formulation



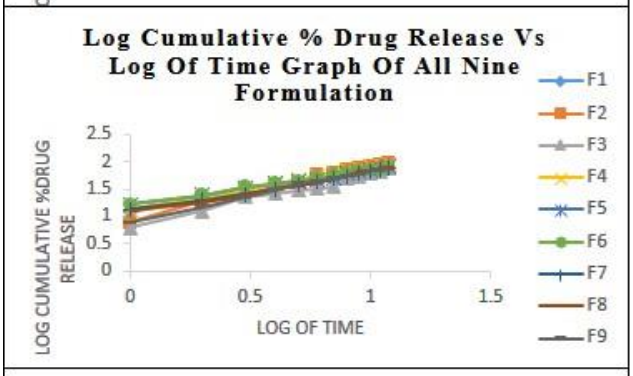
FIRST ORDER RELEASE KINETIC GRAPHS OF ALL NINE FORMULATION

Fig 23 :Log cumulative % drug remain Vs Time graph of all nine Formulation



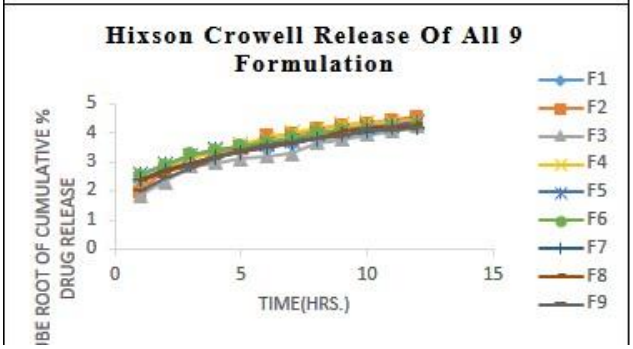
HIGUCHI-MATRIX RELEASE KINETICS GRAPH OF ALL FORMULATION

Fig 24: Cumulative % drug release vs square root of time graph of all nine formulation



KORSMEYER PEPPAS RELEASE KINETICS GRAPH OF ALL FORMULATION

Fig 25 :Log cumulative % drug release vs Log of time graph of All nine Formulation



HIXSON-CROWELL RELEASE OF ALL NINE FORMULATION GRAPH:

Fig 26 :Cube Root Of Cumulative %Drug Reease Vs Time Graph Of All Nine Formulation

RELEASE KINETICS AND MECHANISM DATA OF ALL NINE FORMULATION

Formulation Code	Zero Order		First Order		Higuchi Matrix		Korsmeyer Peppas		Hixson-Crowell	
	R^2	K_0	R^2	K_1	R^2	K_h	R^2	n	R^2	K_{hc}
F1	0.9861	7.9937	0.9127	-0.0497	0.9845	36.428	0.969	0.89	0.9297	0.1815
F2	0.9832	6.0997	0.979	-0.0463	0.9391	27.598	0.927	0.7968	0.915	0.214
F3	0.9863	5.7873	0.9288	-0.0958	0.9846	25.147	0.913	0.6488	0.9137	0.1861
F4	0.9935	7.4046	0.9776	-0.0509	0.979	26.985	0.949	0.8569	0.9699	0.1756
F5	0.9555	5.9901	0.9551	-0.0597	0.9885	32.815	0.976	0.8378	0.9638	0.1627
F6	0.9753	6.3272	0.986	-0.0799	0.9913	25.464	0.933	0.7849	0.9213	0.1446
F7	0.984	6.2104	0.9917	-0.0495	0.9734	28.625	0.973	0.6893	0.9558	0.1519
F8	0.9924	6.473	0.968	-0.057	0.9921	28.019	0.989	0.8238	0.9508	0.1955
F9	0.9906	6.0364	0.9796	-0.0499	0.9811	26.352	0.929	0.7493	0.953	0.1577

In vivo experimentation.[6]

2 groups containing 3 animals in each group were used for performing the experiment. The animals (Male Rabbit, New Zealand White Species) were kept fasting for overnight. Water was given adlibitum during fasting and throughout experiment. Microspheres were swallowed easily without any difficulties. One group containing 3 animals were in Control .Other group was fed with prepared Gymnemic acid floating microspheres (F1, F4,F6,F9 having the drug polymer ratio 1:2.3,1:1.3,1:1.9,1:2.1). Blood samples (1.5ml) were collected from marginal ear vein of animals using xylene into centrifuge tubes containing 0.4ml of 2.5 % (m/v) sodium citrate solution. The same method was followed in all cases at an interval of 30 min, 1 hr., 2, 4, 6, 12, 18 and 24th hr. during study. Absorbance of blood samples (Plasma) were measured in UV-vis spectroscopy (Schimadzu-1700).

In vitro- iv vivo Correlation (IVIVC) According to FDA guidance four levels of IVIVC have been described which are levels A, B, C, and multiple C[54].Here the correlation was established according to Drewe and Grewe (Degree A)[55].The parameters compared were cumulative absorption profile to that of in vitro dissolution i.e. Correlation of the amount of drug dissolved to that of respective fraction of dose absorbed , time taken for 50% dissolution to that of 50% absorbed (T50), In vitro dissolution rate constant (K) Vs. Area Under Curve (AUC) and Mean dissolution time (MDT) versus mean residence time (MRT). [13]

In-vivo pharmacokinetics — study design

In cross over design each rabbit received not more than two formulations in complete study (In each study period one formulation). Each formulation was administered for a total of three times. Each pair of rabbit received same combination of formulations alternatively in each study period. Total study was divided into two study periods. Wash out period was maintained between study periods. Optimized formulations of drugs and pure formulations were taken and finely powdered. Animal dose was calculated based on Km values and about 20mg of drug dose was taken. [7]

Human equivalent dose (mg/kg) × Km value human

Km value of animal = Animal equivalent dose

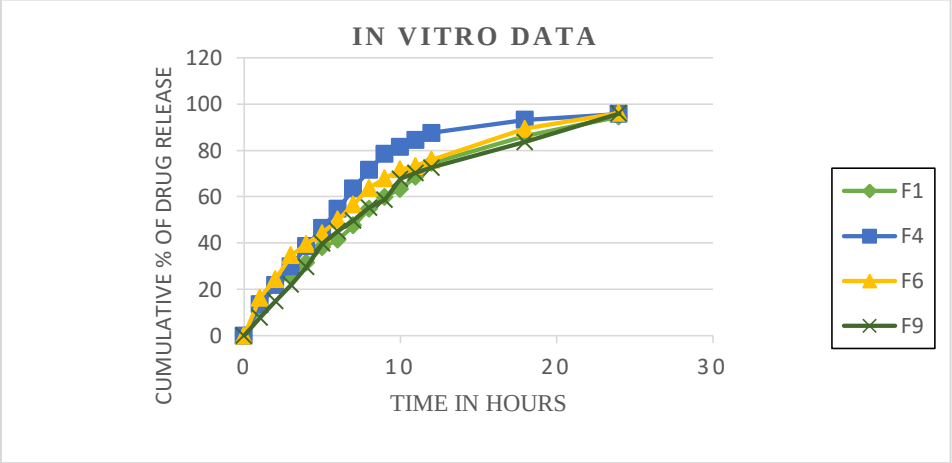
Procedure

Place 1.5 ml of sample (or plasma standard), 2 ml of buffer, and 30 ml of chloroform/isopropanol mixture into a 60-ml separatory funnel. Extract gently for about 5 mm and filter the chloroform through Whatman No. 1 filter paper. Add 3 ml of NaOH to 25 ml of the filtered chloroform and shake gently for 5 mm; use 50-ml round-bottomed centrifuge tubes with ground-glass stoppers. After centrifugation, remove 2.0 ml of the aqueous phase, add 0.1 ml of NH4C1, mix, and determine the ultraviolet absorption spectrum. Use a solution containing NaOH and NH4C1 in the same ratio as the reference solution. If theophylline is present, there will be an absorption peak at 237 nm. Subtract the absorbance (1 cm, 1 g/dl) at 400 nm from that at 200 nm and determine the concentration by comparison with an identically processed plasma standard. Subtracting the absorbance at 300 nm eliminates some baseline errors resulting from traces of endogenous color or turbidity. [8]

IT SHOWS IN VITRO DISSOLUTION PROFILE OF F1, F4, F6 & F9

Time in (Hrs.)	F1	F4	F6	F9
	Concentration			
0	0	0	0	0
1	11.97	13.56	16.39	7.8
2	21.99	21.92	24.29	14.9
3	26.4	29.97	34.7	21.98
4	31.45	38.67	39.67	29.78
5	38.16	46.4	44.31	39.78
6	41.45	54.69	50.1	45.17
7	47.6	63.56	56.73	49.79
8	54.9	71.55	63.87	55.27
9	59.87	78.56	67.89	58.67
10	63.41	81.55	71.78	67.75
11	68.67	84.55	73.29	70.32
12	74.56	87.58	75.89	72.54
18	86.23	93.2	89.36	83.68
24	94.6	95.7	96.17	95.96

IT SHOWS COMPARATIVES CUMULATIVE % OF DRUG RELEASE WITH RESPECT TO TIME (Fig 27)



It Shows Cumulative Percentage of Drug Dissolved Of Different Formulations (In Vivo Data)

Time(Hrs.)	**F1**	**F4**	**F6**	**F9**
	(Concentration in µg/ml)			
0	0	0	0	0
1	1.7	1.9	1.6	2
2	2.6	2.8	2.3	3.2
3	3.2	3.3	3.6	3.9
4	4.1	4.5	4.9	5.1
5	5.5	5.2	5.7	6.1
6	6.1	5.9	6.2	6.4
12	2.8	3.1	2.7	2.9
18	2.4	2.5	2.1	1.9
24	0.9	0.6	0.7	0.5

IT SHOWS CUMULATIVE % F.D ABSORBED OF DIFFERENT FORMULATIONS

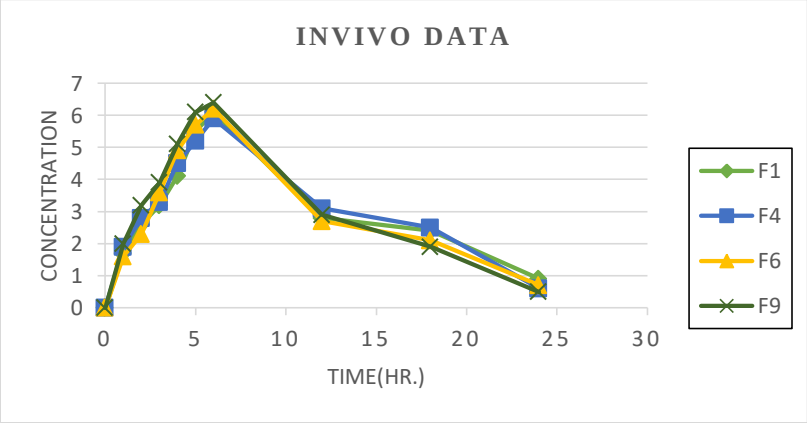
Time in Hr.	F1	F4	F6	F9
	(Concentration)			
0	0	0	0	0
1	10.39	12.96	15.69	6.98
2	19.23	20.32	23.67	15.21
3	25.98	28.87	35.32	22.06
4	30.69	39.42	40.25	28.36
5	38.54	45.93	43.61	38.29
6	42.16	55.48	49.85	44.28
7	49.21	63.89	57.63	50.19
8	56.35	70.64	64.15	54.65
9	60.14	79.12	68.45	59.39
10	62.89	82.28	69.53	68.02
11	70.66	85.26	72.18	69.28
12	76.34	87.58	76.34	71.26
18	93.3	92.8	88.23	81.98
24	93.98	94.78	95.15	93.64

PHARMACOKINETIC PARAMETERS SUCH AS C_{max}, T_{max}, AUC_{0-t}

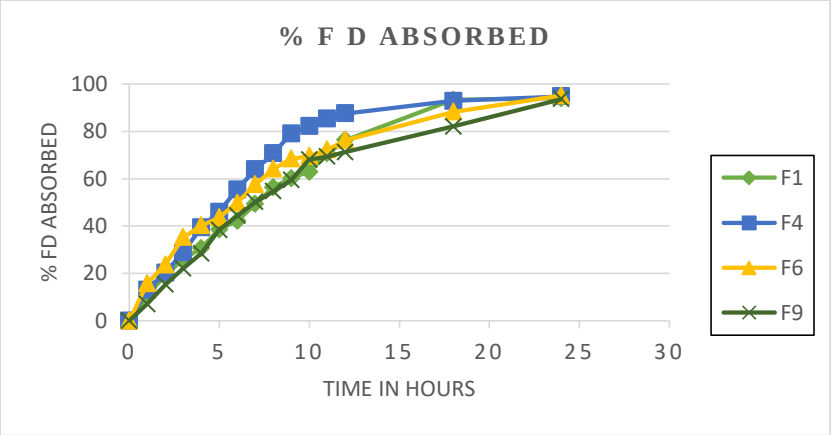
Formulation Code	C_{max} (mcg/ml)	T_{max} (Hrs.)	AUC_{0-t} (µg.h./ml)
F1	6.1	7	64.55
F4	5.9	7	74.35
F6	6.2	7	70.7
F9	6.4	7	75.25

The graphical analysis in Figure 28, 29 and 30 which confirm a good degree of correlation (r^2 =0.9784) and fulfill our objective.

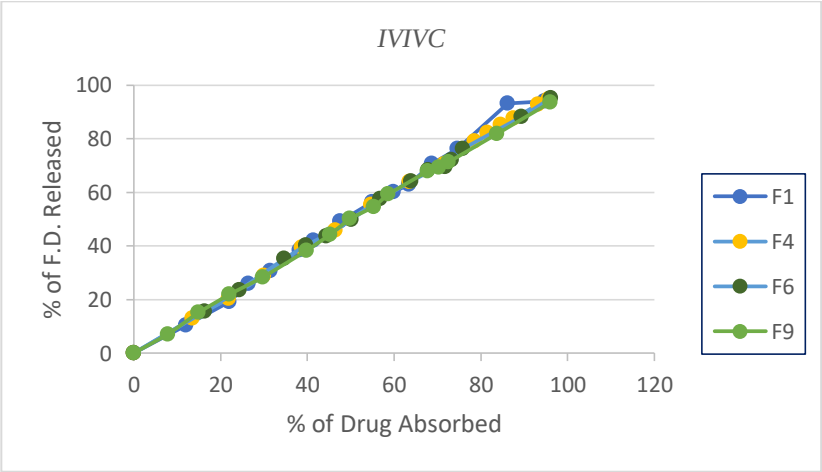
IT SHOWS IN VIVO DATA OF DIFFERENT FORMULATION (Fig 28)



IT SHOWS %FD ABSORBED IN DIFFERENT TIME OF ALL FORMULATION (Fig. 29)



IT SHOWS % FD ABSORBED VS. % F.D. RELEASED OF ALL FORMULATION (Fig.30)



9. CONCLUSION:

The objective of the study is to formulate and evaluate Gymnemic acid floating microspheres by ionotropic gelation method. The Preparation contains nine formulations using different polymers i.e. Sodium Alginate and Microcrystalline cellulose in different ratios. The prepared batches of Gymnemic acid floating microspheres were evaluated for micromeritic studies like bulk density, tapped density, Carr's index (ci), Hauser's ratio, angle of repose, and evaluation studies like in vitro buoyancy, swelling index, drug entrapment efficiency and *invitro* release studies and *IVIVC* level A. The results of Hauser's ratio and angle of repose were found to be 1.11±0.04 to 1.18±0.12 %, 27.58± 0.15 to 29.69± 0.19 respectively. These results show that the formulations have very good flow properties. The drug entrapment efficiency increased from 68.78±0.65 to 77.13±0.78 %, % yield range between 75.9 to 90.11 %, Experimental Drug loading ranges between 27.78 to 55.82, Buoyancy ranges between 64 to 89%, and swelling index ranges between 30.25 to 38.33 %. The percentage of moisture content is in the range of 2.3% to 2.9% .F6 showed the highest value of moisture content which may be due to higher dispersity index and solubility parameter of the polymer used. FTIR spectrum of gymnemic acid physical mixture of gymnemic acid and other excipients, was captured to examine the chemical linkage formed during formulation of floating microsphere .The FTIR spectrum showed the characteristic peaks of pure gymnemic acid powder at 2922.5 cm-1 which signifies presence of C-H Stretching functional group and peak 1635.1 cm-1 shows a presence of C=C Stretching. All the important functional groups. These peaks were remain unchanged or marginal changes in the physical mixture of both components. The SEM photographs of Gymnemic acid microsphere before dissolution shows the spherical and smooth surface whereas after dissolution the pores and crevices were shown which is indicating that the microsphere are showing drug release by erosion mechanism. R2 value of F4 from shown 0.9935 which is following Zero Order drug release and Korsmeyer Peppas (n) value ranging between 0.89 to 0.6488 which indicate

the Non-Fickian transport formulation follows. The formulation of **F4** was chosen as the best optimized floating microsphere of Gymnemic acid formulation with sodium alginate/MCC as the error was minimum (0.01) for the response of the dependable variables. F4 showed good micromeritic properties, entrapment efficiency and releases drug slowly and completely for 12 hours as beads remain in floating condition throughout dissolution study that assures prepared formulation remain floated in stomach without its early passing to lower GIT side.

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