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Research Article

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Design and Optimization of Floating Microspheres Using *Abelmoschus esculentus* Natural Polymer

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Abstract: The main objective of the present research study was to fabricate Vildagliptin floating microspheres by ionotropic gelation using natural polymer, Abelmoschus esculentus obtained from the fruits of Abelmoschus esculentus in combination with sodium alginate. Microspheres were prepared and optimized using central composite rotatable design model using design expert software version 12 .The study is focussed on the interaction effects of the three independent variables, natural polymer concentration, sodium alginate concentration and crosslinker concentration, optimization of formulations response surface methodology was used . drug –excipient compatibility studies were carried out by infrared spectroscopic studies. Infrared spectroscopic studies clearly shown that drug and excipients were compatible. Totally 15 formulations were generated taking 8 factorial points, 6 axial points and 1 centre point. Response surface methodology was used to optimize the formulations**.** To investigate the responses %cumulative drug release, floating time and floating lag time Response surface methodology was used. Polynomial equations and model plots of 3 dimensional model surface plots were generated. Vildagliptin optimized microspheres were formulated and a second order, model quadratic model was used to study influence of formulation factors on response variables. Experimental data of Statistical analysis exhibited good coefficient of regression for cumulative in vitro drug release. Regression F –ratios for the experimental variables were significant. The experimental values and predicted values are agreed. All fifteen formulations exhibited % yield of 94.35-99.99%, particle size of 124-441µm, %swelling index 64.52-89.65%, floating time of 10.16 to 13.16 and floating lag time of 30.31 to 46.98 sec. F4 formulation is optimized based on cumulative % in-vitro drug release at 2nd hour, 12th hour, floating lag time and floating time values. Predicted and observed results are in agreement of 95% confidence intervals. Based on investigation, RSM is the good tool for optimization of formulations.

Key words: Vildagliptin, floating microspheres, Abelmoschus esculentus, natural polymer, optimization, Response surface methodology

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1. INTRODUCTION

Pharmaceutical dosage forms advancements can be related to the utilization of polymers. The drug release pattern is controlled and predicted with the utilization of various pharmaceutical excipients. One of those excipients is natural polymers. Natural polymers are obtained from different sources such as proteins, carbohydrates[1]. The present investigation focussed on design and fabrication of natural polymers in the drug delivery phenomenon. Abelmoschus esculentus is the natural polymer obtained from the fruits of Abelmoschus esculentus. Microspheres are vesicular drug carrier systems consisting of proteins and polymers of biodegradable nature having particle size of 1-1000µm[2]. These are free flowing particulate systems. These are the preferred dosage forms to deliver the drug and to retard the release. In chronic diseases drug administration is on a daily basis. Frequent administration of drugs is not possible in all patients. In this inevitable situation a well organised and fabricated controlled drug delivery makes things easy. Antidiabetic drug administration is one of those situations, to enhance the residence period of short half -life drugs floating drug delivery is silver lining. In this research study, Vildagliptin floating microspheres were prepared using natural polymers. Vildagliptin is a short half- life drug and eliminates quickly from the body in a period of 90 min. it exhibits more stability in the acidic environment and degrades in the basic environment. Abelmoschus esculentus polymer is a macromolecule obtained from Abelmoschus esculentus fruits , natural plant origin. Natural polymers are safe to administer, easily available, biodegradable and bio compatible. From Therapeutic consideration point of view 50mg twice daily Vildagliptin monotherapy is approved for type 2 diabetes mellitus treatment, risk point of view there is no extra risk associated with this. Vildagliptin 50 mg twice daily dosage regimen has a prominent role as disease progresses[3]. Due to direct inhibition of overnight hepatic glucose production[4], Vildagliptin is an interesting option for type 2 diabetes mellitus treatment. In this investigation twice daily dosage regimen is converted to once daily which reduces the frequency of administration. Floating microspheres are the low density micro particles prepared with polymer composites and absorption in the stomach region is promoted by enhancing the residence time. Because of these reasons necessitated the formulation of floating micro beads. Novelty of this work is usage of natural polymer to control the drug release and Design expert software version 12 which is advanced software for designing and optimization of formulations. Gastro retentive drug delivery and its absorption is linked with absorption of drugs in the upper gastric region. It is a well proven fact that the extent of gastrointestinal tract absorption and contact time in the small intestinal mucosa is correlated. The drug release in the upper git is well accomplished by using a floating drug delivery approach. Extended retention ability gives beneficial effects like improved activity span for short half-life drugs, bioavailability of drugs, dosage periodicity reduction and optimized therapy.[5] Amit K.Nagariya et.al. Investigated natural polymers provide potential in the development of gastro retentive floating drug delivery system[6]. Abelmoschus esculentus natural polymer use is significant based on biocompatibility and safety. Okra gum is high viscosity mucilage at low concentrations. It is obtained from the pods of Abelmoschus esculentus. It is a polysaccharide and hydrophilic and it consists of rhamnose, galactose and galacturonic acid. This gum is used in the formulation of sustained release tablets.[7,8] Saravana Kumar. K. et. al. Studied that polymer concentration has effect on drug release and to enhance the drug release in sustained way in floating tablets of glibenclamide which is antidiabetic drug.[9] Wide varieties of natural and synthetic hydrophilic polymers are used in sustained release dosage forms.[10,11] The challenging area in the manufacturing process is development and optimization of dosage forms. [12] Response surface methodology (RSM) is a widely used advanced method for designing formulations, statistical process control. Effort was made towards successful optimization of formulations using design expert software version 12.

2. METHODOLOGY

2.1 Drug excipient compatibility studies

Compatibility must be established between the active ingredient and other excipients to produce a stable efficacious, attractive and safe product. Hence, before producing actual formulation, compatibility of Vildagliptin with Abelmoschus esculentus polymer was tested using the Fourier Transform Infrared Spectroscopy(FT-IR) technique. Fourier Transform Infrared (FT-IR) spectra were obtained by using an FT-IR spectrometer (shimadzu FT-IR 8400) the samples (Vildagliptin and excipients) were previously ground and mixed thoroughly with mixed potassium bromide, an infrared transplant matrix, at 1:5 (sample /KBr) ratio, respectively. The KBr discs were prepared by compressing the powders at a pressure of 5tons for 5minutes in a hydraulic press. Scans were obtained at a resolution of 4Cm $^{-1}$, from 4000-400 cm^{-1}[13].
The FT-IR values were interpreted and are reported.

2.2 Formulation of microspheres using central composite rotatable design CCD

Formulation and optimization of drug loaded microbe utilized central composite rotatable design using design expert (version 12, stat-ease inc) software. In this model three formulation factors with 2 levels were designated as AE: Abelmoschus esculentus polymer concentration (1.5-2.5%), SA: Sodium alginate concentration (3-3.5%) ,C: crosslinking agent concentration calcium chloride(4-5%), and drug concentration kept, 100mg constant. In this experimental data the coefficients of the model are represented by constant terms.

AE- Abelmoschus esculentus polymer concentration
SA- sodium alginate concentration
C- cross- linker concentration
AB- AE &SA polymer interactive term
AC- AE & C interactive term
BC- SA & C interactive term
A^2, B2,C2- quadratic term coefficients

By using the RSM mathematical model, interaction between 2 polymer concentrations, polymer

concentration and cross linker concentration mathematical modeling of the system is made possible. RSM saves time and cost by reducing the number of trials. The obtained experimental data are fitted to a polynomial model at 2nd level. In this investigation design variables effect was determined. Response surface methodology RSM was used to evaluate the influence of formulation factors polymer concentration and cross linker concentration on response variables of cumulative in-vitro drug release at 2nd hour and cumulative in-vitro drug release at 12th hour, floating time and floating lag time. By using this CCRD model, coefficient of regression, analysis of variance and percent coefficient variance were determined. Overall 15 runs were generated taking 8 factorial points, 6 axial points and 1 centre point.

2.3 II Evaluation of microspheres

2.3.1 *In-vitro* evaluation

1. Percentage yield
2. Particle size
3. Swelling studies
4. Floating time
5. Floating lag time
6. In-vitro drug release studies
7. SEM analysis1. Percentage yiel

2.3.2. Percentage yield

The yield was calculated by dividing the weight of the collected beads by the total weight of all the non-volatile components used for the preparation of beads and expressed in terms of percentage[14].

2.3.3. Particle Size Analysis

Particle size analysis of drug-loaded micro particles was performed scanning electron microscopy[15].

2.3.4. Swelling measurement

The extent of swelling was measured in terms of increase in particle size using optical microscopy. The swelling ratios of all formulations of Vildagliptin micro particles were studied. In this test few of micro particles from each formulation were kept in petri dishes containing p^H 1.2. The swelling index (S_w) was calculated according to the following equation[16]:
Swelling index

$$(SW) = \frac{W_t - W_o}{W_o}$$

Where W_o is the initial weight
And W_t is the weight of the swollen micro particles at time *t*.

2.4 *In-Vitro* Dissolution Studies

The physicochemical property of most drugs that has the greatest influence on their absorption characteristics from the GIT is dissolution rate. "The drug is expected to release from the solid dosage forms (granules, tablets, capsules etc.) and immediately go into molecular solution. This process is called `` dissolution". *Apparatus:* - USP type II dissolution apparatus (paddle)

Procedure

The study was carried out in a USP type II dissolution apparatus (paddle). Dissolution fluid consists of 900ml of simulated gastrointestinal fluids of increasing pH namely pH 1.2 (12 hrs) maintained temperature at 37°C ±0.5°C and the paddle was rotated at a constant speed of 50rpm. Aliquots of samples were withdrawn after predetermined periods of time and the same volume of fresh medium was added immediately to the test medium. The withdrawal samples were filtered through a 0.45µm membrane filter. The drug content was determined in the filtrate after appropriate dilution and analysed at 208 nm spectrophotometrically using Shimadzu 1800 UV-visible spectrophotometer[17,18].

Floating properties

2.5 Floating lag time

Weigh 100 mg of beads into a dissolution vessel containing 900 ml of 0.1 N Hcl , PH 1.2 at 37°C ±0.5°C. The time taken by the formulation to emerge onto the surface of dissolution medium is noted as floating or buoyancy lag time [19].

5. Total Floating time

100 mg of the microspheres were weighed and placed in 0.1 N H cl, PH 1.2 at 37°C ±0.5°C , under the influence of 50rpm paddle rotational speed is recorded [20]SEM analysis: The samples were given a conductive coating of 600A° in thick, sputter ion coater. These microspheres were examined for Surface morphology and pore size of optimized batch microspheres(F4) were observed with the sca nning electron microscope (Jeol 6390LA)[21].

3. STATISTICAL ANALYSIS

The obtained experimental data were analysed using Design expert software version 12. Analysis of variance was used and the data were presented as mean ± standard deviation (SD). Confidence, prediction, or tolerance intervals (CI, PI, TI) to its graphical optimization plots.

4. RESULTS AND DISCUSSION

FTIR results

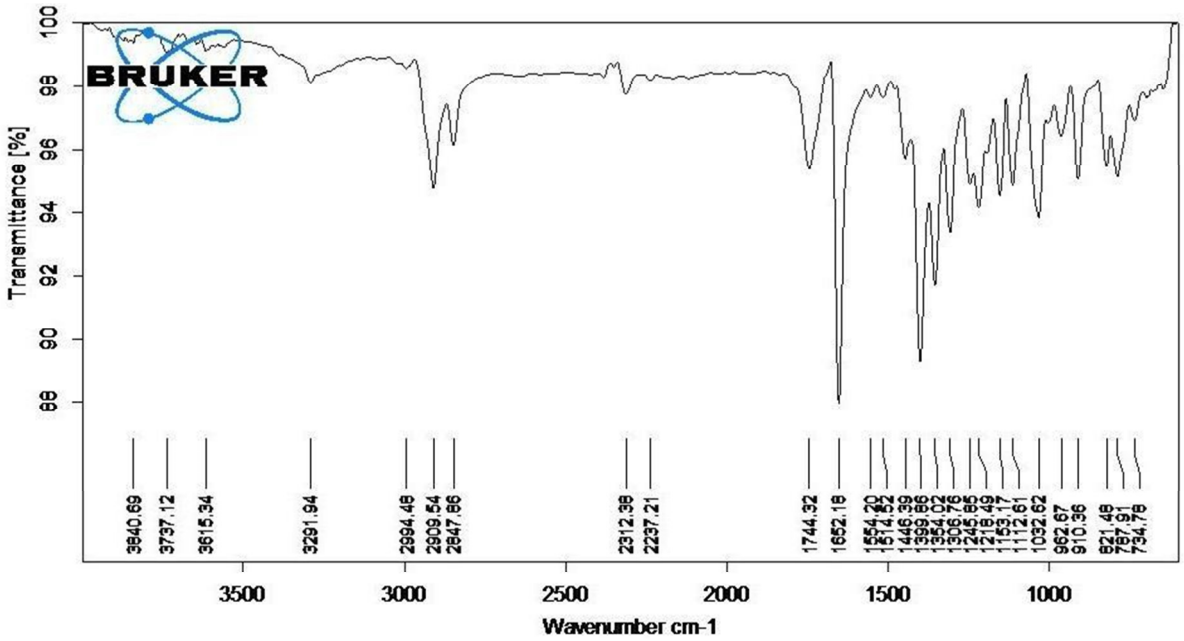


Fig: 1- FTIR Spectrum of Pure Vildagliptin

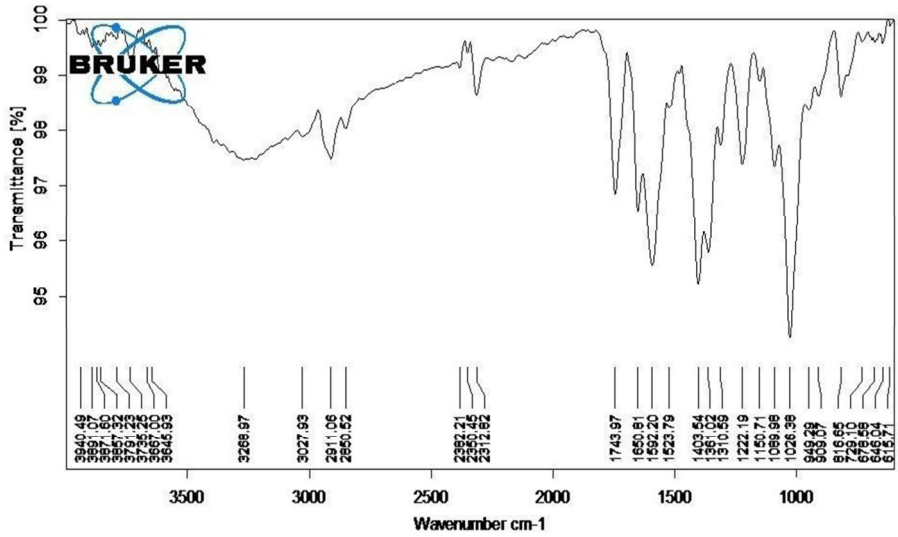


Fig: 2- Vildagliptin+excipients

The standard FTIR spectra of Vildagliptin were compared with the spectrum of Vildagliptin sodium alginate mixture excipients.i.e. Abelmoschus esculentus polymer. The spectrum of Vildagliptin is portrayed by the presence of carbonyl group (c=o) at 1744.32cm^{-1} , the peak of carbonyl group in Vildagliptin +excipients was found to be at 1743.97.00cm^{-1} It was predicted that peak of carbonyl group of Vildagliptin +excipients was similar to the peak of Vildagliptin. The nitrile group(C-N) in the Vildagliptin spectrum is at 2237.21cm$^{-1.}$ The peak of nitrile group in Vildagliptin+ excipients was found to be at 2312.82cm$^{-1.}$ It was interpreted that the peak of the nitrile group in Vildagliptin + excipients is analogous with that of the standard Vildagliptin spectrum. Also the alkane group is located at 2847.86cm^{-1} in standard Vildagliptin spectrum and the alkane group is found at nearest frequency in the spectrum of drug-excipient mixture i.e., at 2846.91cm^{-1}. The alcohol group (O-H) is found at 3615.34cm^{-1} in Vildagliptin spectrum and frequencies of about 3645.93cm^{-1} were observed in case of Vildagliptin –excipients mixture. Thus from the observations of the FTIR spectrum it was concluded that the mixture of drug and drug- excipient natural polymer mixture was found to be the most compatible[22] one with the Vildagliptin spectrum with no well-defined chemical interaction ,as no new peaks were observed. Hence this excipient mixture was selected for the formulation of a floating drug delivery system. In the present investigation Vildagliptin floating microspheres were developed utilizing ionotropic gelation method. These developed formulations were evaluated for various in-vitro parameters. Response surface methodology is a widely used experimental method introduced by box and Wilson. With the help of these statistical and

mathematical optimization techniques, it is used for investigation of relationship between one or more response variables[23].Different formulations were manufactured using Abelmoschus esculentus polymer concentration(1.5-2.5%), sodium alginate concentration(3-3.5%) and calcium chloride concentration (4-5%). these combination formulations were generated using design-expert® version 12 software presented in table 1 .

4.1 Microspheres were prepared using an ionotropic gelation method.

Table 1a: levels of variables for optimization

Factor	Code	Units	Type	minimum	Maximum	Coded low	Coded high	Mean	Standard deviation
A	AE	%	Numeric	1.16	2.84	1 ↔ 1.50	1 ↔ 2.5	2.00	0.4938
B	SA	%	Numeric	2.83	3.67	1 ↔ 3.00	1 ↔ 3.5	3.25	0.2469
C	Cacl2	%	Numeric	3.66	5.34	1 ↔ 4.00	1 ↔ 5.00	4.50	0.4938

Table 1b: Concentration of variables

Run number	Concentration of AE polymer (%)	Concentration of AE polymer (%)	Concentration of AE polymer (%)
	2	3.25	3.66
	2.5	3	4
	1.16	3.25	4.5
	2.84	3.25	4.5
	1.5	3	4
	2	3.25	4.5
	1.5	3.5	5
	2	3.67	4.5
	2	3.25	5.34
	2	2.83	4.5
	2.5	3.5	4
	2.5	3	5
	1.5	3	5
	1.5	3.5	4
	2.5	3.5	5

Table 2: *in-vitro* evaluation parameters

S.no	Formulation Code	% yield	Particle size(µm)	Swelling index	Floating time(hour)	Floating lag time(s)
1.	F1	99.98±0.02	140 ± 0.2	78.32±0.02	12.45±0.032	42.21±0.21
2.	F2	96.36±0.12	290 ± 0.6	89.25±0.03	11.84±0.21	39.21±0.54
3.	F3	97.89±0.23	385 ± 0.4	88.64±0.2	12.7±0.01	36.94±0.005
4.	F4	99.99±0.41	420 ± 0.8	89.65±0.05	12.88±0.01	30.31±0.001
5.	F5	99.99±0.54	441 ±0.6	87.58±0.04	10.86±0.02	44.83±0.003
6.	F6	96.35±0.32	421 ± 0.6	75.68±0.01	13.02±0.23	37.1±0.004
7.	F7	97.12±0.21	240±0.02	65.47±0.21	10.95±0.52	41.05±0.014
8.	F8	94.85±0.22	125±0.02	74.21±0.21	10.64±0.26	36.96±0.005
9.	F9	95.68±0.02	124±0.01	87.98±0.01	13.16±0.24	37.95±0.004
10.	F10	98.65±0.01	213±0.05	85.67±0.02	10.16±0.54	44.21±0.002
11.	F11	97.21±0.01	241±0.14	84.12±0.04	11.02±0.01	36.98±0.05
12.	F12	98.65±0.05	200±0.21	87.24±0.08	11.11±0.01	46.98±0.002
13.	F13	94.35±0.03	214±0.03	75.64±0.05	12.05±0.02	40.08±0.001
14.	F14	98.75±0.03	256±0.06	78.45±0.050	11.98±0.21	38.09±0.005
15.	F15	96.68±0.02	241±0.08	64.52±030	11.84±0.03	35.39±0.003

Values are expressed mean ± SD, n=3

4.2 *Optimization of formulations using surface response methodology*

RSM is a mathematical and statistical tool widely used for optimization[24]. Optimization by RSM method involves three major steps; these are firstly statistically designed experiments, secondly, estimate the coefficients in a mathematical model and finally predicting the response and checking the adequacy of the model within the setup of the experiment[25]. This study is based on RSM to optimize and observe the influence of formulation variables (AE,SA,Cacl2) on response variables like floating lag time, floating time, cumulative in-vitro drug release at 2nd hour and cumulative in-vitro drug release at 12th hrs[27]. Centre composite design, quadratic model were applied to investigate the responses. Statistical model is fitted and response variables are presented in table.3-6.

ANOVA for Quadratic model [26]

Response 1: cumulative drug release at 2nd hour CDR2 (quadratic model)

Table 3. statistical summary of response variables-CDR2

Factors	Sum of Squares	df	Mean Square	F-value	p-value	
Model	449.57	9	49.95	9414.98	< 0.0001	significant
A-AE	179.53	1	179.53	33837.72	< 0.0001	
B-SA	0.0336	1	0.0336	6.33	0.0534	
C-Cacl2	33.67	1	33.67	6345.32	< 0.0001	
AB	5.25	1	5.25	989.30	< 0.0001	
AC	51.00	1	51.00	9613.48	< 0.0001	
BC	27.98	1	27.98	5272.80	< 0.0001	
A^2	13.43	1	13.43	2530.51	< 0.0001	
B^2	105.30	1	105.30	19847.86	< 0.0001	
C^2	1.15	1	1.15	216.61	< 0.0001	
Residual	0.0265	5	0.0053			
Cor Total	449.59	14				

ANOVA for Quadratic model
Response 2: cumulative %drug release at 12th hour(CDR12)

Table 4.statistical summary of response variables-CDR12

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	199.46	9	22.16	58.91	0.0002	significant
A-AE	0.0201	1	0.0201	0.0535	0.8263	
B-SA	2.67	1	2.67	7.11	0.0446	
C-Cacl2	0.0236	1	0.0236	0.0626	0.8123	
AB	57.35	1	57.35	152.44	< 0.0001	
AC	68.68	1	68.68	182.55	< 0.0001	
BC	58.10	1	58.10	154.44	< 0.0001	
A^2	8.38	1	8.38	22.28	0.0052	
B^2	1.69	1	1.69	4.49	0.0877	
C^2	9.13	1	9.13	24.26	0.0044	
Residual	1.88	5	0.3762			
Cor Total	201.34	14				

ANOVA for Quadratic model
Response 3: floating time

Table 5. Statistical summary of response variables- floating lag time

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	12.54	9	1.39	478.46	< 0.0001	significant
A-AE	1.36	1	1.36	468.61	< 0.0001	
B-SA	1.08	1	1.08	370.45	< 0.0001	
C-Cacl2	2.50	1	2.50	858.72	< 0.0001	
AB	3.11	1	3.11	1068.93	< 0.0001	
AC	0.9591	1	0.9591	329.39	< 0.0001	
BC	0.0210	1	0.0210	7.22	0.0435	
A^2	0.0671	1	0.0671	23.06	0.0049	
B^2	1.82	1	1.82	626.61	< 0.0001	
C^2	1.92	1	1.92	659.31	< 0.0001	
Residual	0.0146	5	0.0029			
Cor Total	12.55	14				

ANOVA for Quadratic model

Response 3: floating lag time

Table 6. Statistical summary of response variables-floating time

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	246.37	9	27.37	11950.95	< 0.0001	significant
A-AE	5.22	1	5.22	2278.33	< 0.0001	
B-SA	38.84	1	38.84	16957.20	< 0.0001	
C-Cacl2	1.42	1	1.42	619.00	< 0.0001	
AB	4.90	1	4.90	2138.52	< 0.0001	
AC	39.69	1	39.69	17329.23	< 0.0001	
BC	49.10	1	49.10	21437.35	< 0.0001	
A^2	57.62	1	57.62	25154.56	< 0.0001	
B^2	104.13	1	104.13	45461.11	< 0.0001	
C^2	39.62	1	39.62	17298.03	< 0.0001	
Residual	0.0115	5	0.0023			
Cor Total	246.38	14				

4.3 Effect Of Formulation Variables On Cumulative Invitro Drug Release At 2nd Hour Using RSM

The predicted cumulative in-vitro drug release at 2nd hour values are denoted in the following coded equation as: $CDR2=24.71+3.63A+0.0496B+1.57C+0.81AB-2.52AC-1.87BC+1.49A^2+4.17B^2+0.4357C^2$ The given equation in terms of coded factors can be used to make predictions about responses of given levels of factors that are coded as +1 and low levels are coded as -1. The coded equation is useful for identifying the relevant impact of factors by comparing the factor coefficients. The predicted R2 of CDR2 is 0.9990 and adjusted R 2 is 0.9998. The difference is less than 0.2. Adequate precision AP determining variation of response target, under varying noise conditions, indicating adequate signal, which is greater than 4 and desirable. P-value is less than 0.05 indicating model terms are significant. A, C, AB, AC, BC,A2,B2,C2 are significant terms. Cumulative drug release of F4, F8, F10, F13, F14, and F15 exhibited acceptable results. These exhibited less than 30% drug release in 2 hours duration which indicates not a burst release [1,2,3,4,28]. The effect of formulation variables on the *invitro* drug release at 2nd hour was illustrated in fig .3a, 3b and 3c

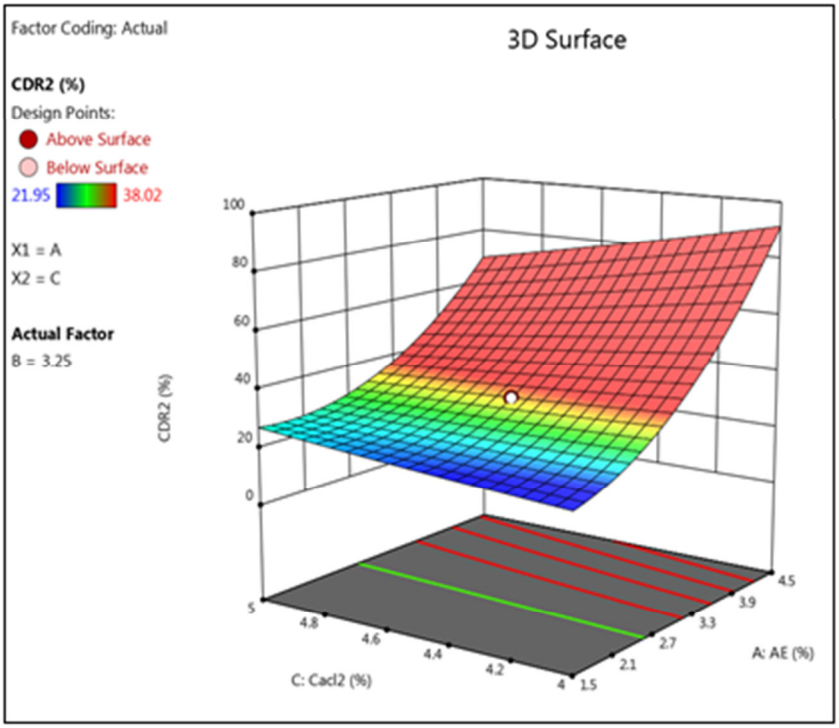


FIG.3a. 3D contour -graphical representation of effect of AE polymer , crosslinker on CDR2

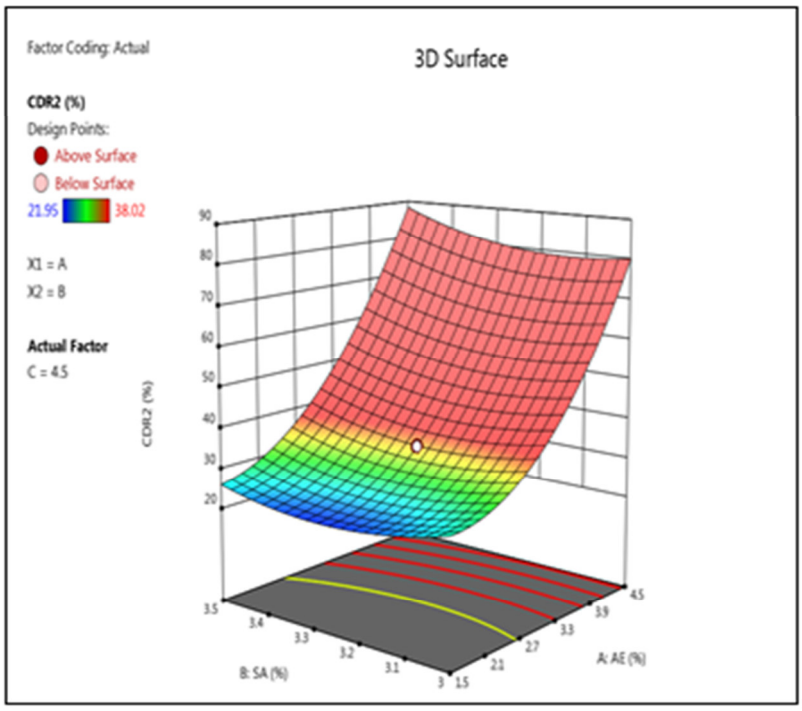
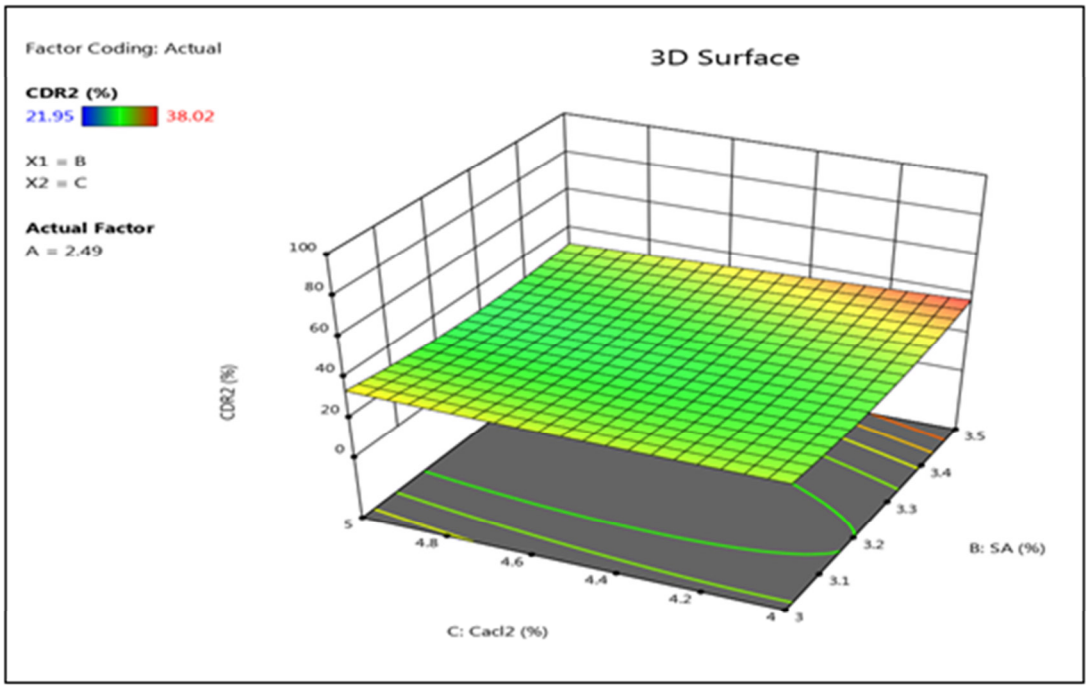


FIG.3b. 3D contour -graphical representation of effect of AE polymer, sodium alginate on CDR2



IG.3c. 3D contour -graphical representation of effect of sodium alginate, cross-linker on CDR2

4.4 *Effect Of Formulation Variables On Cumulative Invitro Drug Release At 12th Hour Using RSM*

The predicted cumulative in-vitro drug release at12th hour values are denoted in the following coded equation as: CDR12=100.01-0.0384 A+0.4425 B+0.0415C-2.68AB+2.93AC+2.7BC-1.18A^2-0.5281B^2-1.23C^2 The Predicted R^2 of 0.9203 is in reasonable agreement with the Adjusted R^2 of 0.9738; i.e. the difference is less than 0.2. Adequacy of Precision measures the signal to noise ratio[29]. A ratio greater than 4 is desirable. The ratio of 24.077 indicates an adequate signal. This model can be used to navigate the design space. Cumulative drug release of F1, F2, F3, F4, F10, F11, F12 exhibited acceptable results. These exhibited 99-100% drug release at the end of 12th hour duration which indicates complete release of the loaded drug which is a desirable result. The effect of formulation variables on cumulative drug release at 12th hour was illustrated in fig. 4a, 4b and 4c.

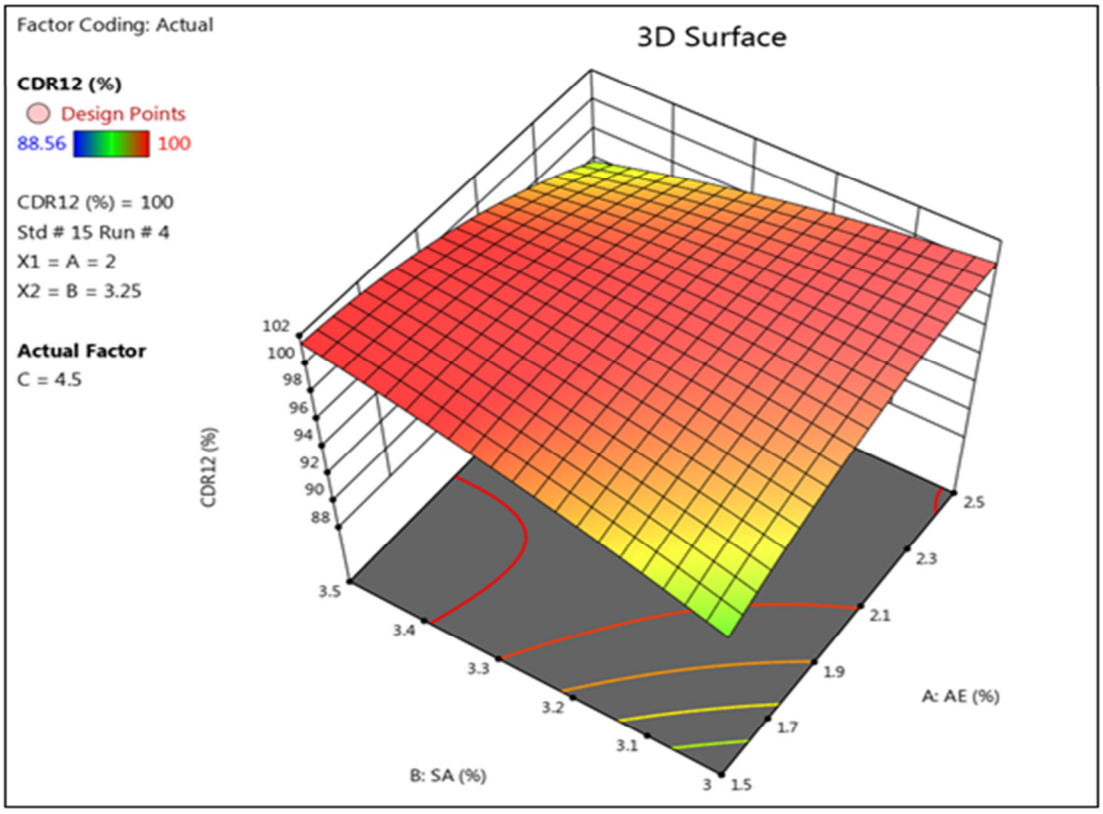


FIG.4a. 3D contour -graphical representation of effect of AE polymer , sodium alginate on CDR12

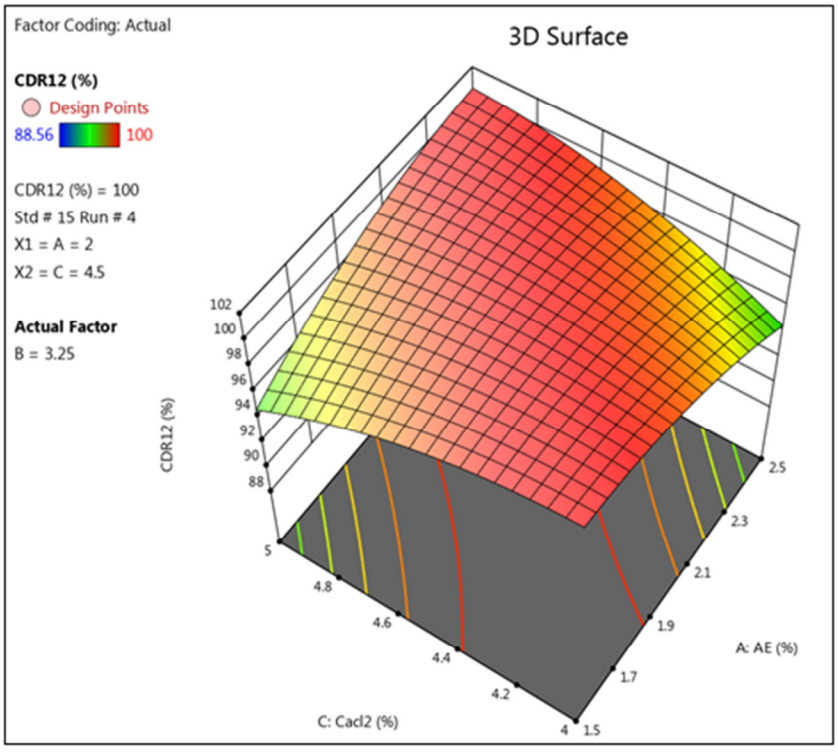


FIG.4b. 3D contour -graphical representation of effect of AE polymer, sodium alginate on CDR12

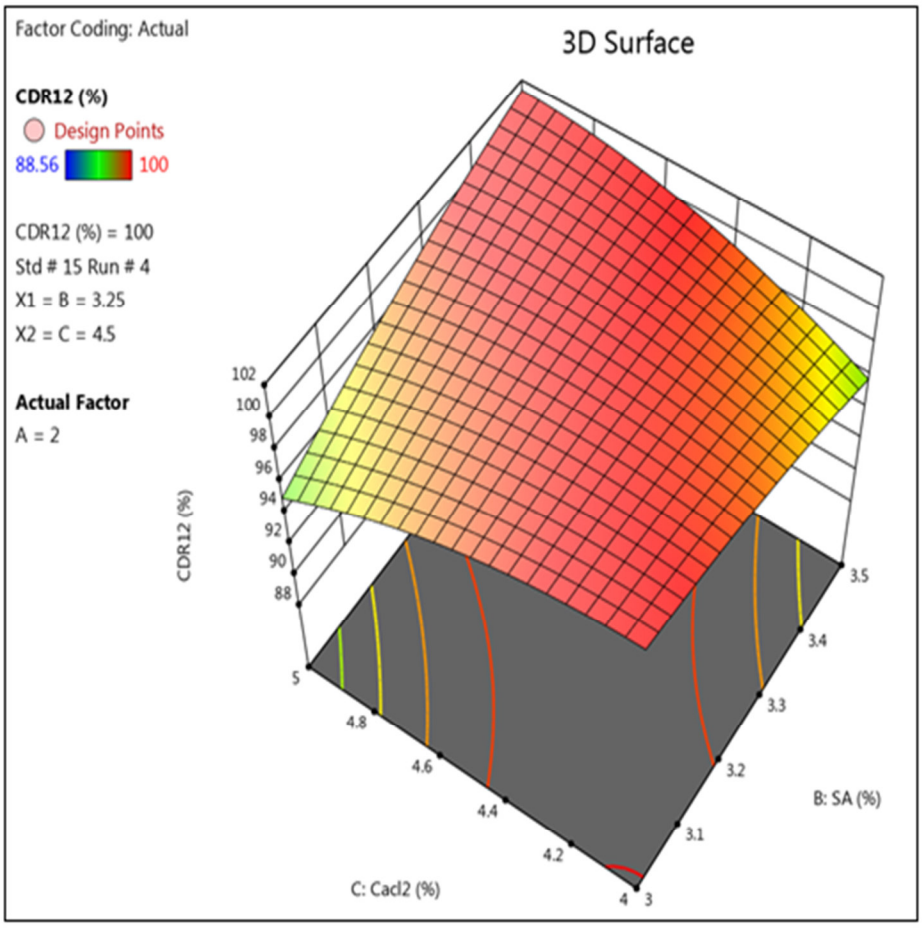


FIG.4c. 3D contour - RSM graphical representation of sodium alginate, crosslinker on CDR12

4.5 Effect Of Formulation Variables floating lag time Using RSM

The predicted floating lag time values are denoted in the following coded equation as: Floating lag time =30.30-0.6182A-1.69B-0.3222C-0.7825AB-2.23AC+2.48BC+3.09A^2 +4.15B^2+2.56C^2 The Predicted R^2 of 0.9987 is in reasonable agreement with the Adjusted R^2 of 0.9999; i.e. the difference is less than 0.2. Adequacy Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 426.578 indicates an adequate signal. This model can be used to navigate the design space. Floating lag time of 30-32 sec is taken as the range in the optimization of formulation. The formulation exhibiting less time to emerge to the surface of the liquid i.e. less than half minute is basis point. In this study short floating lag time and long floating time formulation characterstics taken into selection criterion[30]. F4 formulation exhibited less floating lag time of 30.31 sec. The effect of formulation variables on floating time and floating lag time was presented in fig.5a, 5b, 5c, 6a, 6b and 6c. Using overlay plot.

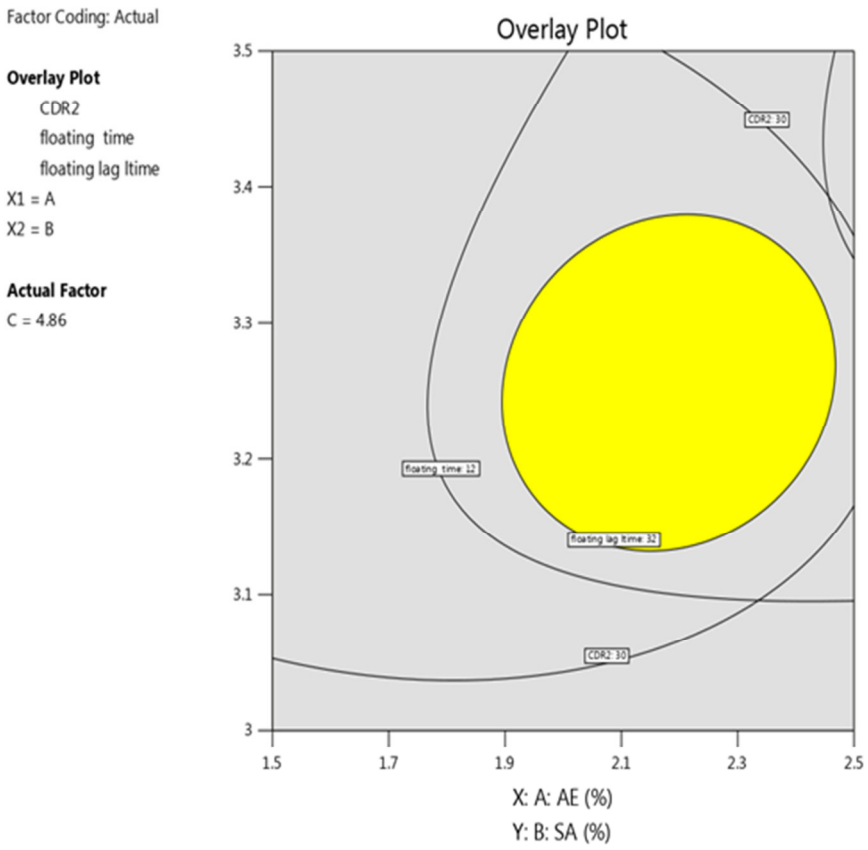


FIG.5a. overlay plot of effect of AE polymer, sodium alginate on floating lag time

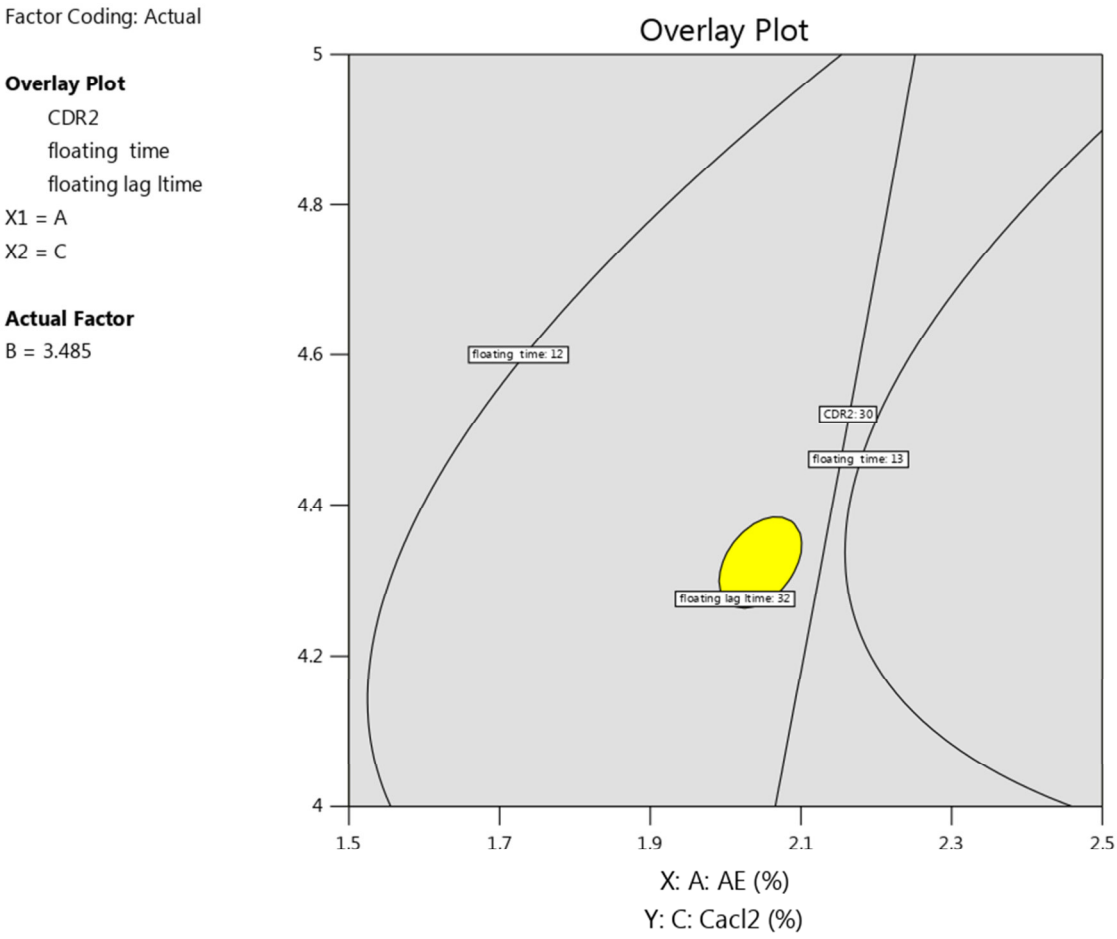


FIG.5b. overlay plot of effect of AE polymer, crosslinker on floating lag time

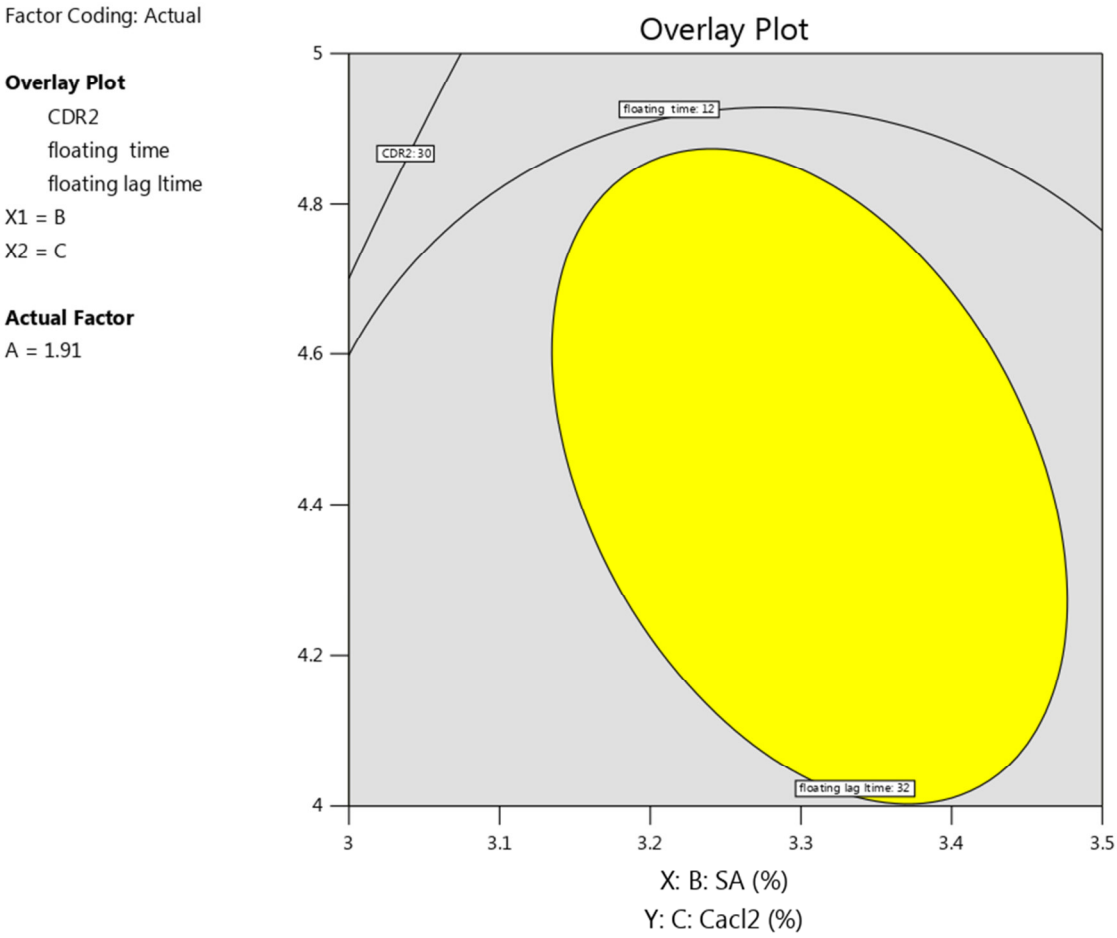


FIG.5c.over lay plot-graphical representation of sodium alginate and crosslinker on floating lag time and floating time

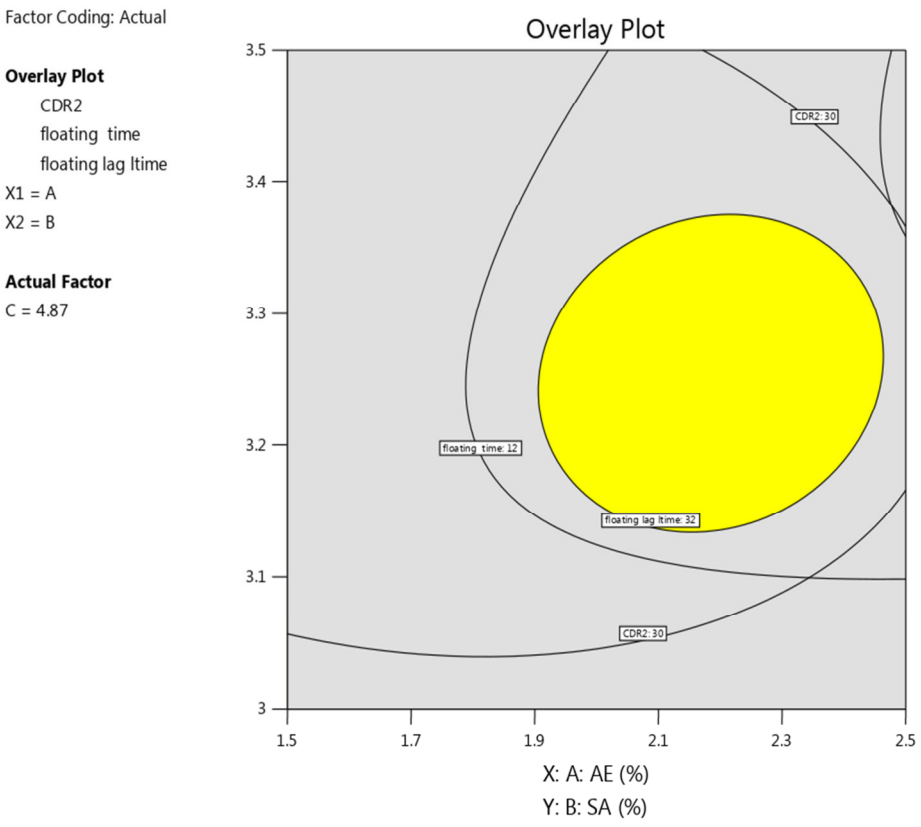


FIG.6a. overlay plot of effect of AE polymer, sodium alginate on floating lag time

Factor Coding: Actual

Overlay Plot

CDR2

floating time

floating lag ltime

X1 = A

X2 = C

Actual Factor

B = 3.48

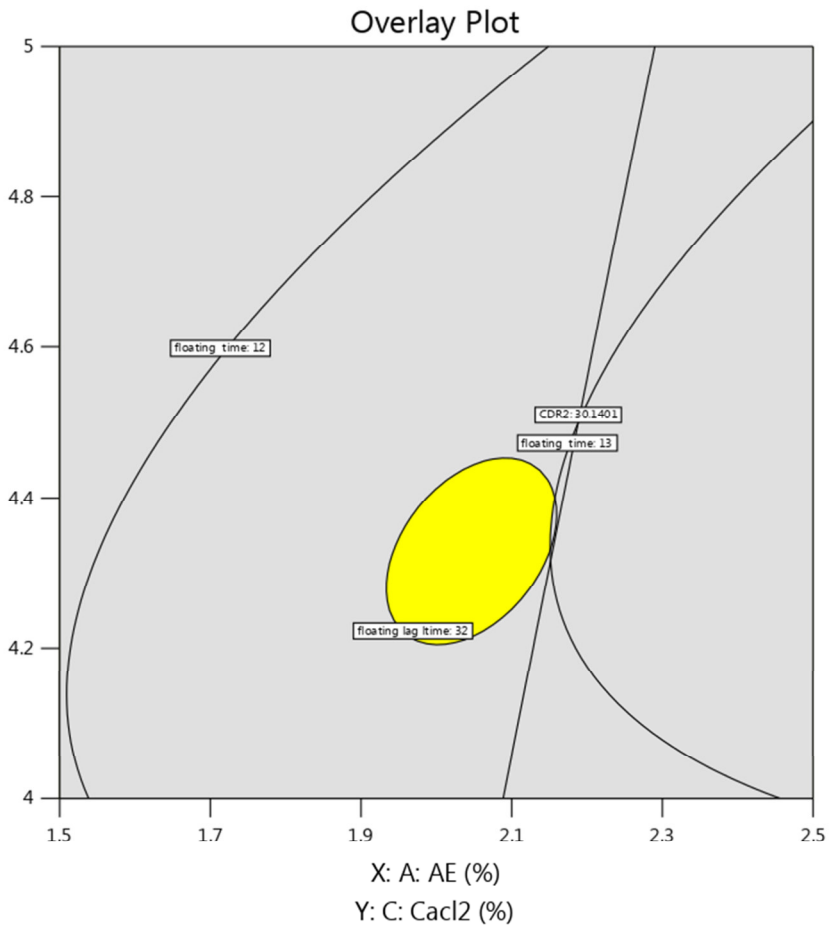


FIG.6b. overlay plot of effect of AE polymer, crosslinker on floating lag time

Factor Coding: Actual

Overlay Plot

CDR2

floating time

floating lag ltime

X1 = B

X2 = C

Actual Factor

A = 1.96

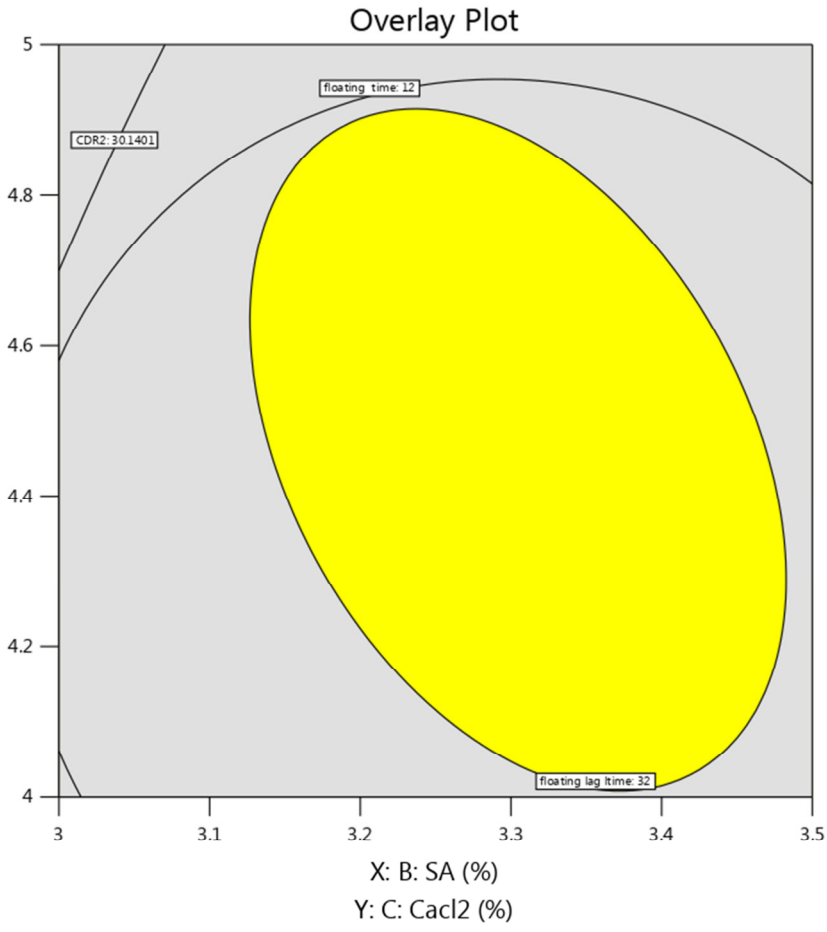


FIG.6c. overlay plot-graphical representation of sodium alginate and crosslinker on floating lag time

4.6 Effect of formulation variables on floating time using RSM

The predicted floating time values are denoted in the following coded equation as: Floating time =12.89-0.6182A-1.69B-0.3222C-0.7825AB-2.23AC+2.48BC+3.09A^2 +4.15B^2+2.56 C^2 The predicted R2 of 0.9710 is in reasonable agreement with the adjusted R^2 of 0.9968; i.e. the difference is less than 0.2.Adequacy precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Ratio of 66.625 indicates adequate signal. This model can be used to navigate the design space.

Formulations F1,F2,F3,F4,F6,F7,F8,F10,F12,F14,F15 exhibited floating time of 12 hours and more than 12 hours of buoyancy on gastric contents are essential features to release the drug and to remain in the gastric environment. The effect of formulation variables on floating time was presented using three dimensional graphical image, fig.7a, 7b and 7c.By observing the surface response 3D contour graphical representations F4 formulation is optimised using numerical and graphical methods

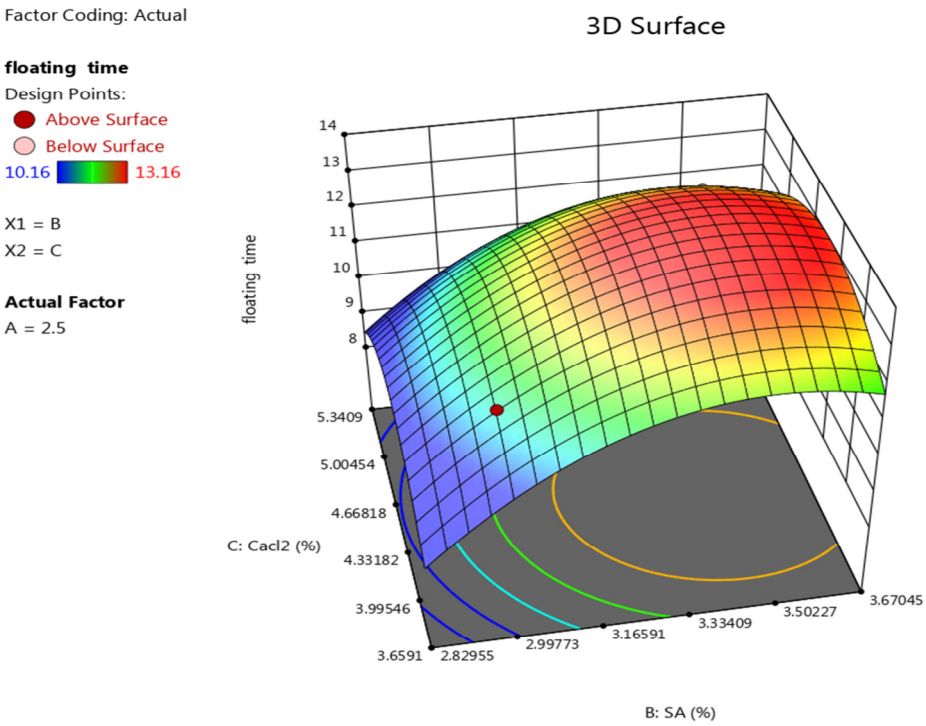


Fig 7a. 3D contour -graphical representation of sodium alginate , crosslinker on floating time

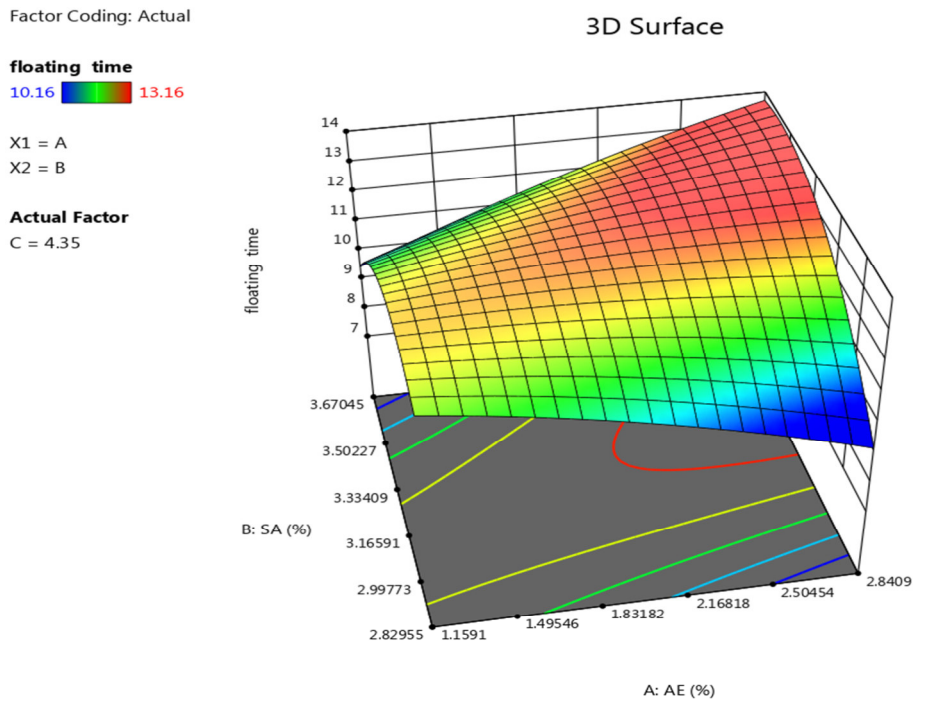


FIG.7b. 3D contour -graphical representation of AE polymer, sodium alginate on floating time

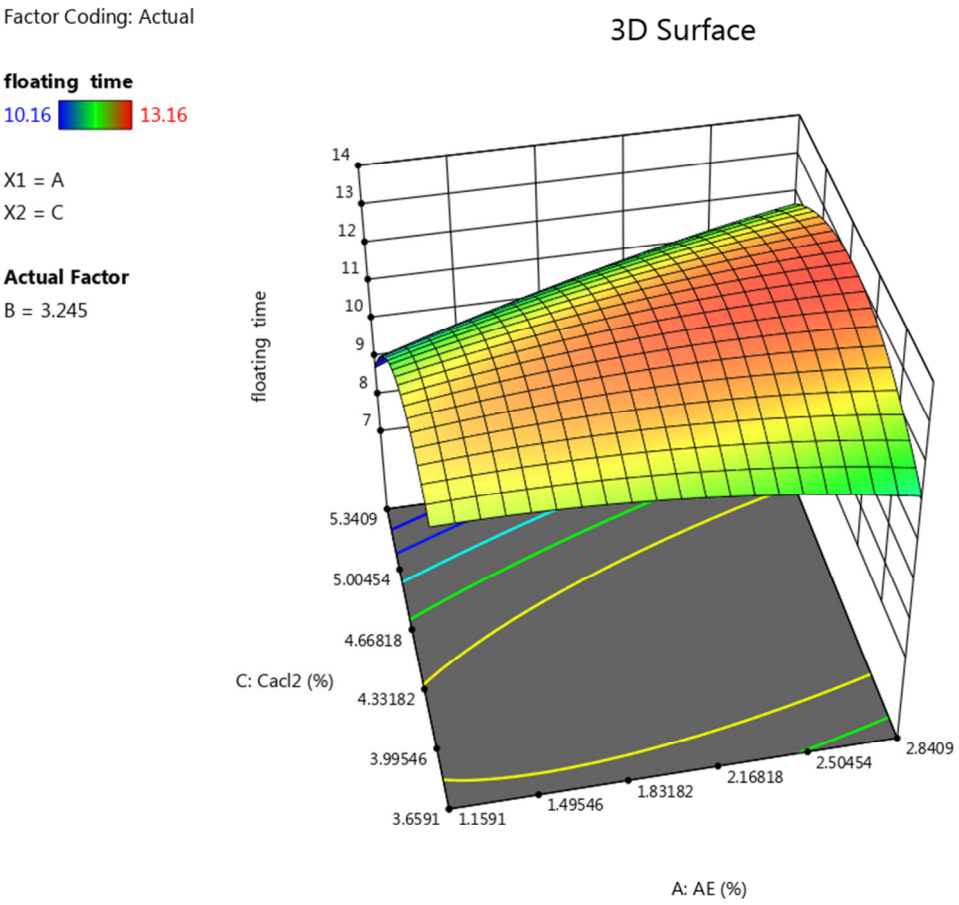
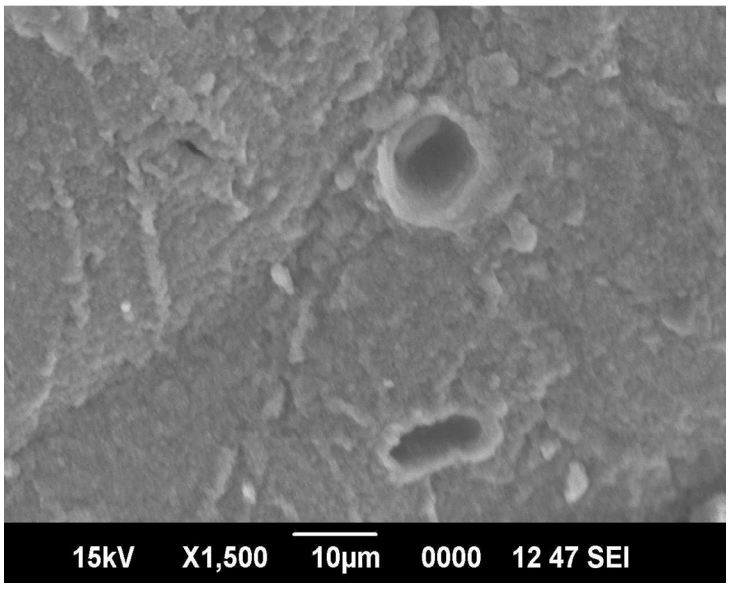


FIG.7C. 3D contour -graphical representation of AE polymer, crosslinker on floating time

4.7 SEM analysis

Optimized formulation (F4) microspheres exhibited pores formed due to processing. Formation of voids might be related to the entrapped fluid formed during the crosslinking and solidification. Pores of 10µm size clearly evidenced in the SEM fig 8. Surface roughness and presence of irregularities can be related to solvent evaporation process. Pore structure is responsible for the drug release pattern in microspheres[31,32].



4.8 Point prediction

Design-Expert® software provides powerful features to add confidence, prediction, or tolerance intervals (CI,PI,TI) to its graphical optimization plots. Selecting optimum formulation (F4), the responses observed (experimental) were compared with the expected ones (predicted) which are in good agreement of 95% confidence interval. This is confirmed for optimum formulation F4 at AE polymer concentration 2%, SA polymer concentration 3.25% and cross linker Cacl2 concentration 4.5%.

4.9 Point Prediction

Two-sided Confidence = 95% Population = 99%

Table 7: observed responses vs predicted responses by point prediction

Run Response	Predicted mean	Predicted median	Observed	Standard deviation	SE mean	95% CI low mean	95% CI for high mean	95% TI for low 99% population	95%TI for high 99% population
CDR2	24.07058	24.7058	24.7	0.0728	0.0724143	24.5196	24.8919	24.1678	25.2437
CDR12	100.014	100.014	100	0.6133	0.609791	98.4462	101.581	95.484	104.543
Floating time	12.8859	12.8859	12.88	0.0539	0.0536461	12.748	13.0238	12.4874	13.2843
Floating lag time	30.3045	30.3045	30.31	0.0478	0.0475807	30.1822	30.4268	29.951	30.6579

Confirmation Location		
AE	SA	Cacl2
2	3.25	4.5

All fifteen formulations exhibited % yield of 94.35-99.99%, particle size of 124-441µm, %swelling index 64.52-89.65%, floating time of 10.16 to 13.16 and floating lag time of 30.31 to 46.98 sec.

5. CONCLUSION

Vildagliptin microspheres were developed by design expert (version 12, stat-eases Inc) software using CCD. 15 Floating Formulations were formulated using Abelmoschus esculentus natural polymer for an extended period of 12 hours. Floating microspheres are evaluated for in-vitro parameters. All fifteen formulations exhibited % yield of 94.35-99.99%, particle size of 124-441µm, %swelling index 64.52-89.65%, floating time of 10.16 to 13.16 and floating lag time of 30.31 to 46.98 sec. F4 formulation is optimized based on cumulative % in-vitro drug release at 2nd hour, 12th hour, floating lag time and floating time values. Optimization of formulations was performed by Response surface methodology method. Statistical analysis of the experimental data exhibited good coefficient of regression, quadratic model. F-ratios of the regression for variables vs response variables have significant values. Predicted and observed results are in agreement of 95% confidence intervals. Based on investigation, RSM is the good tool for optimization of formulations.

6. AUTHORS CONTRIBUTION STATEMENT

Krishnaveni Manubolu did literature review, conceptualised and performed research work. Sreenivasulu Munna contributed for FTIR spectrum analysis and literature work .Kothapalli Bonnothth Chandrasekhar helped in the statistical analysis.All authors discussed the methodology and results and contributed to the final manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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