Formulation, Design, Evaluation And Optimization Of Pregabalin Microspheres

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Abstract:
Pregabalin mucoadhesive microspheres were created and optimised with the use of Box-Behnken process optimisation software. Experimental data were obtained on the quantitative responses of particle size, entrapment effectiveness, and in vitro drug release for various combinations of independent variables, sodium alginate as a release retarding polymer, sodium carboxymethylcellulose as a mucoadhesive polymer, and calcium chloride as a cross-linking agent. The data were found to fit the design model. Polynomial equations could be used to estimate the quantitative impact of these parameters on the responses at various levels, and strong linearity was seen between anticipated and actual response variable values. According to the study's findings, the number of polymers and cross-linking agent had a significant and interactive impact on the responses, particle size, entrapment effectiveness, and in-vitro drug release. The design expert software's point prediction revealed the optimised formulation F3 to be the best formulation. It was discovered that the in-vitro drug release was under control for more than 12 hours and adhered to the Higuchi model. Three dependent variables had RSM validations of 99.76%, 98.78%, and 97%. As a result, it can be said that a three-factor, three-level Box-Behnken design was used to build and optimise a mucoadhesive microsphere for Pregabalin.

Keywords:-Mucoadhesive microspheres , Box-Behnken, validation , cross-linking.

Introduction

The easiest and most practical method of administering medication is frequently thought to be oral administration. The development of stomach-specific word-controlled release drugs has attracted the attention of many researchers in recent years, but this has proven to be a challenging task due to pH differences in different parts of the GIT. It has also been noted that rapid gastro-intestinal transits with drug absorption can lower the administration dose potential. The upper small intestine or the stomach are where most drugs are absorbed. This deal with this issue, researchers put forth a lot of effort to create an oral controlled medication administration system, a type of floating drug delivery system. This innovation is low-density system with a sensitive quality that enables it to pass through the digestive tract.CR formulations, which spend only a brief amount of time in the upper gastrointestinal system. Maximum drug release occurs during the "drug absorption window" when retention in the upper GIT is prolonged. The bioavailability consequently increases.

The health care system has been greatly impacted by drug delivery systems (DDS) that can precisely control release rates or direct medications to a specific body spot. The perfect drug delivery system only delivers the active ingredient to the site of action and administers the medication at a pace determined by the body's requirements throughout the duration of the therapy. By combining the drug with a carrier particle like microspheres, nanoparticles, liposomes, etc. that modifies the drug's release and absorption characteristics, carrier technology thus offers an intelligent method for drug administration [1].

Pregabalin (S) - 3 - amino methyl hexanoic acid, or PGMHA, is a structural analogue of GABA. They make up a significant class of chemicals that are used to treat neuropathic pain and epilepsy. It is a solid made of white crystals. It is soluble in aqueous solutions that are basic and acidic as well as in water. Pregabalin has been researched for usage in several conditions, including postherpetic neuralgia, diabetic neuropathy, postoperative dental pain, and other pain syndromes. It has also been explored as a monotherapy for refractory partial seizures and social anxiety disorders. Pfizer-Global is the creator of pregabalin, which is sold under the trade name Lyrica throughout the world. Pregabalin has a short half-life (5–6.5 hours), which makes it a good option for sustained release formulations. In addition, it lessens side effects, lowers frequency, and improves patient compliance [2].

Small spherical particles known as microspheres have a diameter in the micrometre range (typically between 1 and 1000 micrometres). Sometimes, the term "microparticle" is used to describe microspheres. Different natural and artificial materials can be used to make microspheres. There are commercially accessible glass microspheres, polymer microspheres, and ceramic microspheres. Microspheres: Hollow and Solid have a wide range of densities and are used in various applications as a result. In order to reduce a material's density, hollow microspheres are frequently employed as additives. Depending on their size and construction material, solid microspheres can be used in a wide range of applications. Microspheres made of polyethylene and polystyrene are the two most popular varieties. Because they make procedures like cell sorting and immune precipitations easier, polystyrene microspheres are frequently used in biomedical applications. Microspheres are defined as solid, roughly spherical particles with a diameter ranging from 1 to

1000 micrometres, such as dispersed pharmaceuticals in certain solutions or microcrystalline shapes. Microspheres and microcapsules are frequently used interchangeably [3].

The circulatory system in the blood instantly destroys medication that is simply transmitted in from the gastrointestinal tract (GIT) and has a short half-life. Another solution to this issue is the oral sustained or controlled release (CR), which releases the drug into the gastrointestinal tract (GIT) gradually and maintains a constant level of medication in the plasma for a lengthy period. A good dose formulation is one that consistently maintains the required plasma therapeutic medication concentration during the treatment. Delivering a conventional dosage type in a fixed dose and at a predetermined frequency can accomplish this [4]. An advantage they lack Microcarriers, which are smaller than nanoparticles, move through the interstitium at a range of 100 nm and perform local functions. Transporting encapsulated dangerous compounds is likely possible, and dried microparticles may be referred to be solids rather than liquids. The intake dose is given in several tiny, distinct multiparticulate particles that each hold and release a small portion of the dosage, so the failure of one subunit does not affect the entire dosage [5]. In order to facilitate the release of the medication into the skin, microparticles are utilised in skin applications. These particles make sure that the drug remains localised at the application site and does not needlessly reach the systemic circulation [6]. In order to maintain an effective concentration of therapeutic products in the skin while reducing undesirable side effects, they function as a reservoir that releases an active ingredient over a longer period [7]. As a result, there are fewer cycles of over- and under-medication. It is particularly important for lowering antibiotic resistance while treating infectious disorders. Additionally, these distribution techniques can improve product integration or safety [8,9].

Mucoadhesive microspheres

The addition of mucoadhesive properties to microspheres has additional benefits, such as efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drug to the absorption site achieved by anchoring plant lectin. Mucoadhesive microspheres are of 1-1000 mm in diameter and consist entirely of a mucoadhesive polymer or have an outer coating The possibility of localised as well as systemic controlled drug release is provided by mucoadhesive microspheres' ability to bind to any mucosal tissue, including those present in the eye, nasal cavity, urinary tract, and gastrointestinal tract [10].

AIM AND OBJECTIVES

Aim

Formulation, Design, Evaluation and Optimization of Pregabalin Microspheres.

Objectives

To enhance bioavailability of drug.
To develop a gastroretentive microsphere of pregabalin to decrease dosing frequency.
To increase upper gastro intestinal tract retention.
To improve absorption.

Drug profile (Pregabalin)

Pregabalin does not bind to benzodiazepine or GABA receptors directly, even though it is a structural derivative of the inhibitory neurotransmitter GABA.

Half-life: The elimination half-life of pregabalin is 6.3 hours

Material

M Sea Pharmaceuticals Pvt Ltd gave me pregabalin as a gift (India) whereas sodium alginate, sodium carboxymethylcellulose, and calcium chloride were from CDH Chemicals, New Delhi (India). All other reagents were of analytical grade.

Preliminary trials

Based on an earlier observation that was reported elsewhere, microsphere formulations were created by adjusting the concentration of polymers and the cross-linking agent in different ratios. While a higher concentration of calcium chloride increased the effectiveness of drug entrapment, a lower concentration of sodium alginate produced a less viscous gel and smaller microsphere particle size [11,12].

Preparation of formulation

Pregabalin was added to the dispersion after sodium alginate and sodium carboxymethylcellulose were thoroughly mixed to generate a viscous dispersion. A needle with a 0.45 mm diameter was used to add the resulting dispersion to calcium chloride while it was being continuously stirred on a magnetic stirrer. The droplets were kept in the solution for 20 to 25 minutes to test their stiffness. The microsphere was collected, dried in an oven, and then filtered [13]. Utilising Box-Behnken experimental design, the formulations were created, and statistical screening was used to produce the optimised formulation. Particle size, surface morphology, drug entrapment effectiveness, in vitro drug release, and in vitro mucoadhesion were all assessed across 17 rounds of the experiment.

Experimental design

For examining quadratic response surfaces and creating second order polynomial models using Design Expert®, a three-factor, three-level design is appropriate. Table 1 lists the independent and dependent variables as well as their low, medium, and high levels. Following is the polynomial equation produced by this experimental strategy:

$Y_0 = b_0 + b_1 A + b_2 B + b_3 C + b_{12} AB + b_{13} AC + b_{23} BC + b_{11} A^2 + b_{22} B^2 + b_{33} C^2$,

where Y_0 remains the dependent variable, consistent toward also particle size (Y_1) before drug entrapment efficiency (Y_2) or else in vitro drug release (Y_3), then A, B then C remain the independent variables on behalf of sodium alginate, sodium carboxymethyl cellulose then calcium chloride concentrations, correspondingly. b_0 remains a continual; b_1 , b_2 then b_3 remain the constants interpreting the linear weight of A, B then C, correspondingly; b_{12} , b_{13} then b_{23} remain the constants interpreting the connections amid the variable star; then b_{11} , b_{22} then b_{33} of the constants translating the quadratic effect of A, B then C. Linear then second-order polynomials remained tailored towards the new information towards get the reversion equations, then their experiential then foretold replies remain assumed in Table 2.

Table 1: Levels of process variables rummage-sale in experiments

Factor	Process parameter	Levels		
		Low (-1)	Medium (0)	High (+)
Independent variables				
A	Sodium alginate (mg)	300	600	900
B	Sodium carboxymethyl-cellulose (mg)	150	300	450
C	Calcium chloride (% w/v)	1	3	5
Dependant variables				
Y1	Particle size			
Y2	Drug entrapment efficiency			
Y3	In-vitro drug release			

Table 2: Experimental strategy of Box – Behnken design by coded values of process variables sideways by the observed value then predicted value of replies

Runs	Batch	Independent variables			Dependent variables					
					Actual			Predicted		
		A	B	C	Y1	Y2	Y3	Y1	Y2	Y3
1	F1	-1	-1	0	460.60	64.40	81.64	453.86	64.80	81.37
2	F2	+1	-1	0	541.22	70.00	75.80	546.32	68.54	75.88
3	F3	-1	+1	0	481.20	62.39	78.50	476.11	63.85	78.42
4	F4	+1	+1	0	540.70	67.88	70.50	547.24	67.47	70.77
5	F5	-1	0	-1	569.43	63.30	75.12	570.67	62.28	75.12
6	F6	+1	-1	0	543.00	67.32	74.72	532.71	68.17	74.37
7	F7	-1	0	+1	489.20	73.48	83.79	499.59	72.62	84.14
8	F8	+1	0	+1	702.20	72.98	71.75	700.96	74.00	71.75
9	F9	0	-1	-1	493.26	70.45	76.25	498.56	70.86	76.52
10	F10	0	+1	0	512.80	70.90	77.42	525.65	70.56	77.50
11	F11	0	-1	+1	557.60	79.25	84.80	558.63	79.69	84.72
12	F12	0	+1	+1	563.93	81.30	75.95	544.50	78.10	75.68
13	F13	0	0	0	561.00	80.85	78.22	544.50	80.81	78.56
14	F14	0	0	0	539.00	82.00	76.70	544.50	80.81	78.56
15	F15	0	0	0	526.00	81.75	79.17	544.50	80.81	78.56
16	F16	0	0	0	557.00	77.98	79.40	544.50	80.81	78.56
17	F17	0	0	0	539.00	79.87	79.30	544.50	80.81	78.56

Surface morphology and particle size characterization

A scanning electron microscope operating at 20 kV was used to examine the microspheres' surface morphology and form. Using an optical microscope with an ocular micrometre, the diameters of the microspheres were measured. Using a stage micrometre, the ocular micrometre was calibrated. 100 microspheres in all were examined, and the mean diameter was reported.

Drug entrapment efficiency

The mucoadhesive microspheres were dissolved in a tiny amount of a suitable solvent in a volumetric flask, and the medication was extracted into 0.1N HCl by hourly shaking for 24 hours. Following a 24-hour period, the solution was filtered, and the filtrate was examined using 211 nm ultraviolet spectroscopy [14,15]. The following equation was used to calculate the percentage of Pregabalin entrapment:

% Drug Entrapment = Amount of drug in known amount of microsphere × 100/ Initial drug load

In-vitro mucoadhesion

When compared to non-bioadhesive ethyl cellulose microspheres, the mucoadhesive property of microspheres was assessed by the method developed by Ranga Rao and Buri using stomach mucosa isolated from mice [16]. Mice were slaughtered following a 24-hour fast, and their stomachs were immediately dissected. On a glass plate, the stomach mucosa was glued before being rubbed with physiological saline. The mucosa was held at high relative humidity at room temperature for 30 minutes while a fixed number of microspheres were dispersed uniformly across the surface. The stomach mucosa was washed with simulated gastric fluid (0.1 N HCl, pH 1.2) for five minutes at a rate of 22 ml/min while remaining at a 45° angle. The number of microspheres still present on the mucosa's surface was counted, and the proportion of them was computed [17].

Percentage of mucoadhesion = No of microspheres remaining × 100/ total no of microspheres applied

In-vitro drug release

Drug release study remained approved available cutting-edge a six-basket USP XXIV dissolution apparatus type I (model – 8000, Lab India Analytical Instruments Pvt. Ltd., Mumbai, INDIA) revolving on 100 rpm upheld on 37 ± 0.5 ° C; 0.1N HCl (pH 1.2) remained rummage-sale by way of dissolution medium (900 ml) toward simulate gastric environment. After appropriate dilution, aliquots were taken out at predetermined intervals up to 12 hours and subjected to ultraviolet spectroscopic analysis at a max of 211 nm. To maintain sink conditions, the removed volume was substituted with an equivalent volume of new medium. Three copies of each experiment were carried out [18-20].

Drug release kinetics and transport mechanism

To evaluate the kinetics of drug release, the drug release data were fitted to the Higuchi model (cumulative% drug released vs square root of time), the first-order (log cumulative% drug retained versus time), and the zero-order (cumulative% drug release versus time). To further describe the drug release mechanism, the data were evaluated using the Korsmeyer-Peppas model to establish the value of the release exponent, n [21,22].

Statistical analysis

Results were calculated and presented as the mean standard deviation of three determinations. All statistical analysis was performed using Windows Design Expert 7.1 software. Statistical analysis was completed using the Response Surface Methodology (RSM) and the Design of Experiments (DOE) (Box-Behnken Design) technique. ANOVA was used to statistically examine the effects of the microsphere's components on particle size, entrapment effectiveness, and drug release. The alterations remained measured important on a equal of $p<0.05$.

Formulation optimization through response analysis

The polynomial equation created by the optimisation software was validated using an ANOVA application. The statistically significant coefficients and R squared values for a total of seventeen runs (F1 - F17) were assessed. By confirming the findings across the full experimental region, the composition of the optimised formulation was discovered. To validate the proposed experimental design and polynomial equations, three best formulations (OF-1, OF-2, and OF-3) were chosen. The expected ideal formulations were created and tested for a range of reactions. The actual and predicted values of the answers were plotted using linear regression after the observed values and predicted values were compared

Results and Discussion

Preformulation Study

API characterization

Table: Organoleptic properties of Pregabalin

Sr. No.	Name of property	Specification
1.	Colour	White
2.	Odour	Unpleasant
3.	Nature	Crystalline

Identification of pure drug

Melting Point

Table: Melting point of Pregabalin

Sr. No.	Obtained range (ºC)	Mean value (ºC)	Reference value
1.	175	176.66 ℃	176-178 ℃
2.	177		
3.	178		

Melting point of Pregabalin was found to be 176.66 ℃, which is in range as given in literature (176-178˚C). Hence the drug can be stated as pure.

UV Spectroscopy

Determination of λ max

Accurately weighed 1 mg of drug was transferred to 100 ml of volumetric flask add dissolved in methanol and volume was made up to 100 ml and the solution was scanned on UV spectrometer in the range 200-400nm.

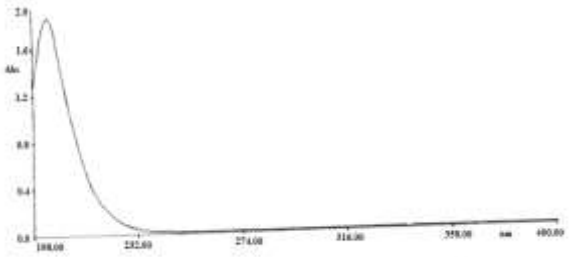

Fig. UV Spectrum of Pregabalin

An absorption maximum was found to be at 211 nm. Hence 211 nm was selected as λ max for further studies

Calibration curve of Pregabalin in methanol

The stock solution for the standard drug of 1 mg was prepared using 100 ml of methanol. The maximum absorbance for the drug solution of 10 mcg/ml was found to be at 211 nm. The linearity was found between the concentration range of 10-35 mcg/ml for UV spectroscopy**.**

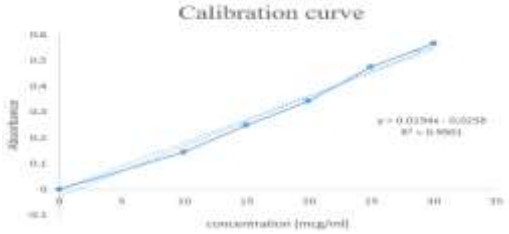

Fig. Calibration curve of Pregabalin in Methanol.

Solubility study

Pregabalin is a BCS class I drug i.e., highly soluble, and highly permeable drug that's why solubility study is not mandatory.

Drug and excipient interaction study

A. Fourier Transformation Infrared Spectroscopy (FTIR)

FTIR spectrum of Pregabalin was shown in following Fig. revealed that the characteristic peaks representing the presence of functional groups claim by its chemical structure. From this we can consider that the Pregabalin was of pure quality.

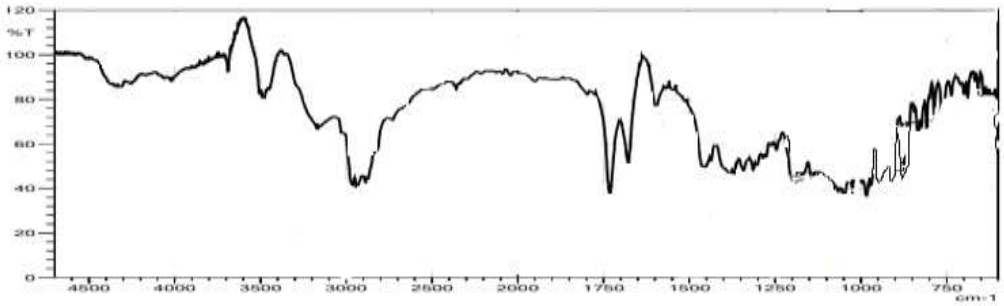

Fig.4: FTIR spectra of Pregabalin

After interpretation of FT-IR Spectrum of Pregabalin, it was concluded that all the characteristic peaks corresponding to the functional group present in the molecular structure of Pregabalin were found within the reference range and confirming its identity.

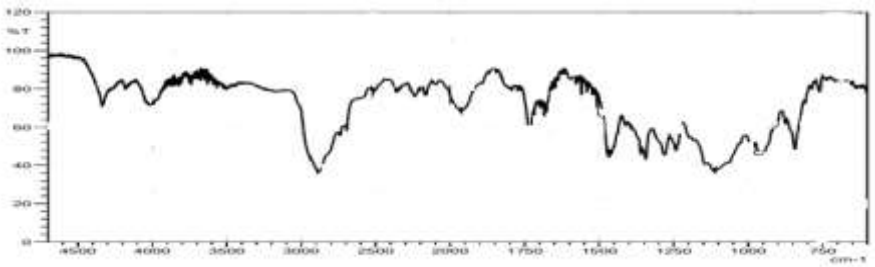

Fig. FTIR spectra of physical mixture (Drug + Polymer)

After interpretation of FT-IR Spectrum of polymer and its physical mixture with drug pregabalin, it was concluded that all the characteristic peaks corresponding to the functional group present in molecular structure of Pregabalin were not found intact within the reference range, confirming its reactivity with polymer. This interaction further supports the selection of polymer.

B. Differential Scanning Calorimetric analysis (DSC)

The thermal analysis of Pregabalin was studied by using DSC as shown in figures respectively. The Pregabalin shows an endothermic peak at approximately 175℃ and it corresponds to its melting point (fig.).

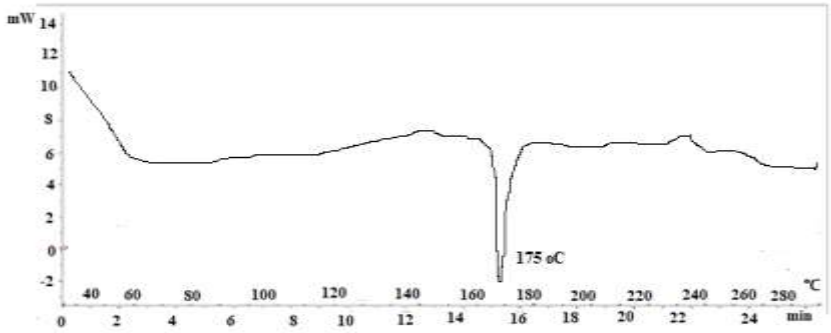

Fig. DSC thermogram of Pregabalin

Preliminary trials

Different ratios of polymers and cross-linking agents were used to create microspheres. Because fewer polymers produced microspheres with irregular shapes, sodium alginate and sodium CMC contents were determined to be at 300–900 mg and 150–450 mg, respectively, with the medium level at 600–300 mg. To manage the degree of cross-linking between the acid group of alginates and the bivalent cation Ca 2+, it was agreed that the calcium chloride amount should be between 1 - 5 % w/v.

Formulation optimization

For the three-factor, three-level experimental design, seventeen formulations were created and evaluated. Particle size, entrapment effectiveness, and in vitro drug release were the investigated responses. In contrast to microspheres created with medium and high concentrations of polymers (+ level), which were larger and more spherical, microspheres prepared with low concentration of polymers (- level) were rough and uneven in shape due to inadequate molecular packing and cross-linking. The availability of reacting/binding sites for the cross-linking cations varied, which led to differences in the microspheres' sizes and morphologies at various polymeric concentrations. Smooth, spherical, and smaller microspheres that were densely packed and distinct were produced when the cross-linking agent amount was raised (Figure 1)

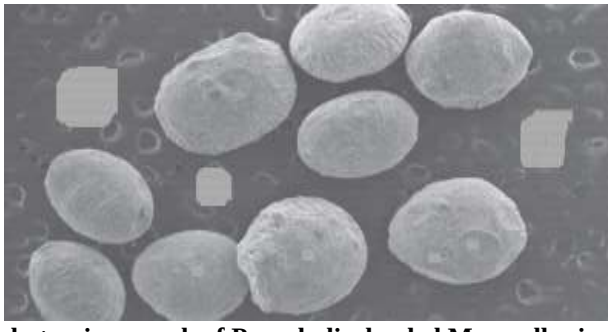

Fig. 1: SEM photomicrograph of Pregabalin-loaded Mucoadhesive microsphere

We also conducted a more in-depth analysis of how polymer concentration affected drug entrapment effectiveness. Both polymers had an impact on the effectiveness of the drug entrapment, although in an opposite way. In contrast to sodium CMC, sodium alginate's quantity directly correlated with entrapment effectiveness, whereas the latter was inversely correlated. The formulation that had a low sodium alginate concentration and a greater sodium CMC concentration (F3) displayed the lowest DEE at 62.39. Formulation with low quantities of sodium alginate and sodium CMC both (F1) demonstrated 64.40% drug entrapment, which was significantly higher than the minimum and lower than the maximum. The formulation F15 (81.75%), which had all the variables at zero, had the highest level of entrapment efficiency.
Since the drug has a window for absorption in the stomach, drug release studies were simulated in an acidic environment. In the formulation containing the highest concentration of polymers (+1 level), the least amount of drug release was seen. The formulations with lower sodium CMC levels but equivalent amounts of sodium alginate demonstrated improved drug release at 74.72% and 75.80%. The formulation F11 (84.80%) with sodium alginate and sodium CMC at 0 and -1 level, respectively, and cross-linking agent at +1 level showed the highest drug release. In F7, which contained sodium alginate and sodium CMC at levels of -1 and 0, respectively, as well as a higher level of the cross-linking agent, a similarly high amount of drug release was achieved. It may be assumed that both polymers were influencing drug release and were balancing each other out in that regard.

To determine the ideal levels of various independent process parameters using the Box-Behnken design, an experimental design with seventeen runs for three factors at three levels was created. Table 2 displays the observed responses along with the projected values for the proposed formulas. The measured sizes, entrapment rates, and drug release values fall within the ranges of 460.60 to 702.20 um, 62.39 to 82.00%, and 70.50 to 84.80%, respectively. Using Design Expert® 7.1 software, the responses were simultaneously fitted to linear, two-factor interaction (2FI), cubic, and quadratic models.

Along with the regression equation, the values of R-squared, Adj-squared, Pred R-squared, SD, and% CV are displayed in Table 3. The quadratic model was chosen since it had a higher adjusted R-squared value because the cubic model was aliased because there weren't enough design points to estimate the coefficients. Table 4 shows the ANOVA data for the various responses, and all statistically significant ($p < 0.05$) coefficients are taken into account in the equations.

Table 3: Regression analysis for response Y_1 , Y_2 and Y_3

Models	R-squared	Adjusted R-squared	Predicted R-squared	SD	%CV	Remarks
Response (Y_1)						
1. Linear	0.4041	0.280	- 1958	45.65	7.00	-
2. Second order	0.7310	0.5757	- 0.146	34.86	5.4	-
3. Quadratic	0.9611	0.9241	0.7106	14.50	2.12	Suggested
4. Cubic	0.9711	0.9148	N/A	15.43	2.25	-
Response (Y_2)						
1. Linear	0.2063	0.0343	- 0.2486	6.61	8.30	-
2. Second order	0.2132	-0.2332	- 1.3927	7.51	9.56	-
3. Quadratic	0.9639	0.95	0.7685	1.57	1.33	Suggested
4. Cubic	0.9763	0.9349	N/A	1.5	1.23	-
Response (Y_3)						
1. Linear	0.6114	0.535	0.24	2.45	3.35	-
2. Second order	0.8802	0.8140	0.6021	1.56	2.06	-
3. Quadratic	0.9642	0.9312	0.9140	0.92	1.20	Suggested
4. Cubic	0.9667	0.8970	N/A	1.15	1.50	-

A positive number indicates good optimisation, while a negative value illustrates an inverse link between the factor and the answer, according to the optimisation plan. It is clear that the three independent variables sodium alginate concentration (A), sodium CMC concentration (B), and calcium chloride concentration (C) all interact with one another to affect the three estimated responses, such as particle size (Y1), drug entrapment effectiveness (Y2), and drug release (Y3).

Table 4: Analysis of variance of calculated model

Result of the ANOVA	Particle size (µm)	DEE	In-vitro drug release
Regression			
Sum of squares	41995.31	717.42	219.25
Degree of freedom (df)	8	8	8
Mean squares	4678.14	81.72	25.26
F- value	25.24	29.89	30.50
P	0.0001	0.0001	0.0001

Residual			
Sum of squares	1174.71	20.58	5.66
Degree of freedom (df)	6	6	6
Mean squares	180.95	2.9	0.83
Lack of fi t test			
Sum of squares	443.6	9.28	0.57
Degree of freedom (df)	3	3	3
Mean squares	148.5	3.10	0.20
F- value	0.72	1.3	0.15
P	0.6047	0.4266	0.9195
Correlation coefficient (R^2)	0.9613	0.9637	0.9644
Correlation of variation (%CV)	2.12	2.33	1.20

Response surface analysis

Figures 2a–2c, Figures 3a–3c, and Figures 4a–4c, for responses Y1–Y3 correspondingly, illustrate the three-dimensional (3D) surface plots that were created for each of the three replies.

Response Y_1 : Effect on Particle size

The model proposes the following equation for particle size –

Particle Size: + 473.48640 - 0.15346A + 1.13267B - 88.36014C - 0.0104AB + 0.088610AC - 0.012007BC + 0.02166A^2 - 0.09246B^2 + .181926C^2

where A remains the concentration of sodium alginate; B remains the concentration of sodium CMC, then C remains the concentration of calcium chloride. The situation remained designated through model F-value (25.24) that here remains 0.01 % accidental that such a " Model F-Value "

Factor A, or the amount of sodium alginate, had a more noticeable impact on particle size. The "Lack of Fit F-value" of 0.72 indicates that the lack of fit was not significant. The " Pred R - squared " of 0.6047 and the " Adj R - squared " of 0.9613 are reasonably in accord. The value of 24.766 for "Adeq Precision" indicates that there is an acceptable signal, and the model can be utilised to explore the design space. The response surface plot in Figures 2a–2c illustrates how several independent variables affect the particle size of microspheres. The findings demonstrated that an increase in polymer concentration led to an increase in microsphere particle size. In our analysis, formulation F8 had the largest particle size, which was 702.20 um (at calcium chloride (+1), sodium alginate (+1), and sodium CMC (0)). A higher quantity of calcium chloride and sodium alginate may be the cause of this. The concentration of the cross-linking agent increases as sodium alginate's droplet size increases, leading to the creation of a bigger mesh structure.

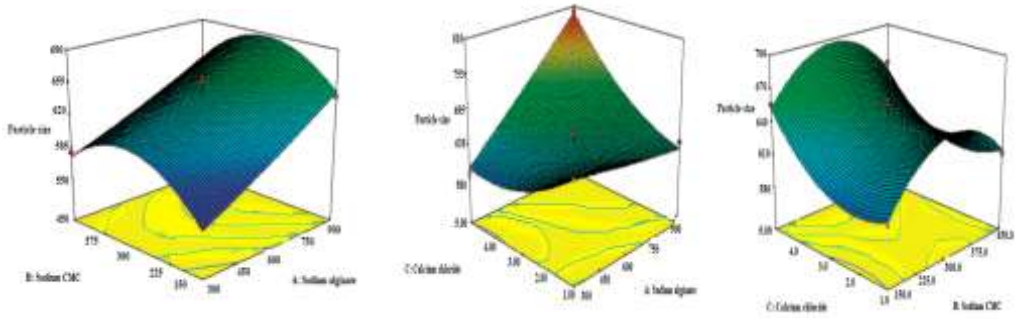

Fig. 2: The effect of independent variables on response Y_1 (particle size): (a) Effect of B and A; (b) Effect of C and A; and (c) Effect of C and B

Response Y_2 : Effect on drug entrapment efficiency

The model proposes the following equation for drug entrapment efficiency

Drug Entrapment Efficiency = 4.00583 + 0.13560A + 0.11022B + 4.38877C - 0.0045AB - 0.0818AC - 0.1416BC - 0.0105A^2 - 0.02577B^2 - 0.24105C^2

where A remains the concentration of sodium alginate, B remains the concentration of sodium CMC, then C remains the concentration of calcium chloride. The model remained important meanwhile the Model F-value remained 29.89 (P = 0.0001). Here remains solitary 0.01% opportunity of this Model F-Value owing towards noise. Model rapports A, C, A^2 , then B^2 remain meaningfully designated through standards of " Prob > F " < 0.0500. The "Lack of Fit F-value" of 1.2 indicates that noise has a 42.66% chance of causing this "Lack of Fit F-value" to occur. The "pred R-squared" and the "adj R-squared" have reasonable agreement (0.4266 and 0.9637, respectively). The value of 13.342 for "Adeq Precision" denotes a signal that is sufficient for the model to use to move about the design space. Figures 3a–3c are response surface plots that illustrate how various independent variables affect the proportion of drug entrapment. Maximum entrapment efficiency was demonstrated by formulations F13- F17, ranging from 77.98 to 80.81% (all factors are at zero level), while minimum entrapment efficiency was demonstrated by formulation F3 (sodium alginate at -1 level, sodium

CMC at +1 level, and calcium chloride at 0 level). This suggests that the volume of the cross-linked network is present in smaller amounts at lower sodium alginate concentrations, which affects the effectiveness of entrapment negatively.

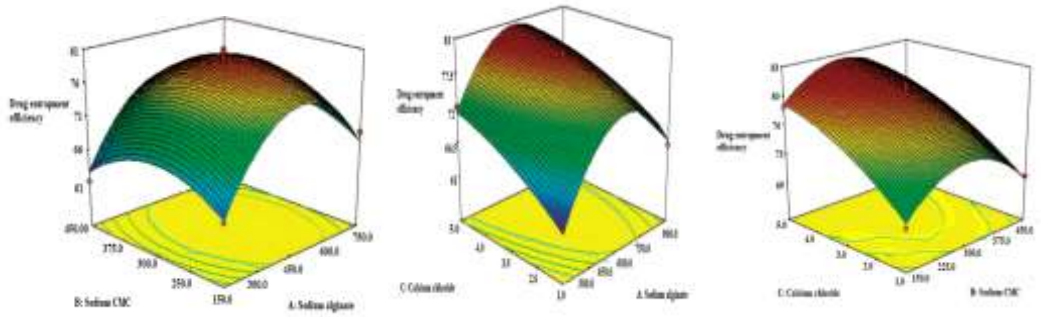

Fig. 3: The effect of independent variables on response Y_2 (drug entrapment efficiency): (a) Effect of B and A; (b) Effect of C and A; and (c) Effect of C and B

Response Y_3 : Effect on in-vitro drug release:

In vitro drug release: + 59.20481 + 0.035287A + 0.014623B + 6.38225C - 0.0081AB - 0.2417AC - 0.4157BC - $0.0158A^2 + 0.0173B^2 - 0.027563C^2$

where A remains the concentration of sodium alginate, B remains the concentration of sodium CMC, C remains the concentration of calcium chloride.

The model remained meaningfully designated through Model F-value of 25.26 (P = 0.0001). Here remains individual 0.01% coincidental that this " Model F-Value " might happen owing toward noise. Model terms A, B, C, AC, BC, then A^2 remained suggestively designated through standards of " Prob > F " < 0.0500. The amount of the cross-linking agent as well as the polymer had a significant impact on the in vitro drug release. The "Lack of Fit F-value" of 0.15 did not significantly indicate the lack of fit. A " Lack of Fit F-value " (very high) could arise due to noise with a 93.95% probability. The " Pred R - squared " of 0.9195 and the " Adj R - squared " of 0.9644 are reasonably in accord. A sufficient signal to use the model to move about the design space is indicated by " Adeq Precision " at 19.063.

Figures 4a–4c are response surface plots that illustrate how several independent variables affect in vitro drug release. Formulations F1, F7, F11, F15, F16, and F17 all demonstrated the highest levels of in vitro drug release, with respective values of 81.64%, 83.79%, 84.80%, 79.17%, 79.40%, and 79.30%. This demonstrated a significant relationship between the concentration of the polymer and the cross-linking agent and the in-vitro drug release.

High values of R^2 were seen in the linear correlation graphs between the experimental and predicted values for each of the three responses. The ranges of the R^2 values for replies Y1, Y2, and Y3 were, respectively, 0.9593 to 0.9613, 0.9622 to 0.9637, and 0.9612 to 0.9644. The situation designates the outstanding goodness of fit on $p < 0.0001$. Thus, the present study's high predictive ability of the RSM is demonstrated by the low magnitudes of error as well as the significance of R^2 values.

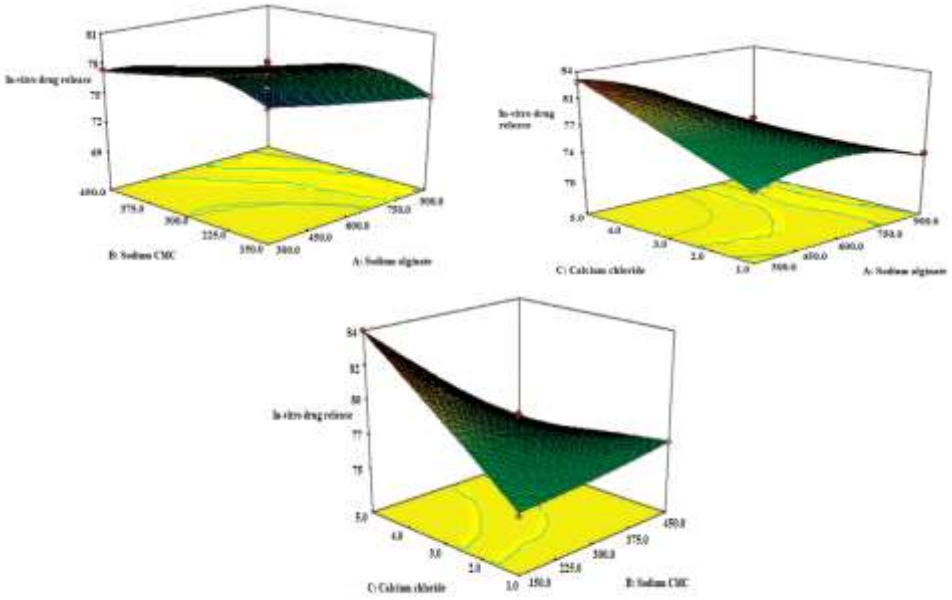

Fig. 4: The effect of independent variables on response Y_3 (Cumulative % in vitro drug release): (a) Effect of B and A; (b) Effect of C and A; and (c) Effect of C and B

Selection of optimized formulations using point prediction method

The optimal preparations remained designated toward attain the optimal standards of apiece response, that remains, particle size (Y1), drug entrapment efficiency (Y2) then % in vitro drug release (Y3). The restraints aimed at the retort Y1 , Y2 then Y3 remained originate toward remain cutting-edge the variety $580.31 \leq Y\ 1 \geq 618.22$ µm, $73.03 \leq Y\ 2 \geq 80.66$ % and $80.33 \leq Y\ 3 \geq 83.73$ %. Three equations were chosen at random based on the prediction, and the answers for particle size, entrapment effectiveness, and percent cumulative drug release were assessed. All three checkpoint formulations used in the RSM validation were found to be within acceptable bounds. Table 5 displays the breakdown of the best check point formulations, their anticipated and actual values for all replies, and the % error. It was discovered that the percentage prediction inaccuracy ranged from -4.65% to +1.33%

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Table 5: Composition of checkpoint formulations with predicted and observed values of responses

Formulation code	Optimized formulation composition			Response variable	Observed value	Predicted value	Percentage of error
	A	B	C				
Optimized F1	382.98	164.06	3.92	Y_1 Y_2 Y_3	580.31 73.03 80.33	574.35 72.09 84.2	1.07 1.33 -4.65
Optimized F2	593.79	301.90	4.63	Y_1 Y_2 Y_3	677.5 80.2 80.44	686 83.04 80.3	-1.28 -3.46 0.30
Optimized F3	472.17	168.12	4.96	Y_1 Y_2 Y_3	618.22 80.66 83.73	617.7 80.8 85.5	0.1 -0.32 -2.00

Point predictions made by the design expert software indicated that the optimal results would have a polymer concentration of sodium alginate 472.17 mg, sodium carboxymethyl cellulose 168.12 mg, and calcium chloride 4.96 (% w/v) as a cross-linking agent, as well as a particle size of 618.22 um, drug entrapment efficiency of 80.66%, and cumulative in vitro drug release of 83.73%.

Mucoadhesion

The optimised formulations shown adequate adhesiveness, according to the mucoadhesion test results. In comparison to non-mucoadhesive ethylcellulose microspheres, formulation OF-3 demonstrated good mucoadhesive properties (82 ± 0.50%) up to 10 h.

In-vitro drug release

The amount of drug release considerably decreased with an increase in the concentration of polymers, according to an in-vitro drug release research ($P < 0.05$). It can be explained by an increase in the polymeric matrix's viscosity, which leads to larger microspheres. As a result, the drug's diffusional path length lengthens. The medication release from the microspheres was also delayed when the cross-linking agent concentration increased ($p < 0.05$). Smaller cavities were created as a result of a higher cross-linking agent concentration, which lowers the ability of microspheres to swell. As a result, the effective surface area for drug diffusion decreases. It was discovered that 83.62 mg of medication was cumulatively released from the optimised formulation, OF-3. The release behaviour of OF-3 is shown in Figure 5.

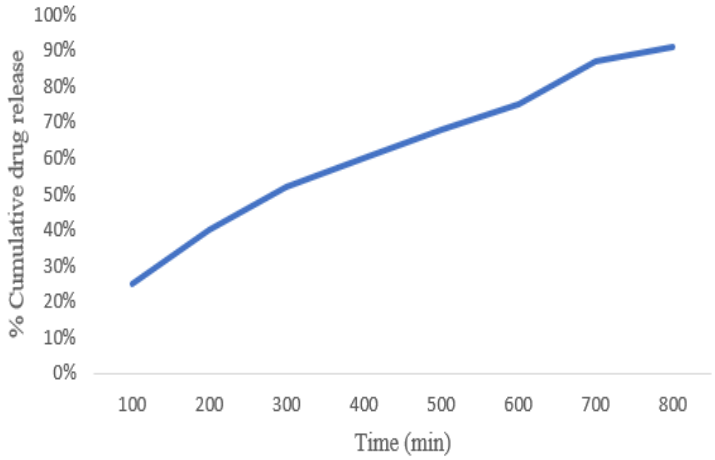

Fig. 5: In-vitro release profile of optimized formulation OF3

Release mechanism

To ascertain the release kinetics, zero-order, first-order, and Higuchi models were each used to in-vitro drug release data for optimised F3. The drug transport pathway was further described using the Korsmeyer-Peppas equation (Table 6).

Table 6: Release kinetics and transport mechanism of optimized formulations (OF-1, OF-2, and OF-3)

Model	Equation	Parameters	Formulation code		
			OF1	OF2	OF3
Zero order	m_0 -m = kt	r^2	0.736	0.715	0.669
First order	In m = kt	r^2	0.879	0.874	0.989
Higuchi ' s model	m_0 -m = $kt^{1/2}$	r^2	0.887	0.88	0.996
Korsmeyer-Peppas	log m_0 –m = log k +n log t	r^2	0.884	0.864	0.980
Diffusion coefficient	-	N	0.686	0.690	0.663

The finest fit remained prophesied through Higuchi model (r^2 , 0.996) than through zero-order (r^2 , 0.669) then first-order (r^2 , 0.989) replicas. This obviously designates that the release of pregabalin after the expressed mucoadhesive microspheres remains diffusion controlled.

The investigational information remained additional practical toward the Korsmeyer-Peppas equation toward describe the conveyance device. The worth of release advocate (n) aimed at the future model remained 0.663 aimed at adjusted preparation OF3 ($0.5 < n < 1$), signifying drug release measured through swelling then relaxation of polymer. The chosen formulation demonstrated diffusion-controlled drug release through polymer swelling and relaxation. Area of diffusion, diffusion path length, porosity, tortuosity, concentration gradient, and diffusion coefficient are important variables in the diffusion process. The utilised polymers were hydrophilic, meaning they swelled when they came into touch with a liquid. Reduced concentration gradient hinders drug release, but swollen polymer matrix has enhanced porosity, decreased route length, and reduced tortuosity. The optimised formulation has an optimised balance, which keeps the amount of medicine entering the biological system for absorption constant.

SUMMARY AND CONCLUSION

Pregabalin mucoadhesive microspheres were created and optimised with the use of Box-Behnken process optimisation software. Experimental data were obtained on the quantitative responses of particle size, entrapment effectiveness, and in vitro drug release for various combinations of independent variables, sodium alginate as a release retarding polymer, sodium carboxymethylcellulose as a mucoadhesive polymer, and calcium chloride as a cross-linking agent. The data were found to fit the design model. Polynomial equations could be used to estimate the quantitative impact of these parameters on the responses at various levels, and strong linearity was seen between anticipated and actual response variable values. According to the study's findings, the number of polymers and cross-linking agent had a significant and interactive impact on the responses, particle size, entrapment effectiveness, and in-vitro drug release. The design expert software's point prediction revealed the optimised formulation F3 to be the best formulation. It was discovered that the in-vitro drug release was under control for more than 12 hours and adhered to the Higuchi model. Three dependent variables had RSM validations of 99.76%, 98.78%, and 97%. As a result, it can be said that a three-factor, three-level Box-Behnken design was used to build and optimise a mucoadhesive microsphere for Pregabalin.

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