RESEARCH ARTICLE

QbD Based Formulation Development of Delayed-Release Beads for Better Management of Nocturnal Asthma

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ABSTRACT

The present study discusses about the quality by design (QbD) based formulation development and characterization of delayed release beads for better management of Nocturnal Asthma. Ionotropic gelation technique was used to create Budisonide-loaded pectin-alginate beads, and calcium chloride was added as a crosslinking agent. The central composite design model was used to optimize the beads. The response of beads was 91.9861 % for loading efficiency, 0.998 mm for bead size, and 360 min for time required for 90% drug release. The optimal formulation variables for a formulation were found to be 4.14 mg of pectin, 1.82 mg of alginate, 14.36% of $CaCl_2$, and 5.99 hours of cross-linking time. PEC-ALG beads showed no release in 0.1 N HCl (pH 1.2). In contrast, increased Ca^{2+} and Na^{+} ion exchange as well as solvent penetration into the pectin-alginate network have been linked to the quick release from cross-linked beads in phosphate buffer pH 7.4. FTIR results showed that there was no interaction between the medication and the polymers, as evidenced by the significant peaks of BUD detected in the beads. An *in vitro* study of the toxicity of beads on A549 cell lines revealed significantly higher cell viability than the group of cells treated with pure BUD.BUD loaded PEC-ALG beads could have potential for chrono modulated delivery system for targeting nocturnal asthma.

Keywords: Chronotherapy; Delayed Release Beads; Quality by design; Nocturnal Asthma.

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Conflict of interest: None

INTRODUCTION

The use of targeted and controlled drug delivery has surpassed conventional dosage forms in recent years. In order to target the precise site, this system has focused on a continuous, variable, and sustained drug delivery system.[1] The term "chrono modulated drug delivery system" refers to such a system.[2] It is also referred to as a pulsatile drug delivery system or a chrono modulated drug delivery system. Two-thirds of asthma patients have nocturnal asthma, which is characterized by an increase in symptoms such as wheezing, tightness in the chest, increased responsiveness of the airways, and worsening of lung function during the night between midnight and 8:00 am, and particularly around 4:00 am, these symptoms manifest. Therefore, since the patient is asleep, taking medication at midnight is inconvenient[2]. For the best course of treatment, maintaining a steady drug level is not always necessary. A medication should only be administered in the bare minimum amount necessary in terms of BCS classification, it is in class[3]. The intention was to have a 6-hour lag time, meaning that the medication is taken at bedtime and released into the system at 4:00 am, or after 6 hours.[3] By coordinating drug delivery with disease patterns and circadian rhythms, chrono-modulated drug delivery systems have become a viable strategy for improving therapeutic outcomes. Numerous physiological processes, such as drug absorption, distribution, metabolism, and elimination, are regulated by the circadian rhythm.[4] With chrono-modulated drug delivery systems, drugs are released under controlled conditions at predetermined intervals to correspond with the body's natural rhythm. Nocturnal asthma, that affects two-thirds of asthma patients, is typified by an increase in symptoms like wheezing, tightness in the chest, increased airway reactivity, and a decline in lung function at night. In particular, at 4:00 am, and between midnight and 8:00 am, these symptoms manifest. Several strategies have been explored for designing chrono-modulated drug delivery systems, such as implantable devices, transdermal patches, and oral formulations with specialized release profiles. These systems incorporate various mechanisms, including pH-sensitive coatings, osmotic pumps, and programmable electronics, to achieve the desired temporal release pattern.[5-7] The benefits of chrono-modulated drug delivery systems are extensive. By targeting drug administration during specific periods of disease activity or maximum need, therapeutic concentration levels can be optimized, leading to improved patient outcomes. Furthermore, by minimizing drug exposure during inactive disease phases, side effects and drug resistance can potentially be reduced.[8] This study focused on developing chrono-modulated drug delivery systems for targeting nocturnal asthma.[9]

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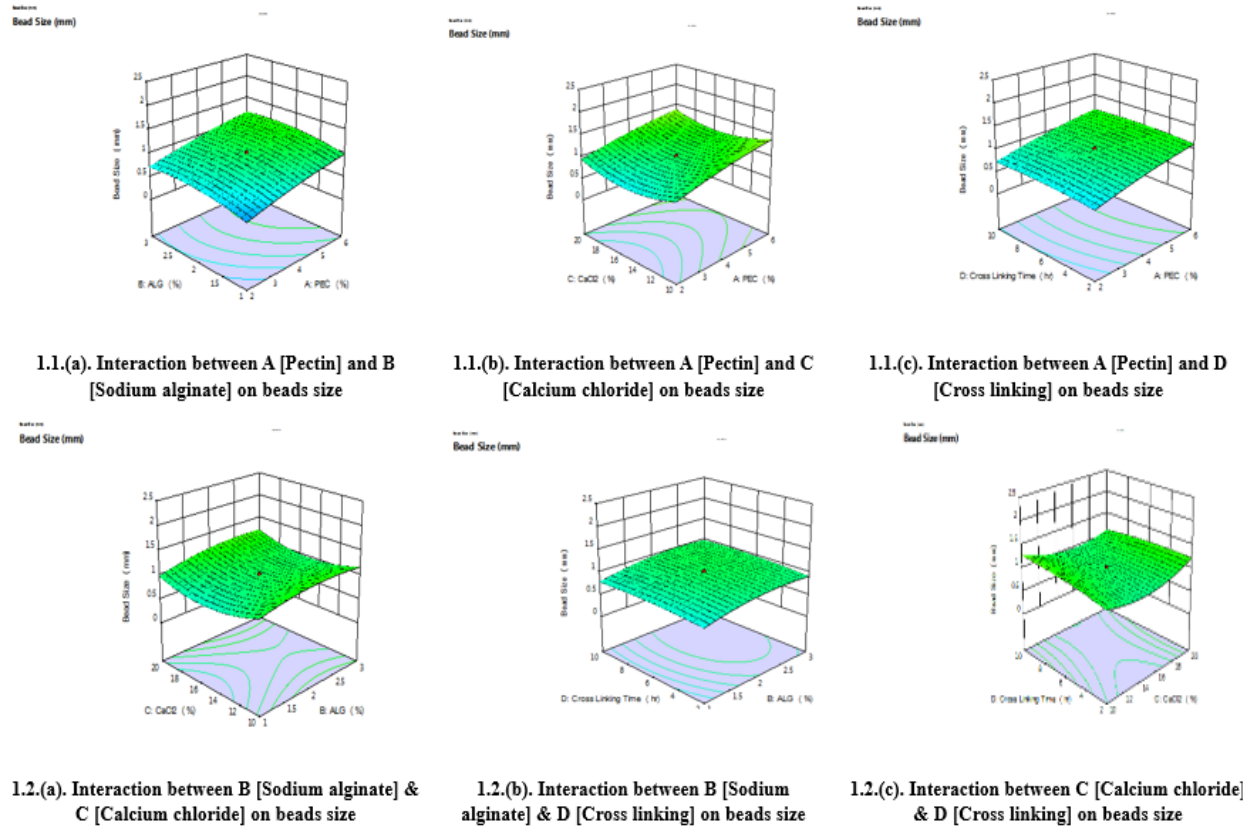
The quick in vitro release of the entrapped material is a significant drawback of alginate, one of the most widely utilized natural biopolymers as a delivery method for low molecular weight phytochemicals.

Since alginate aqueous solutions have extremely high viscosities even at low concentrations, the matrix material for encapsulation can be employed at a maximum concentration of roughly 2-4%, for example, in the case of



1.1.(a). Interaction between A [Pectin] and B [Sodium alginate] on beads size

1.1.(b). Interaction between A [Pectin] and C [Calcium chloride] on beads size

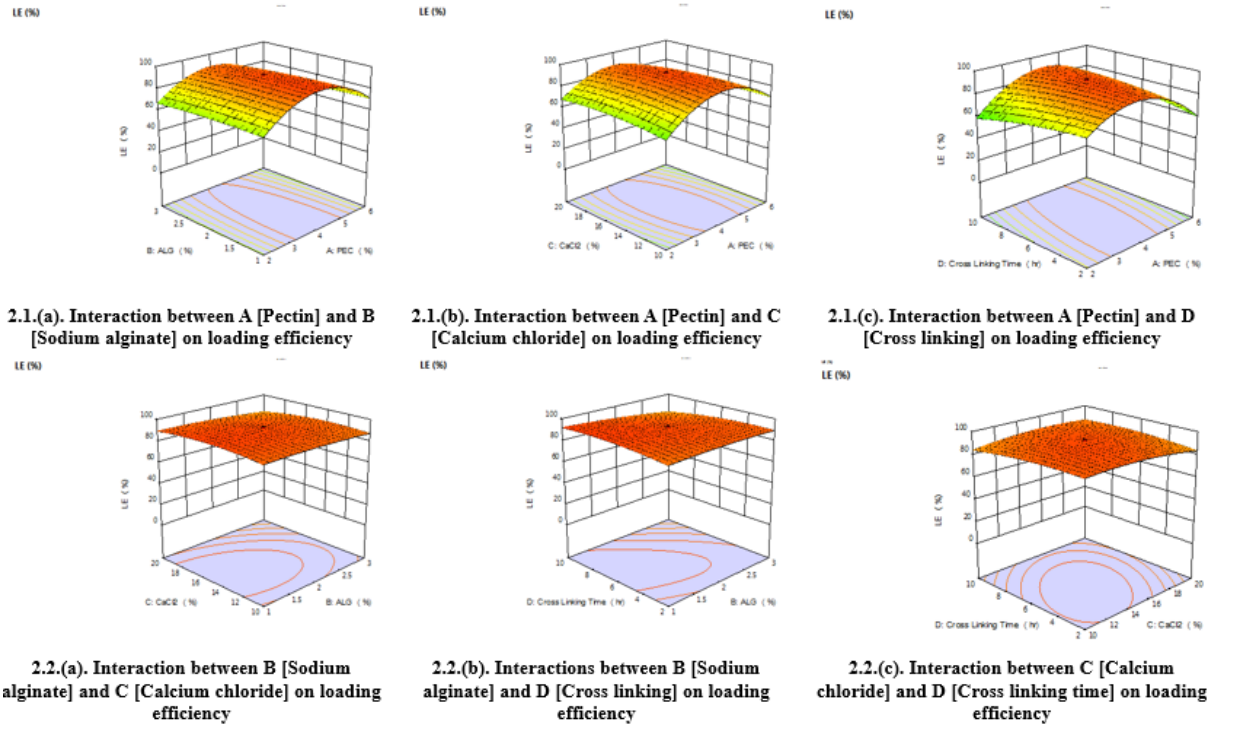
1.1.(c). Interaction between A [Pectin] and D [Cross linking] on beads size

1.2.(a). Interaction between B [Sodium alginate] & C [Calcium chloride] on beads size

1.2.(b). Interaction between B [Sodium alginate] & D [Cross linking] on beads size

1.2.(c). Interaction between C [Calcium chloride] & D [Cross linking] on beads size

Figure 1. Effect of the Independent Variable on Bead Size (mm)



2.1.(a). Interaction between A [Pectin] and B [Sodium alginate] on loading efficiency

2.1.(b). Interaction between A [Pectin] and C [Calcium chloride] on loading efficiency

2.1.(c). Interaction between A [Pectin] and D [Cross linking] on loading efficiency

2.2.(a). Interaction between B [Sodium alginate] and C [Calcium chloride] on loading efficiency

2.2.(b). Interactions between B [Sodium alginate] and D [Cross linking] on loading efficiency

2.2.(c). Interaction between C [Calcium chloride] and D [Cross linking time] on loading efficiency

Figure 2. Effect of the process parameters on the Loading Efficiency of the beads

Ca-alginate. Consequently, the resultant gel network lacks the required barrier effect and has a low density. As a result, a strategy for developing novel techniques to produce water-insoluble microparticles with decreased porosity and delayed release of entrapped materials is needed.[10,11] Alginate can be used in conjunction with complementing plant-derived biopolymers, like Based on the literature, the present work aims on design and development of chrono-modulated drug delivery systems using alginate-pectin combination polymers with ionotropic gelation technique.[12]

MATERIALS AND METHODS

Optimization of Delayed Released Beads (Design of the Experiment [DOE])

As a possible colon administration technique, bud-beads have been developed and designed with the best possible formulation using the response surface methodology.[13] The Central Composite Design approach was used to optimize the parameters that make up the BUD-Beads formulation using Design Expert Software version 11 (Stat-Ease, Minneapolis, MN, USA) (https://www.statease.com/docs/v11/ viewed on April 28, 2024). Pectin (X1), sodium alginate (X2), calcium chloride (X3), and cross-linking [x4] were the four formulation components (independent variables) that were put to use. Three responses (dependent variables) were studied in order to determine the best formulation: the time required for drug 90% release (Y3), loading efficiency (Y2), and bead size (Y1). The experimental design consisted of a randomized sequence, six replication cases with central points, six axial points, and eight designated factorial points. To test the method's repeatability, the focal point was iterated five times.[14] The response surface regression technique was employed to assess the information. A polynomial model was chosen by taking into consideration the significant terms ($p < 0.05$), coefficient of variance, least significant lack of fit, and multiple correlation coefficients that were obtained from the Design Expert tool. According to the independent variables, Table 1 shows the highest and lowest levels.

Preparation of Ca^{++} ion Cross-linked Pectin-Alginate

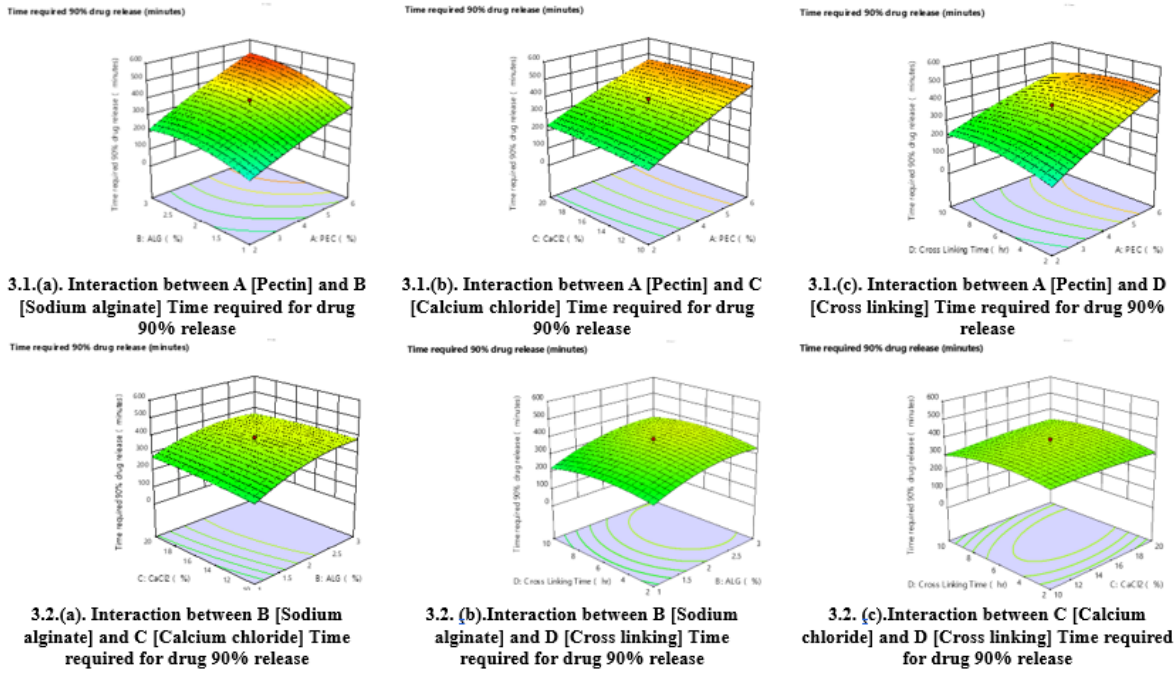


Figure 3. Effect of the process parameters on the Time required for drug 90% release

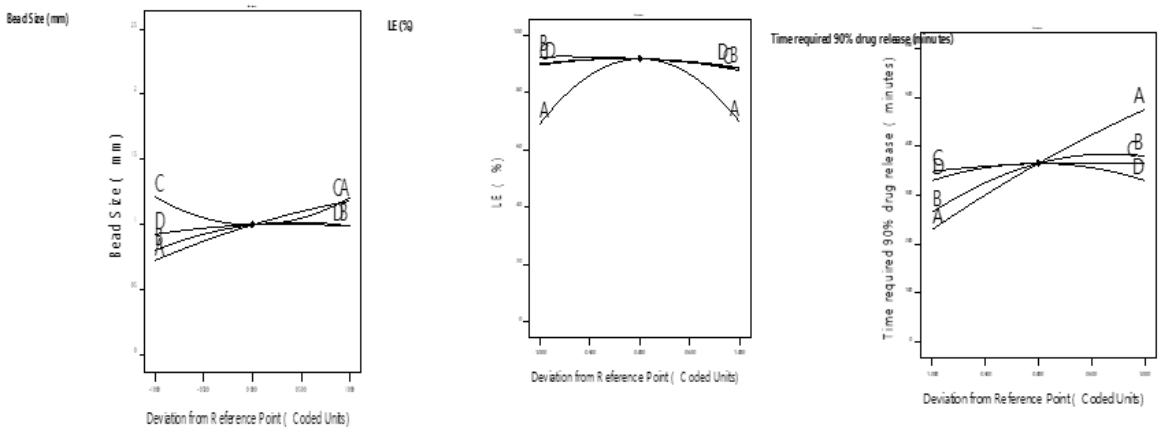


Figure 4. Pertubation Graph a) Bead Size b) Loading efficiency c) Time required 90% drug release

Beads

In double-distilled water, sodium alginate and pectin were dissolved at concentrations of 1-3.0% w/v and 2–6% w/v, respectively. Then, while stirring, precisely weighed BUD (drug: polymer, 1:1) was added. The dispersions were gently stirred at room temperature and added to a 250 ml solution of CaCl2 (at concentrations of 0.05 M and 0.20 M) using a syringe (no. 23) with a drop-in grate set at 1 ml/min. The calcium pec-alginate beads that had formed were left to stand in the gelling solution for two to ten

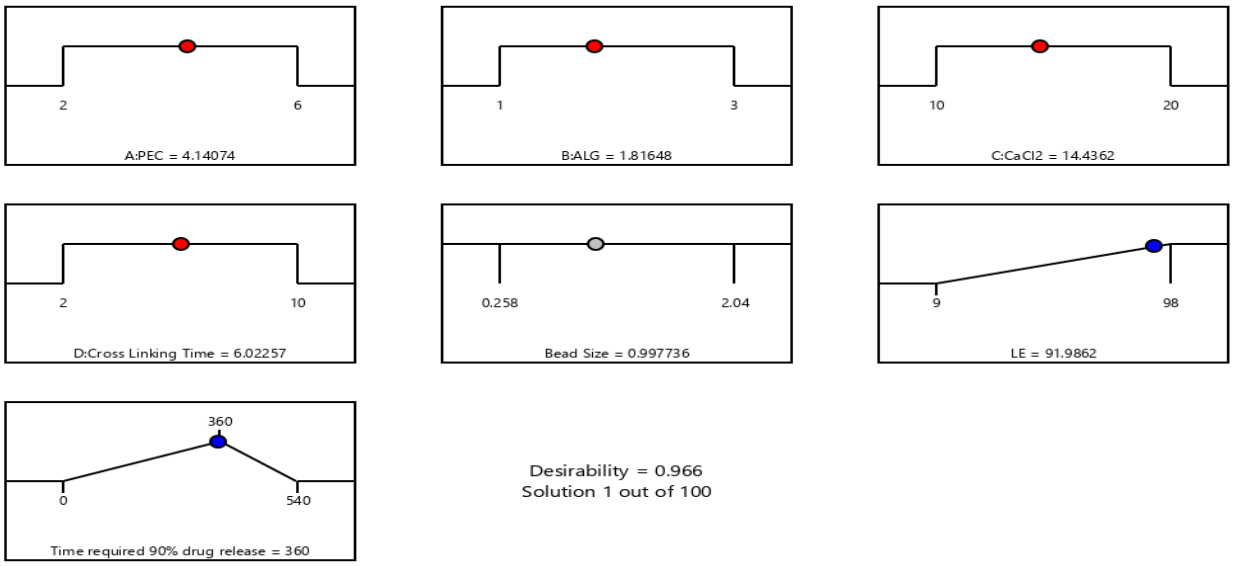


Figure 5. Observed values of optimal condition

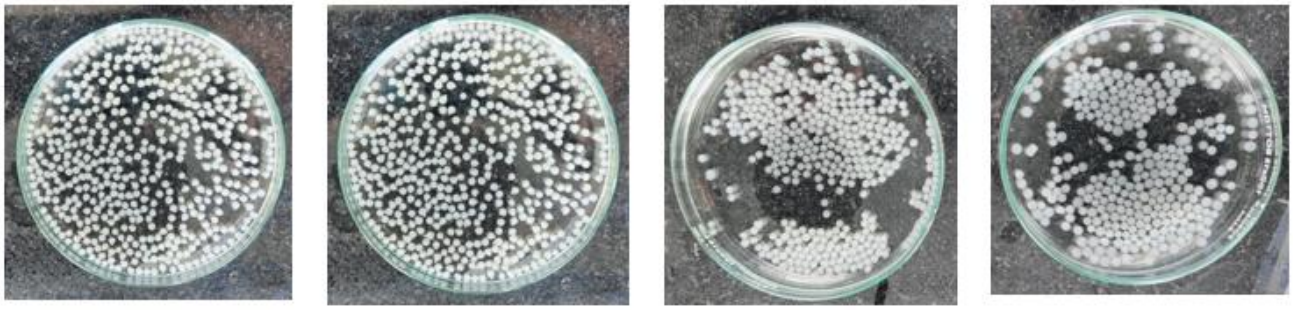


Figure 6. Formulation of Budesonide-PEC-ALG Beads.

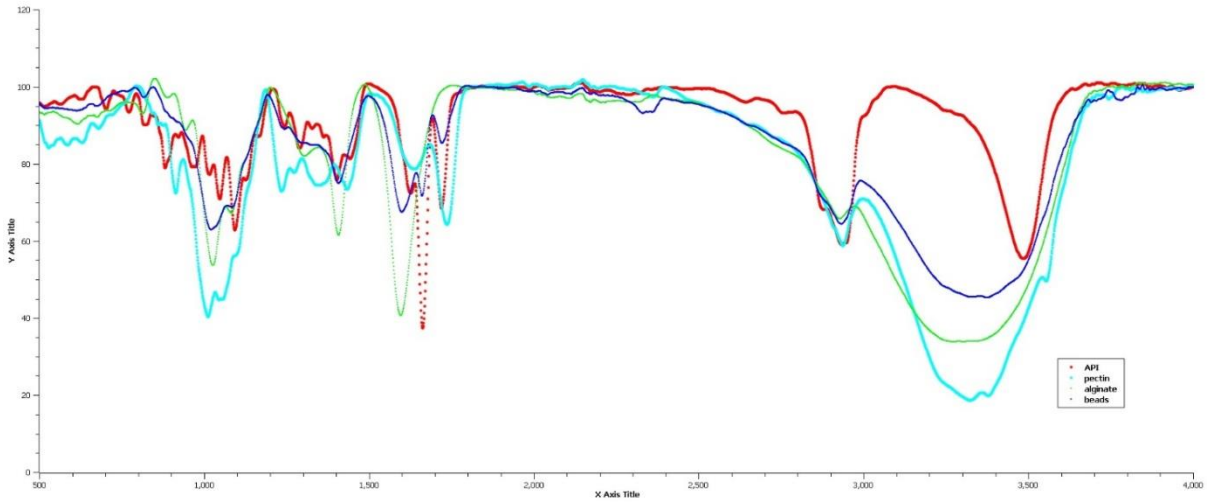


Figure 7. The combined graphical image of FT-IR. The line which indicatesred color is pure API,the line sky bluecolor indicates pectin, theline green colorindicates alginate and blue color indicate beads. Overall prepared beads show the combined graph.

hours. After that, they were filtered out and given two successive washes in 100 milliliters of double-distilled water. The beads were then baked for 24 hours at 50°C to dry them out.[15]

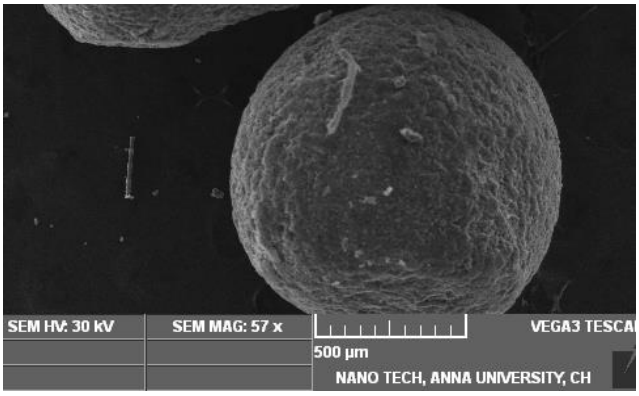


Figure 8. SEM image of Delayed release beads

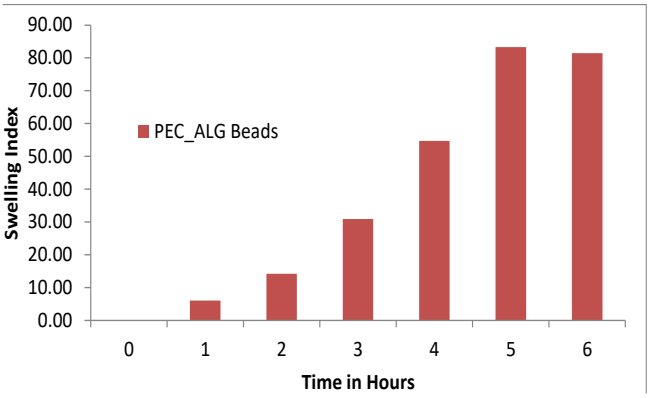


Figure 9. Swelling index bar graph

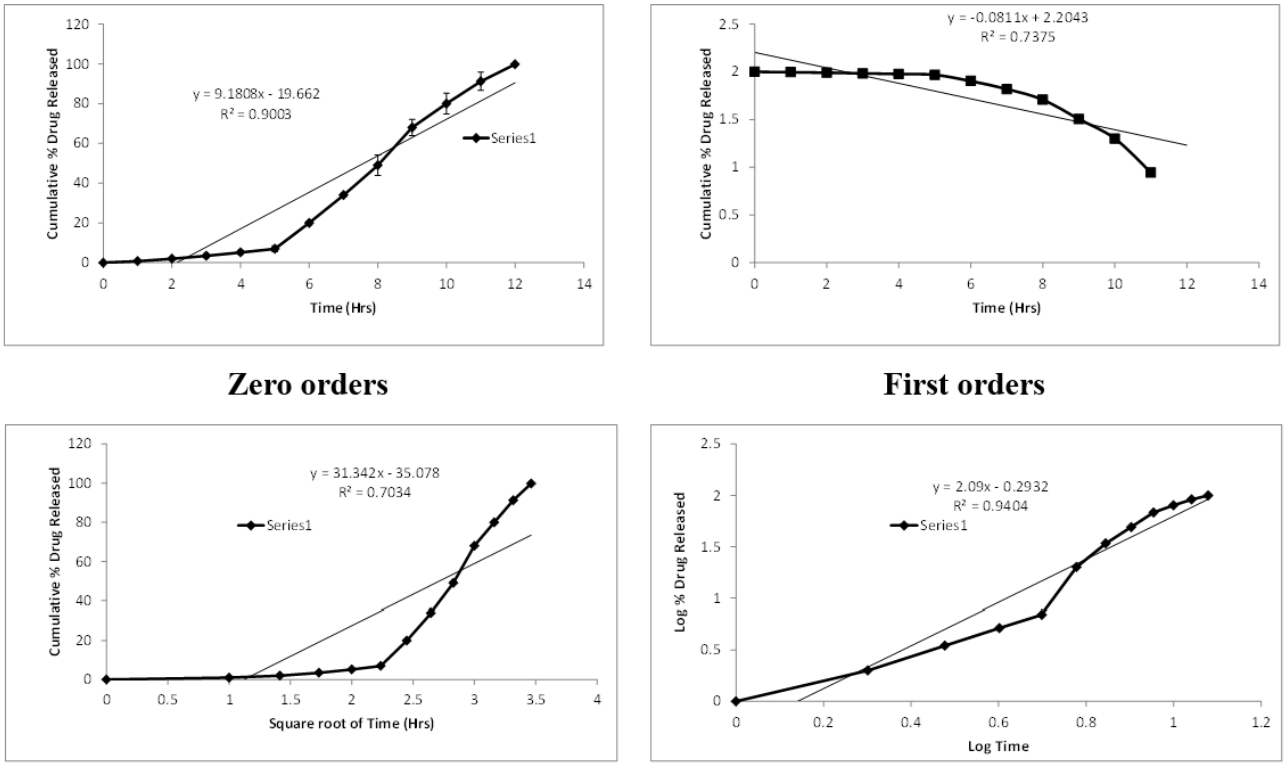


Figure 10. Release kinetics of Budesonide from PEC-ALG Beads.

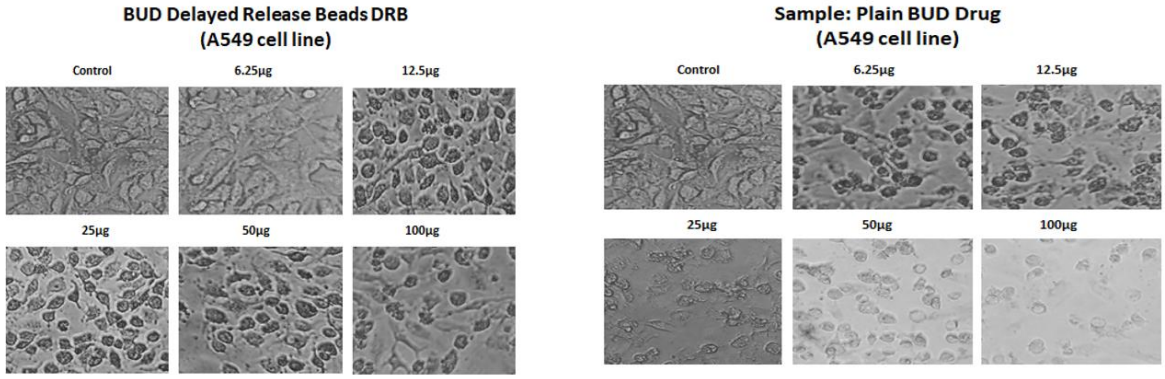


Figure 11.*In vitro* Cytotoxicity Assay of optimized beads a) Budesonide DRB (A549 cell lines) and b) Plain Budesonide drug (A549 cell lines)

Practical Yield

After the last beads were gathered, the practical yield was calculated using the equation

Practical yield = [(practical weight of beads)/(theoretical weight of beads)] × 100.

Encapsulation Efficiency

Every batch of designed beads was individually weighed at 50 mg and added to 100 mL of pH 6.8 phosphate buffer containing 0.5% w/v Tween 80. After that, BUD was released when the beads were broken down by ultrasonication. Whatman's filter paper (diameter 0.45 µm) was used to filter the samples, and a UV spectrophotometer was used to examine them at 240 nm.

Encapsulation efficiency was determined by the below given formula[15],

Encapsulation efficiency = (Actual drug loading/ Theoretical drug loading) × 100

Measurement of Beads Size

A ten-piece string BUD-Beads were chosen at random. Each type of bead was measured for size and form using a computerized vernier caliper (Mitutoyo, Kawasaki, Japan). Ten beads were chosen at random to establish the mean diameter of the beads. Each bead's length and width were measured, and its diameter was used to compute the mean.

Scanning Electron Microscopy (SEM)

Double-sided adhesive was used to adhere the beads on the plate, and gold spray was used afterwards. Using an accelerating voltage of 10 kV, the morphological features of the samples (Supra 55, Zeiss, Oberkochen, Germany) were examined and captured on camera.[16]

Fourier Transform Infrared Spectroscopy (FT-IR)

The drug's physical interaction and the surface chemistry of the polymers were determined by FT IR spectra. The samples were combined with KBr and compressed into transparent tablets. A Jasco 6700 FT-IR spectrometer was used to analyze the samples for FT-IR spectrometry across the 4000-400 cm-1 range[17](Figure 7).

Swelling Index

Using a digital vernier caliper, 10 BUD's length and width-The weights of the beads were calculated after they were measured. After being placed in 20 milliliters of pH 1.2 buffers, each type of bead was stirred for two hours at 50 rpm and 37 °C.to mimic stomach conditions. Beads were transferred to a pH 7.4medium after two hours.[14] For six hours, the beads were examined in the medium for signs of swelling, erosion, and water absorption. Each wet bead's moisture content was removed, and then the bead was carefully wiped over a dry Petri dish until no visible moisture remained on the dish's surface in order to determine the weight. The bead was then

$$SI = \frac{St - Si}{Si} x100$$

Where Si = initial PEC-ALG dry bead weight, and St = wet PEC-ALG bead weight at time t (hours). (Figure-9).

***In vitro* Drug Release Studies**

For the dissolution investigation, a USP Type II (paddle type) dissolving apparatus was used, with a paddle speed of 75 rpm and a temperature of 37±0.5 °C. In order to simulate gastrointestinal conditions, the core cross-linked beads were first tested for dissolution in 500 mL of 0.1N HCl (pH 1.2) with 0.5% w/v tween 80, and then for further hours in 900 mL of phosphate buffer pH 6.8 with 0.5% w/v tween 80. The amount of medication was taken into account when placing beads into size 1 hard gelatin capsules.[15-17] At predetermined intervals, five milliliters of the sample were removed, replaced with fresh matching media, and examined at two distinct wavelengths using a UV-Visible spectrophotometer at 240 nm in 0.1N HCl and 240 nm in phosphate buffer.

***In vitro* drug release kinetics analysis**

To identify which mathematical model best matched the drug release profile, the received release data were fixed into numerous models, including the (i) Zero Order, (ii) First Order, (iii) Higuchi, Korsemeyer-Peppas, and (iv) Hixson Crowell models.[18] The release from systems where the particle diameter and surface area change is described by the equation. Data from in vitro drug release studies were plotted as the cube root of the drug percentage remaining in the matrix versus time to study the release kinetics. The result is a straight line.

***In vitro* cell line study**

Preparation of test solutions

For MTT assay, serial two-fold dilutions (6.25 – 100 µg) were prepared from this assay.

Cell lines and culture medium

Penicillin (100 IU/mL), streptomycin (100 µg / mL), and 10% inactivated fetal bovine serum (FBS) were added to DMEM media containing the A549 cell line until it reached confluency. The culture was kept in a humidified environment with 5% CO2 at 37 ℃.

Procedure

The monolayer cell culture was trypsin zed and the cell count was adjusted to 1.0 x 105 cells/mL using the proper media containing 10% FBS. In each well of the 96-well microtiter plate, 100 µL of the diluted cell suspension (1 x 104 cells/well) was added[19-21]. After 24 hours, when a

Table 1. Formulation factors for preparation of bud-beads and corresponding level

Factor Variable	Name of Parameters	High	Medium	Low	Alpha(+)	Alpha(-)
A	Pectin	6	4	2	8	0
B	Sodium Alginate	3	2	1	4	0
C	Calcium Chloride	20	15	10	25	5
d	Cross linking time	10	6	2	14	-2
RESPONSE		Response			Units	
Y1		Beads size			Mm	
Y2		Loading efficiency			%	
Y3		Time required 90 % drug release			minutes	

partial monolayer had formed, 100 µL of different test sample concentrations were added to the partial monolayer in microtiter plates, the supernatant was disposed of, and the monolayer was once more washed with medium. The plate was then incubated for a full day at 37℃ with 5% CO2 in the air. After the test solutions were removed, 20 µL of MTT (2 mg/1 mL of MTT in PBS) was added to each well.

RESULTS AND DISCUSSIONS

The BUD-Beads for this study were developed using Stat-Ease Design Expert software version 11 and the response surface methodology. The main compound model was used to investigate the primary formulation variables for the experimental design that influence the responses of the Bead size (Y1), loading efficiency (Y2), and time required for 90% drug release (Y3) of the Pectin, Alginate, and Beads. The research study looked to determine whether various amounts of cholesterol, Chitosan, and surfactants affected the previously mentioned responses. In accordance with the main compound layout, twenty equations in a three-factor, three-response format with six in central locations were created. Furthermore, the responses were assessed using analysis of variance (ANOVA), as well as the matching response. A uniform and regulated spherical bead size is crucial for understanding the behaviour of drug release in the GIT area. As a result, it was thought that the goal of optimizing size should be circular naturally and have a minimal value. After considering dual straight regression equations, the Y1 result can be written as follows:

$$\boldsymbol{BeadSize}\ (\boldsymbol{Y_1}) = +\mathbf{850.00} - \mathbf{35}\boldsymbol{A} - \mathbf{48.75}\boldsymbol{B} + \mathbf{1.25}\boldsymbol{C} + \mathbf{42.50}\boldsymbol{AB} + \mathbf{27.50}\boldsymbol{AC}$$

Table2. Optimization using CCD Design

Std	Run	Factor 1	Factor 2	Factor 3	Factor 4	Response 1	Response 2	Response 3
		A: PEC	B: ALG	C: CaCl2	D: Cross Linking Time	Bead Size	LE	Time required 90% drug release
		%	%	%	hr	mm	%	minutes
7	1	2	3	20	2	0.86	66	180
5	2	2	1	20	2	0.79	60	120
10	3	6	1	10	10	1.21	72	240
1	4	2	1	10	2	0.6	61	90
24	5	4	2	15	14	1.02	92	240
13	6	2	1	20	10	0.69	70	270
16	7	6	3	20	10	1.08	58	480
15	8	2	3	20	10	0.76	42	180
11	9	2	3	10	10	0.99	40	180
28	10	4	2	15	6	0.89	90	390
14	11	6	1	20	10	0.97	60	240
8	12	6	3	20	2	1.42	49	480
2	13	6	1	10	2	1.21	65	420
26	14	4	2	15	6	1.02	92	360
22	15	4	2	25	6	2.04	86	300
12	16	6	3	10	10	1.38	67	480
25	17	4	2	15	6	1.02	92	360
23	18	4	2	15	-2	0.84	87	120
6	19	6	1	20	2	0.976	51	420
4	20	6	3	10	2	0.923	49	480
27	21	4	2	15	6	1.02	92	360
17	22	0	2	15	6	0.258	10	0
18	23	8	2	15	6	1.58	9	540
9	24	2	1	10	10	0.856	44	120
21	25	4	2	5	6	1.85	87	270
20	26	4	4	15	6	1.01	90	300
30	27	4	2	15	6	1.02	92	360
3	28	2	3	10	2	0.798	79	240
29	29	4	2	15	6	1.02	92	360
19	30	4	0	15	6	0.368	98	0

Table 3: Optimized formulation based on desirability

Factor	Name	Level	Low Level	High Level	Coding
A	PEC	4.14	2.00	6.00	Actual
B	ALG	1.82	1.0000	3.00	Actual
C	CaCl2	14.36	10.00	20.00	Actual
D	Cross Linking Time	5.99	2.00	10.00	Actual

Table 4: Experimental and predicted values under optimal conditions for Oil Entrapped Beads

Solution 1 of 99 Response	Predicted Mean	Predicted Median	Observed	Std Dev	SE Mean
Bead Size	0.998432	0.998432	1.02	0.178968	0.0725966
LE	91.9861	91.9861	90.56	9.69966	3.93457
Time required 90% drug release	360	360	360	68	28

$$-10.00BC + 45A^2 + 27.5B^2 + 52.5C$$

In contrast to beads with more spherical morphology and medium and high polymer concentrations (+ level), low concentration beads (-level) have rough and uneven shapes because of inadequate molecular packing and cross-linking. As the PEC and SA concentrations were increased this was the case, as demonstrated by the response surface plots relating bead size (Figure1). The variations in the size and morphology of the beads with different crystalline levels of PEC and SA were caused by changes in the availability of reacting/binding sites for cross-linking cations (Ca^{2+}). When the cross-linking agent content was increased with SA, the result was smooth, spherical, well-packed beads with a distinct structure; however, similar material in this instance of CS resulted [21].

Effect of the process parameters on the Loading Efficiency of the beads (Y2)

Using polynomial equation, the impact of Examined were the primary and compound Impact of separate variables on EE. An ANOVA revealed that the effect was statistically significant (P < 0.0001).

$$EE\ (Y_1) = +82.00 + 7.25A + 1.63B + 1.13C + 2.00AB - 2.50AC - 0.25BC - 3.88A^2 - 5.63B^2 - 2.62C^2$$

The model's predictive power was demonstrated by the model fitting criterion of R2 coefficient calculation. The Y2 (LE) score for each trial batch displayed a strong R-squared of 0.9976. The equation shows that the independent variables A, B, C, and D have "p" < 0.05, which significantly affects the LE. The findings unequivocally demonstrate that the interaction between the Ca2+ ion and the COO groups in both polymers may have led to the development of a "egg-box" structure in PEC within alginate beads (Figure2). This can, as predicted, lessen the issue of drug leaking during bead manufacturing. Particle size variations in response to simultaneous changes in two independent variables were demonstrated by the interaction terms (AB, AC, and BC). The coefficient values in the given equation.[21]

Time required for drug 90% release

Regardless of time, every bead delivered 3-4% of the medication in an acidic media. Another benefit of the modest drug release in the acidic medium is that it helps lessen BUD's gastrointestinal discomfort. Following this lag, a pulse with full drug release in phosphate buffer occurred in 360 minutes. The PEC-ALG beads exhibited a notable delay in drug release at an acidic pH, which could potentially be attributed to the drug's insolubility with pectin. Calcium pectinate may protonate into an insoluble form with decreased swelling at an acidic ph. It is possible to relate the second pulsed release stage in pH 7.4 phosphate buffer to the calcium pectinate gel's fast expansion and gel relaxing at an alkaline ph. There was a notable impact of matrix beads on the time needed for 90% drug release[22-23] (Figure 3).

Optimization of Formulation Factors

The second-order polynomial equations explain the model in terms of the coded parameters, and the central composite design chose A four-dimensional equation. To link the answers and the Separated factors. The multiple correlation coefficient (R2) and confidence interval (P) of the revised model were employed for assessment. The equations for (LE), (size), and (DR) have R2 values of 0.9707, 0.9690, and 0.9718, respectively, as shown in Table 2. An R2 level above 0.80. Indicates the method of regression used is suitable and is considered a good fit model. This suggests that the model was unable to account for only 2% of the response variation and that the process parameters under investigation account for more than 98% of the diversity in the features of pectin, alginate, and beads (Figure 4).

ANOVA

After the responses were collected, they were adjusted, and an ANOVA was performed in order to estimate the R2 based on the Central Composite Design. Table provides the adjusted and predicted R2, precision, percentage CV, and P values.[24]

Predicted and observed values

After obtaining and tabulating the experimental and predicted values, the percentage error of the responses was also obtained (Figure 5).

Optimization of PEC-ALG beads factors

A quadratic model that connects the independent parameters and the DRB (response) was chosen by the Central composite design. The second-order polynomial equation, which is shown in Table 3&4, describes the quadratic model in terms of the coded parameters. The

multiple correlation coefficient (R2) and confidence interval (P) of the revised model were employed for assessment. The R2 values for Equation (Size), (LE), and TR were 0.9707, 0.9690, and 0.9718, in that order. An R^2 value of higher than 0.09 indicates that the applied coefficient regression model is suitable and is considered a good fit model. This suggests that the model was unable to account for all but 2% of the response variation, and that the process factors under investigation account for more than 98% of the diversity in DRB features.

Preparation of Ca^{++} ^{ion} Cross-linked Pectin-Alginate Beads

The ideal formulation variables for a formulation that is optimized following to the CCD model and it was found that 4.14 mg of Pectin, 1.82 mg of Alginate, and 14.36 % of Cacl2 and 5.99 hours of cross-linking time using predicted values that are 91.9861 % for Loading Efficiency, 0.998 mm for Bead Size and 360 min for time needed for 90% BUD release(Figure 6).The observed values were strikingly comparable to the projected values, confirming the validation and dependability of the CCD model used for DRB formulation optimized. This is why the % error was so low.

The loading efficiency (EE%) of the PEC-SA beads was calculated, and the result was observed to be 91%. The practical yield of the beads was discovered to be 95%. According to the SEM photomicrograph of the complete bead structure and the cross-sectional view of the optimized formulation displayed in the figures, respectively, the BUD-entrapped beads had a spherical shape and a rough, porous exterior surface that helped the PEC-Alginate beads release the drug under control.[25] A web of tiny fissures and cracks covers the exterior surfaces; these may have formed as a result of the polymeric gel's drying process and water molecules moving around shrinking as a result. It is evident that the polymers' successful crosslinking with Ca2+ resulted in the spheres' uniform creation. These beads' surface did not exhibit any drug crystals, suggesting that the pectin-alginate matrix contained a finely distributed drug. The scanning electron microscopy study reveals that optimized beads were found to be spherical in shape. The size of the beads was ranged from 0.798 – 1.30 mm and their mean value was found 1.02 mm of PEC-ALG matrix delayed released beads (Figure.8).

Swelling index

PEC-ALG bead swelling behavior was compared at two distinct pH values (1.2 and 7.4) to simulate how the beads in the GIT change. Two distinct solutions were used to study the characteristics of bead swelling: one at pH 1.2 for two hours, and the other at pH 7.4 at 37°C.The impact of PEC on the optimized beads' degree of swelling is depicted in (Figure 8). After two hours of incubation in 0.1 M HCl, the percentage weight gain was measured to estimate the swelling index. Because of the ionized carboxylic groups, which are highly hydrophilic, beads showed a maximum degree of swelling at pH 7.4, and as the PEC ratio rose, the swelling index dramatically rose as well.[26]

***In vitro* release study**

Ionotropic Ally gelled PEC-ALG beads release BUD in vitro in a pulsatile manner (Figure 10). After two hours in the alkaline dissolving medium (phosphate buffer; pH 7.4), there was a faster release of BUD in comparison to the acidic dissolution media (pH 1.2; 0.1 N HCl). This could be because the medication released in the alkaline media was relatively higher due to the beads' tendency to expand faster in the alkaline medium than in the acidic one. It is likely that the ionized carboxylic acid groups of the pectin backbone repelled each other electrostatically produced a significant swelling force in an alkaline medium. The PEC-ALG beads in an alkaline environment were expected to grow following liquid uptake during the first drug, which then diffused through the hydrated viscous layer. The dissolution profiles revealed that the beads released the medication slowly at first, but then more quickly (Figure 10). The overall findings indicate that the dried beads did not swell in the stomach. When the beads were transported to the colon, they began to expand and served as a matrix for chrono-modulated drug release.[27]

***In vitro* Cytotoxicity Assay of optimized beads**

The MTT method is accurate, user-friendly, and yields repeatable results. 3- [4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide, or MTT, is the primary component. It is a tetrazolium salt that dissolves in water and, when mixed with salt solutions or other media without phenol red, yields a yellowish solution. After MTT is dissolved, it becomes an insoluble purple formazan (Figure 11).

CONCLUSION

Beads of BUD loaded pectin-alginate beads were made by ionotropic gelation, in which calcium chloride was added as a crosslinking agent. FT-IR analysis revealed significant peaks of BUD was found in beads, indicates good compatibility between drug and polymers. Sodium alginate was used to prepare the pectin alginate beads, which were then optimized using the central composite design model. 4.14 mg of pectin, 1.82 mg of alginate, 14.36% of cacl2, and 5.99 hours of cross-linking time were found to be the appropriate formulation variables. The response of the beads was 91.9861 % for loading efficiency, 0.998 mm for bead size, and 360 minutes for the amount of time needed for 90% drug release. SEM images revealed that the polymers' successful crosslinking with Ca2+ resulted in the sphere-shaped of beads with uniform distribution. A maximum degree of swelling was observed in the bead swelling characteristics at pH 7.4, and the results revealed that a significant higher in the PEC ratio was accompanied by a significant increase in the swelling index. PEC-ALG beads released in vitro in an alkaline medium may swell upon liquid absorption as the first medication pass through the diffusion mechanism into the high degree hydrated and leads to viscous layer. The dissolution of PEC-ALG beads profiles revealed beads first released the medication more slowly and then released it more quickly. An *In vitro* study of the toxicity of beads on A549 cell lines revealed significantly higher cell viability than the group of cells treated with pure

BUD.BUD loaded PEC-ALG beads could have potential for chronomodulated delivery system for targeting nocturnal asthma. Further *in-vivo* animal studies are needed to prove the novel concept.

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