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Research Article

Formulation and Optimization of Clarithromycin Loaded with Pullulan Acetate Microsphere for Sustained Release by Response Surface Methodology

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Abstract

The aim of this investigation was to develop and optimize Claritromycin loaded with pullulan acetate for sustained release application by response surface methodology based on factorial design with reduced 2FI Model. Total 8 formulations (F1, F2, F3, F4, F5, F6, F7 and F8) were prepared, out of which F5 was found to be best formulation for the sustained release of the Clarithromycin. Further, Various parameters like RPM, Time, Drug (Clarithromycin) Concentration and Pullulan acetate concentration were optimized with respect to F5. It was found that RPM and Time were having more effect as compared to Clarithromycin and Pullulan acetate concentration for sustained release of the drug. The 3D response plot were drawn and optimum interactions were selected by feasibility and grid searches. The observed responses were coincided well with the predicted values by the experimental design. The optimized formulation F5 showed prolonged sustained release of Clarithromycin over 7 hours. The release profile of the drug loaded microsphere reveals that pH of the medium was influencing it at *in vitro* condition. In the optimized conditions, the sustained release can be reached up to 9 h. Therefore Pullulan acetate loaded with Clarithromycin is a useful polymerised materials for the development and formulation of pH sensitive drug.

Keywords: Clarithromycin; Pullulan acetate; 2FI model; Formulation; Sustained release

Introduction

Helicobacter pylori are spiral-shaped bacteria that grow in the digestive tract and have a tendency to attack the stomach lining. *H. pylori* infections are usually harmless, but they are responsible for the majority of ulcers in the stomach and small intestine. It is associated with the development of serious gastro duodenal disease including peptic ulcers, gastric lymphoma and acute chronic gastritis [1-3]. This is because of the low concentration of the antibiotic reaching the bacteria under the mucosa, instability of the drug in the low pH of gastric fluid and short residence time of the antibiotic in the stomach. Triple therapy for treatment of *H. pylori* includes Proton pump inhibitor, Clarithromycin (500 mg), and Metronidazole (400 mg) or Amoxicillin (1 g) twice a day. Clarithromycin is semisynthetic macrolide antibiotic derived from erythromycin which is active against a variety of microorganisms. It is effective against Mycobacterium avium complex (MAC) and is used for the treatment of *H. pylori*-associated peptic ulcer disease [4-6]. One way to improve the efficacy in eradicating the infection is to deliver the antibiotic locally in the stomach. Better stability and longer residence time will allow more of the antibiotic to penetrate through the gastric mucus layer to act on *H. pylori* [7-9]. Pullulan is a good water soluble neutral linear polysaccharide consisting of α-1,6 linked maltotriose residues. It is broadly used as a food additive and is considered to be safe for human use. In present days material scientist are paying more and more attention to process the inorganic crystallization studies within a largely formulated organic matrix of biologically synthesised compounds like Pullulan. They are generally used for various types of *in-vitro* and *in-vivo* drug delivery studies along with different types of therapeutic formulations etc. Pullulan is a hydrophilic polysaccharide by nature, so it is very essential to change the hydrophilic nature into a hydrophobic for its applications in the controlled drug delivery system which can be done by the substitution reaction (generally acetylation reaction). The best suitable acetylated-pullulan is Pullulan acetate, which was used for carrying the drug as polymers in previous studies done by various authors [10].

In the development of any pharmaceutical product for sustained release ability, an important issue is to design a formulation with optimized quality in a short time period and minimum number of trials [11-13]. The response surface methodology has been commonly used for designing and optimization of different pharmaceutical formulations, which requires minimum experimentation. Thus, it is less time-consuming and cost-effective than the conventional methods of formulating dosage forms. Based on the design of experiments, response surface methodology encompasses the generation of polynomial equations and of the response over the experimental domain to determine the optimum formulation [14,15].

The main objective of the current investigation was to develop Clarithromycin sustained release formulaion loaded with a hydrophobic polymer, pullulan acetate by using response surface methodology. A computer-aided optimization technique using factorial design (4-Factors, 2-Levels) was employed to investigate the effect of the amounts of various parameters namely, RPM, pH, Pullulan acetate concentration and Clarithromycin concentration in order to find optimum combination for maximum release of the Clarithromycin from the polymer.

Materials and Methods

Chemicals

The Clarithromycin (CLN) was purchased from Square Pharmaceuticals Ltd. and Dichloromethane was taken from Sigma Chemical Co. (USA). Formamide, Pyridine (Chemical grade) and acetic anhydride were purchased from Hi media, India. Pullulan was purchased from TCI Chemicals, India.

Synthesis of pullulan acetate from pullulan

The Pullulan acetate was synthesized from pullulan as per the standard method [16] given by Mishra and Suneetha. The synthesis of Pullulan acetates was done as follows; in which 2.5 g of pullulan was suspended in 25 ml of formamide solution and dissolved by vigorous stirring at 400 rpm. After that 60 ml of pyridine with 150 ml of acetic

anhydride were added to this suspension which was stirred at 500 rpm for 2 days. The synthesized Pullulan acetate was extracted after reprecipitation from 250 ml of water.

Drug loading procedure

50 mg of pullulan acetate was made to dissolve in 5 mL of dichloromethane and to this solution 80 mg of Clarithromycin was added. Then, the solution was homogenised at gentle rpm in the room temperature until it was dissolved completely. In order to prepare the microspheres, this homogenised solution was dropped very slowly into 50 ml of double distilled water with stirred conditions in the homogeniser again to evaporate the organic solvent for 2 h at room temperature. Clarithromycin-loaded microspheres were obtained by centrifugation at 10,000 rpm, in 4°C for 15 min and then subjected to be dried at 70°C for 1 h to get the final microsphere samples. To get the amount of drug that had been loaded, the freeze-dried pullulan acetate microspheres were suspended in methanol, vigorously stirred for 30 min and then sonicated for 10 min. The resulting solution was centrifuged at 12,000 rpm for 20 min and the supernatant was taken for the measurement of drug concentration using UV spectrophotometer.

In-vitro release study

The release study of the drug loaded microspheres was carried out as follows; 50 mg of Clarithromycin-loaded microspheres in 1 ml of 0.15M phosphate buffered saline (PBS; pH 7.4) or HCI in PBS (pH 1.2) were put into two different dialysis tube which was introduced into 100 ml of respective buffer and was kept in a stirrer at gentle rpm at room temperature. At specific time intervals, the released sample was taken out to find the concentration of the released drug and replaced respectively with freshly prepared buffer. The concentration of the released drug was estimated by using UV spectrophotometer at 319 nm.

Experimental design for optimization

Different parameters like Time, RPM, Pullulan acetate concentration and Clarithromycin concentration were considered for the efficiency to release the loaded drug in the designed buffer. The whole analysis was done with Design Expert Software Version 7.0. In the Table 1 the low value and high value for the each factor has given. The design model was reduced 2FI model with 2 levels and 0 centre point. Total 8 runs were framed and each run was corresponded to different formulation such as F1, F2, F3, F4, F5, F6, F7 and F8 (Table 2).

Results and Discussion

In-vitro release study

Various parameters were optimized by the Design Expert Software Version 7.0. In the Table 3 total 8 formulations (F1…..F8) and their corresponding drug, Clarithromycin release % has been summarized.

From the Table 3 and Figure 1, it was found that the F5 was the optimum formulation, in which maximum amount of the drug was released could be reached more than 90% from the polymeric material. Hence F5 was considered for further study in the statistical optimization.

Statistical optimization

Various combinations of different levels of the factors were generated by the Design Expert statistical software and according to it the experiments were conducted. The drug release % was tabulated for respective trials (Table 4). From the experiment it was found that F5 was showing maximum release of the Clarithromycin from the polymeric material. As per the results obtained from the experiment, it was fitted to Reduced 2FI Model. In this model, one factor will not be counted for the analysis by the Design Expert Software. The model summary has been summarized in Table 5. A negative "Pred R-Squared" implies that the overall mean is a better predictor of response than the current model. In addition to that "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. In this model the ratio of 4.971 indicates an adequate signal. There fore, this model can be used to navigate the design space.

In general, a model fits the data well if the differences between the observed values and the model's predicted values are small and unbiased. In general, the higher the R-squared, the better the model fits the data. Here, R-squared value was found to be 82.38%. Therefore, this model can be used for the study.

ANOVA for selected factorial model

The "Model F-value" of 3.51 implies the model is not significant relative to the noise. There is 16.53% chance that a "Model F-value" this large could occur due to noise. Values of "Prob>F" less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model (Table 6).

Factor	Name	Units	Low Actual	High Actual	Mean	Std. Dev.
A	Pullulan Acetate	mg/mL	1.5	3.5	2.5	1
B	Time	Minute	10	30	20	10
C	RPM		500	15000	7750	7250
D	Drug Concentration	mg/mL	0.5	1.5	1	0.5

Table 1: Design of various Factors for the Analysis.

Formulations	Run	A: Pululan Acetate (mg/mL)	B: Time (Minute)	C: RPM	D: Clarithromycin Concentration (mg/mL)
F1	1	3.5	10	15000	0.5
F2	2	3.5	30	15000	1.5
F3	3	1.5	10	500	0.5
F4	4	1.5	10	15000	1.5
F5	5	1.5	30	15000	0.5
F6	6	3.5	30	500	0.5
F7	7	1.5	30	500	1.5
F8	8	3.5	10	500	1.5

Table 2: Various combinations of the factors.

Time in Hour	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	25	35	5	20	43	38	18	8
2	45	55	22	38	68	62	34	22
3	57	68	34	50	77	70	50	37
4	70	82	45	65	92	86	67	40
5	75	85	55	68	95	90	70	45
6	76	86	54	69	95	89	71	45
7	74	84	56	69	96	92	71	46

Table 3: Different formulations and the corresponding release profile (The values are the average of 3 different set of experiment and was made round off).

Formulations	Run	A: Pululan Acetate (mg/mL)	B: Time (Minute)	C: RPM	D: Clarithromycin Concentration (mg/mL)	Drug Release (%)
F1	1	3.5	10	15000	0.5	75
F2	2	3.5	30	15000	1.5	85
F3	3	1.5	10	500	0.5	55
F4	4	1.5	10	15000	1.5	68
F5	5	1.5	30	15000	0.5	95
F6	6	3.5	30	500	0.5	90
F7	7	1.5	30	500	1.5	70
F8	8	3.5	10	500	1.5	45

Table 4: Design Summary of different factors with actual response.

Std. Dev.	11.00568		**R-Squared**	0.82385
Mean	72.875		**Adj R-Squared**	0.588984
C.V. %	15.10213		**Pred R-Squared**	-0.25262
PRESS	2584		**Adeq Precision**	4.970833

Table 5: Model summary.

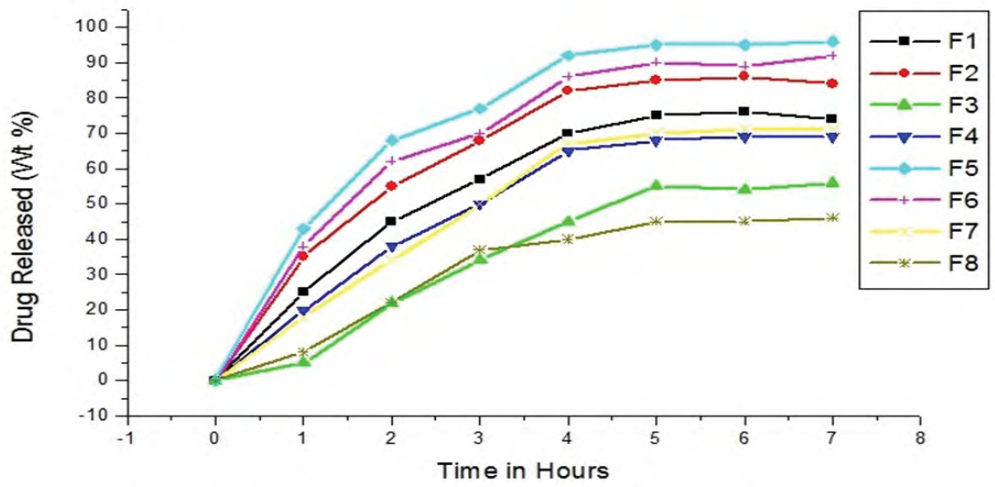

Figure 1: Release profile of different formulations (The values are the average of 3 different set of experiment).

Final equation in terms of coded factors

The different coefficient of the factors with standard error and digree of freedom have been summarized in the Table 7. The final equation in terms of codded factors has given below:

Drug Release (%)=72.875+0.875 × **A**+12.125 × **B**+7.875 × **C**+1.625 × **A** × **B**

The final equation in terms of actual factors has given below:

Drug Release (%)=46.14439655-2.375 × Pullulan Acetate+0.80625 × Time+0.001086207 × RPM+0.1625 × Pullulan Acetate × Time

Interaction of different factors

Pullulan acetate and time: The interaction between Pullulan Acetate and Time was studied, in which it was found that when time of cross linking with the polymer increased, the drug release also increased. But, when Pullulan acetate concentration increased the drug released % was affected less as comparison to time (Figure 2a).

RPM and time: From the Figure 2b, it was found that, both RPM and Time has positive effect on the drug released. But time had more effect as comparison to RPM. Hence, the time of cross linking of drug Clarithromycin with pullulan acetate affects the % of drug release.

Drug concentration and RPM: From the Figure 2c and 2d it was found that RPM had more effect on the release than the drug concentration. When RPM increased, the drug released from the polymer was also increased. In addition to that, the drug loading concentration had no effect on the % of drug release.

Normal plot: All the factors having positive effect and negative effect for % of release of drug was analyzed through normal plot with Standardized effect on X-axis and Normality % Probability on Y-axis. In the Figure 3, it was found that factor A, B, C and AB having positive effect. In Shapiro-Wiki test, the W-value was found to be 0.947 and p-value was found to be 0.554. As all the points lied on the diagonal, the specified model was fitted nicely for this study.

Conclusions

In this study, we have tried to develop and optimize Claritromycin loaded with pullulan acetate for sustained release application by response surface methodology based on factorial design with reduced

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob>F	
Model	1699.5	4	424.875	3.50774	0.1653	not significant
A-Pullulan Acetate	6.125	1	6.125	0.050568	0.8365	
B-Time	1176.125	1	1176.125	9.71001	0.0526	
C-RPM	496.125	1	496.125	4.095975	0.1361	
AB	21.125	1	21.125	0.174407	0.7043	
Residual	363.375	3	121.125			
Cor Total	2062.875	7				

Table 6: ANOVA for the model terms.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High
Intercept	72.875	1	3.891096	60.49179	85.25821
A-Pullulan Acetate	0.875	1	3.891096	-11.5082	13.25821
B-Time	12.125	1	3.891096	-0.25821	24.50821
C-RPM	7.875	1	3.891096	-4.50821	20.25821
AB	1.625	1	3.891096	-10.7582	14.00821

Table 7: Estimation of coefficient of different factors.

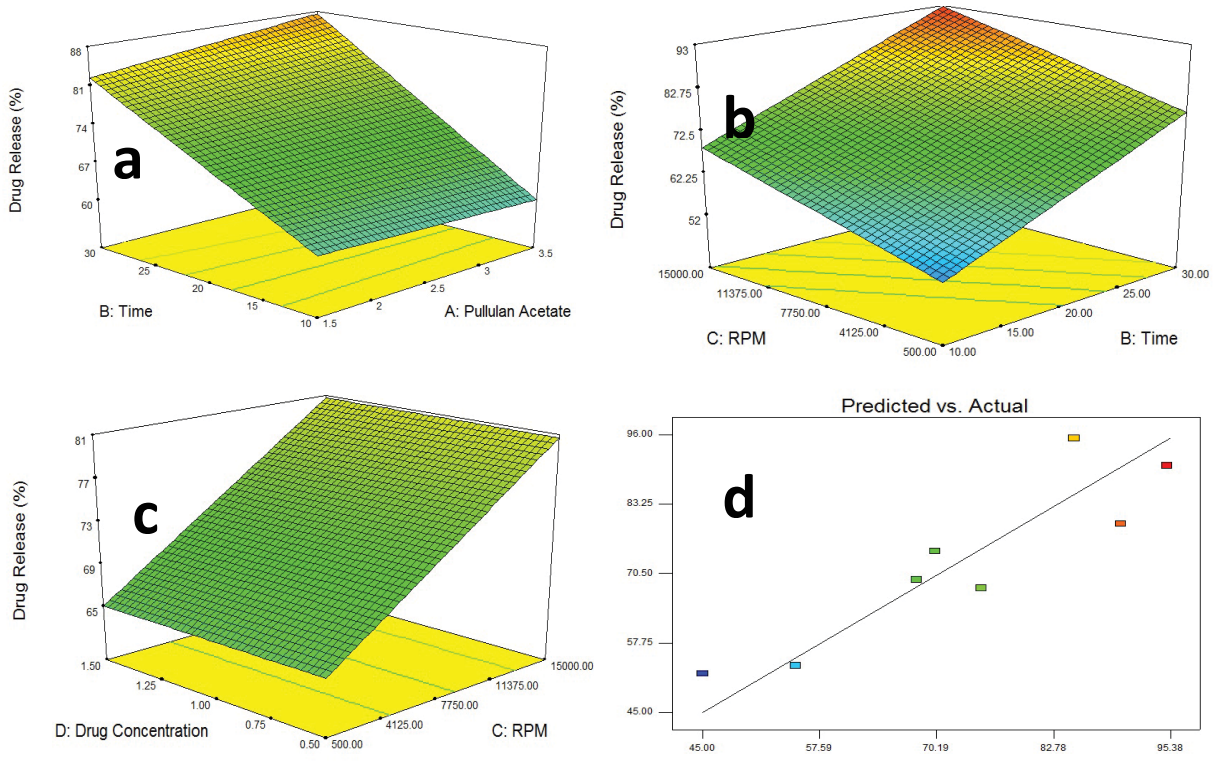

Figure 2: Effects of various factors on drug release (a, b, c) and Interaction between predicted values and actual values (d).

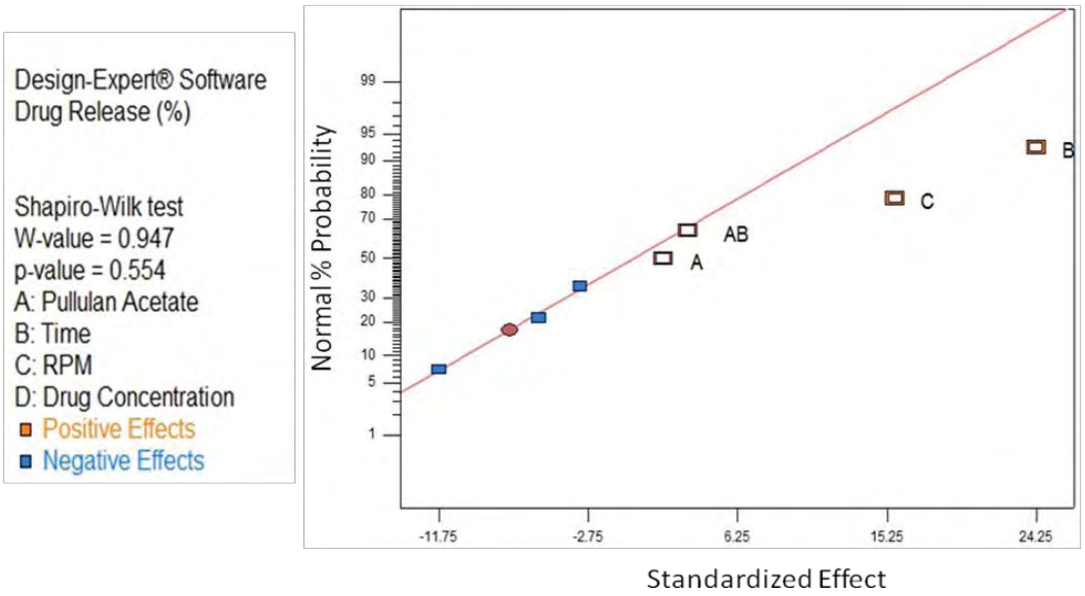

Figure 3: Normal plot to find out the factors having positive effects and negative effects.

2FI Model. Out of 8-formulaion, the 5th formulation (F5) was found to be optimum for the maximum release of the drug (more than 90%). This model was fitted with this study very nicely. From the 3-D plot, it was found that RPM, Time and Pullulan acetate concentration have positive effect on the release of the drug.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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