OPTIMIZATION OF RHAMNOGALACTURONAN EXTRACTION FROM LINSEED USING RSM AND DESIGNING A pH-RESPONSIVE TABLET FORMULATION FOR SUSTAINED RELEASE OF CIPROFLOXACIN

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Received February 15, 2025

Herein, we reported the optimization of the isolation parameters to obtain the maximum yield of the linseed mucilage (LSM) through a statistical technique, *i.e*., response surface methodology by applying Box-Behnken Design (RSM-BBD). The aqueous isolation yield of LSM was optimized by studying the influence of temperature (T, °C), pH, seed-to-water contact time (t, h), and seed-to-water ratio (r, w/v). The maximum yield of LSM, *i.e*., 12.89%, was found at T of 71.9 °C, pH of 7.09, t of 6.24 h, and r of 1:24.50 w/v, according to RSM-BBD. The pH-responsive swelling of the LSM-based tablet formulation (M3), in buffers of pH 1.2, 6.8, and 7.4, and the swelling-deswelling (on-off switching) profile of M3 in the buffers of pH 7.4 and 1.2, respectively, were investigated. The results revealed that formulation M3 swelled at pH 6.8 and 7.4, but expressed minor swelling at pH 1.2. Moreover, formulation M3 showed swelling-deswelling behavior at pH 7.4 and 1.2, respectively. The LSM sustained the release of ciprofloxacin for 24 h and followed the first-order release kinetics as well as a non-Fickian diffusion mechanism. Concluding, the LSM can be considered as a pH-responsive sustained drug release material.

Keywords: linseed mucilage, response surface methodology, polysaccharides, on-off switching, sustained drug release

INTRODUCTION

Plant seed-based mucilages are most readily available in nature and are considered economical, biodegradable, biocompatible, and non-toxic.[1] They closely resemble the cellular structure of humans and hence are becoming the most demandable materials for practical applications in drug delivery systems (DDSs), with and without chemical modification.[2,3] Linseed (*Linum usitatissimum* L.; syn: flax, linum, alsi) is one of the oldest crops cultivated in America, Europe, and Asia. Linseeds have a delicious and nutty flavor, and a crunchy and chewable texture.[4] Due to the mucilaginous nature, both linseeds and their mucilage are beneficially utilized in the food, cosmetic, and pharmaceutical industries. Other than these applications, linseeds are used for the manufacturing of inkjet, varnishes, and curing agents for the hard foundations of roads.[5]

Linseed mucilage, *i.e*., LSM, contains a rhamnogalacturonan backbone, with a higher and lower molecular weight fractions of 1510 kDa and 341 kDa, respectively.[6,7] LSM is a non-toxic, biocompatible, hydrophilic, superporous, and pH-responsive material.[8,9] Particularly, the pH-responsive behavior of LSM makes it of great interest for drug delivery systems. Many conventional drug delivery systems or smart materials rely on passive diffusion or lack precise control over release in response to environmental changes. In contrast, pH-responsive systems offer targeted and controlled behavior, particularly useful in biological environments where pH varies (*e.g*., in the gastrointestinal tract, the pH of the

stomach, small intestine, and colon ranges between 1-3, 6-7, and 7-8, respectively; also, pH varies in tumor tissue *vs*. normal tissue, or intracellular *vs.* extracellular compartments). It has also been studied that such chemically modifiable biomaterials like LSM have a wide range of industrial and pharmaceutical applications.[10] Therefore, it can be considered a new source of food hydrocolloids and can have substantial applications in improving the quality of food products. Hence, the optimization of the ideal conditions to achieve the maximum LSM yield is vital to explore through any suitable statistical model.

The aqueous hot-water extraction method is the most widely used method for extracting mucilages from plant sources, particularly seeds.[11] The extraction process is greatly influenced by the extraction conditions.[12] The most vital factors that affect the mucilage isolation process are pH, temperature, nature of solvents, seed-to-water contact time, salt contents, seed-to-water ratio, and interactions between solvents and mucilages.[13] For statistical and mathematical validation of the extraction yield data, the response surface methodology (RSM) for designing experiments has been proven a very useful technique in cases when the experimental process is based on multivariable independent parameters. Moreover, RSM has been reported as an effective method for minimizing the time and cost incurred due to a large number of experimental runs.[14]

The conditions for the extraction of mucilage from seeds of *Salvia spinosa*,[15] *Mimosa pudica*,[16] *Cydonia oblonga*,[17] *Artemisia vulgaris*,[18] *Salvia hispanica*,[19] and *Hyptis suaveolens*[20] have been optimized using RSM. However, no study on the optimization conditions for the extraction of mucilage from linseeds has been reported so far.

This study hypothesizes that optimizing linseed mucilage (LSM) extraction using RSM-Box Behnken Design (BBD) will enable the development of a pH-responsive LSM-based ciprofloxacin tablet with on-off swelling behavior. Therefore, we attempted to extract LSM using hot-water extraction by varying pH, temperature (T, °C), seed-to-water contact time (t, h), and seed-to-water ratio (r, w/v), and optimizing the ideal conditions using RSM. Moreover, we aimed to prepare an LSM-based sustained-release tablet formulation of ciprofloxacin and also explored the pH-responsive swelling and swelling-deswelling (on-off switching) behavior of the newly designed tablet formulation.

EXPERIMENTAL

Materials

Linseeds were bought from a local market in Sargodha, Pakistan. The seeds were manually cleaned, dried, and stored. The analytical grade chemicals, *i.e*., *n*-hexane, NaOH, HCl, KCl, NaCl and KH_2PO_4, were acquired from Sigma-Aldrich, Hamburg, Germany. Microcrystalline cellulose (MC) was procured from Sigma-Aldrich Co., (St. Louis, MO, USA). Moreover, deionized water (DW) was used during all experiments.

Isolation of LSM and yield calculation

The LSM was isolated according to the procedure reported earlier.[8] Linseeds were soaked in DW for 48 h at room temperature and then heated for 0.5 h at 70 °C. The swollen linseeds were placed on a nylon mesh and LSM was separated with the help of a spatula by rubbing over the mesh. The LSM was washed and purified with *n*-hexane and DW in replicates. The LSM was then spread on a glass slide and kept in a vacuum oven for drying, maintaining the temperature at 60 °C for 24 h. The dried LSM was pulverized to get fine powder and stored in a container. The yield (%) of LSM was calculated using Equation (1):[21]

$$\text{Extraction yield of LSM (\%)} = \frac{\text{Weight of extracted LSM in dry form}}{\text{Weight of linseeds used for extraction}} \times 100 \quad (1)$$

Statistical optimization of experimental design

Preliminarily, the effect of four different variables, *i.e*., pH of 1-10, T of 25-110 °C, r of 1:5-1:35 w/v, and t of 1-12 h, on the yield of LSM was examined using RSM-BBD. Based on the preliminary studies, three different levels, such as low (-1), moderate (0), and high (+1), for each independent variable (pH, T, r, and t) were selected to assess the effect of two parameters in binary form on the yield of LSM. For designing RSM-BBD, the selected levels for each parameter were: pH of 6, 7, and 8, T of 50, 70, and 90 °C, r of 1:10, 1:20, and 1:30 w/v, t of 3, 6, and 9 h. The statistical analysis of isolation conditions for the extraction yield data of LSM was appraised through RSM-BBD using Design-Expert version 11.1.2.1 (Stat-Ease Inc., Minneapolis, USA).

Therefore, average values of the data of extraction yield were put into a second-order polynomial equation (Eq. (2)) to evaluate the response variables as well as the statistical significance of the model design:

$$\text{Yield of LSM (\%)} = \mho_o + \mho_1 pH + \mho_2 T + \mho_3 r + \mho_4 t + \mho_{11} pH^2 + \mho_{22} T^2 + \mho_{33} r^2 + \mho_{44} t^2 + \mho_1\mho_2 pHT + \mho_1\mho_3 pHr + \mho_1\mho_4 pHt + \mho_2\mho_3 Tr + \mho_2\mho_4 Tt + \mho_3\mho_4 rt \quad (2)$$

where $\mho_o$ is the coefficient of regression for intercept, $\mho_1$, $\mho_2$, $\mho_3$, and $\mho_4$ are the coefficient of regression for linearity, $\mho_{11}$, $\mho_{22}$, $\mho_{33}$, and $\mho_{44}$ are the coefficient of regression for squared interaction, and $\mho_1\mho_2$, $\mho_1\mho_3$, $\mho_1\mho_4$, $\mho_2\mho_3$, $\mho_2\mho_4$, and $\mho_3\mho_4$ are the coefficient of regression for interaction terms.

From the second-order polynomial equation, the values of the theoretical yield of LSM were calculated

and compared with the experimental yield of LSM by plotting the graph between experimental *vs.* theoretical yields to check the model adequacy, suitability, and reliability. These features for RSM-BBD were also determined using analysis of variance (ANOVA), *i.e.*, by knowing *p*- and *F*-values, the coefficient of variance (C.V., %), lack of significance, predicted error sum of squares (PRESS), standard error (SE), adequate precision (ADP), predicted-R^2 (R^2-predicted), adjusted-R^2 (R^2-adjusted), and regression coefficient (R^2). Two-dimensional contour (2D-C) plots and three-dimensional response surface (3D-RS) were obtained from the Design Expert to pinpoint best experimental conditions to get the maximum yield of LSM.

Compatibility studies

The Fourier transform infrared spectroscopic analysis (FTIR, KBr method) of LSM, ciprofloxacin, and a mixture of all the ingredients of LSM and ciprofloxacin-based tablet formulation (M3) was performed on a Prestige-21 FTIR (Shimadzu, Japan) to determine the compatibility among all the ingredients. Each of the three samples was separately mixed with KBr before being pressed into a thin pellet under hydraulic pressure. The thin pellet was then dried at 50 °C before recording the spectra between 4000-400 cm^{-1}.

Tablet preparation

An LSM-based sustained-release tablet formulation of ciprofloxacin was prepared through the wet granulation method. In a mortar and pestle, LSM, ciprofloxacin, MC, and TG were thoroughly mixed and homogenized. The dry mixture was granulated by adding a few drops of DW to get a damp mass and dried at 50 °C for 6 h in a vacuum oven. Finally, the obtained dried mass was passed through a sieve no. 20 and pressed into tablet form using a 9 mm flat face punch. Tablet hardness was kept in the range from 5.5 to 8.5 kg/cm^2. Using a similar procedure, three tablets, *i.e.*, M1, M2, and M3 were prepared with different concentrations of LSM (Table 1).

Table 1
Composition of tablets

Composition of tablets	M1	M2	M3
LSM	150	200	250
MC	140	90	40
Ciprofloxacin	100	100	100
TG	10	10	10
Net weight	400	400	400

pH-responsive dynamic swelling studies

Formulation M3 was selected for the evaluation of dynamic swelling properties because of the presence of the highest gel content. The swelling studies were performed in a buffer of pH 1.2, 6.8, and 7.4, using the tea bag method.[22] Three preweighed tea bags were used to pack the tablets of formulation M3. The tea bags were hung in the corresponding buffers. The tea bag from each medium was frequently removed and the swelling capacity of M3 was calculated using Equation (3):

$$\text{Swelling capacity } (g/g) \text{ of LSM} = \frac{Ws - Wo - We}{Wo} \quad (3)$$

where W_s (g) is the weight of M3 in swollen form enclosed in a wet tea bag, W_o (g) is the weight of M3 in dry form, and W_e *(g)* is the weight of a wet empty tea bag.

pH-responsive on-off switching studies

The on-off switching behavior of M3 was studied in the buffer of pH 7.4 and 1.2, respectively, using the tea bag method at room temperature. Accurately weighed M3 was added in a tea bag and then suspended in a buffer of pH 7.4 (100 mL) for 1 h. After every 15 min, the swollen M3 enclosed in the tea bag was removed from the beaker, and swelling capacity was noted using Equation (3). After 1 h, the same tea bag was shifted to another beaker having a buffer of pH 1.2 for 1 h, and its deswelling capacity was determined periodically using Equation (3). The swelling and deswelling studies were carried out for three cycles.

***In vitro* ciprofloxacin release studies from M3**

The ciprofloxacin release study from formulation M3 was conducted in the buffer of pH 7.4 using a USP Dissolution Apparatus II. A tablet of formulation M3 was put in a dissolution vessel having 900 mL buffer of pH 7.4. The sample (5 mL) was collected from the vessel with the help of a pipette after predetermined time intervals. The sample was filtered using filter paper and the rest of the dissolution medium was restored with a buffer of pH 7.4. The sample was analyzed at a wavelength of 278 nm using a UV/Vis spectrophotometer (Shimadzu, Japan). A calibration curve was drawn to calculate the cumulative drug release (%). The release study was performed in triplicate and average values were represented.

Drug release kinetics and mechanism

The kinetics and mechanism of ciprofloxacin release from formulation M3 tablets were analyzed by putting ciprofloxacin release data to the equation of first-order kinetics (Eq. (4)) and Korsmeyer-Peppas models (Eq. (5)), respectively:[23-27]

$$\log Q = \log Q_0 - \left(\frac{K_1 t}{2.303}\right) \quad (4)$$

where Q_o is the initial drug concentration in M3, Q_o is the drug concentration remaining, and K_1 is the first-order kinetic rate constant;

$$M_t / M_\infty = k_p t^n \quad (5)$$

where M_t/M_∞ is the amount of drug release in percentage after time t, n is the diffusion exponent, and k_p is the rate constant for the Korsmeyer-Peppas kinetics model. Measurements of the diffusion coefficient (n) provided information regarding the mechanism of drug release.

RESULTS AND DISCUSSION

Isolation of LSM

Upon soaking the linseeds in hot water, the hydrophilic nature of the mucilage (LSM) permitted the water molecules to enter the seeds usually through the pores. As a result, the seeds swelled and the mucilage was released. The mucilage was purified to remove polar and non-polar impurities, and centrifuged to isolate the LSM. After drying, the LSM appeared as a light brown powder. Figure 1 (e-h) shows different steps of the isolation of LSH. The yield (%) of LSM (dry powder) was initially calculated using preliminary studies, followed by optimizing the yield of LSM by applying RSM-BBD.

Optimization of isolation conditions

Effect of pH

The effect of pH was assessed on the yield of LSM at a constant T of 70 °C, r of 1:20 w/v, and t of 6 h. As the yield of LSM was found less than 4% at pH of 1-3, therefore, the pH range of 4-10 was selected and plotted (Fig. 1(a)). The yield of LSM increased by increasing the pH from 4 to 7, and reached to highest value of 12.55% at a pH of 7. However, beyond this value, the yield of LSM began to decrease and reached 7.79% at a pH of 10 (Fig. 1(a)). At a pH of 7 (neutral), the swelling of LSM was greater as compared to acidic and basic pHs. However, the decrease in the yield of LSM after a pH of 7 might be due to the conversion of insoluble polysaccharides of LSM to soluble ones.[17] Comparatively, the yield of LSM at acidic pH (pH of 6) was greater, *i.e*., 8.91%, as compared to basic pH (pH of 10), *i.e*., 7.79%, owing to the breakdown/hydrolysis of the insoluble polysaccharides of LSM to soluble ones at higher pH.[15]

Effect of temperature

The temperature of the extraction media was varied between 25-110 °C at a constant pH of 7, r of 1:20 w/v, and t of 6 h to isolate the LSM. The yield of LSM was found to be 4.35% at T of 25 °C, which tended to increase up to 12.58% at T of 70 °C. After T of 70 °C, the yield of LSM was decreased to 8.13% at T of 110 °C (Fig. 1(b)). This phenomenon of increase in the yield of LSM by increasing the T was due to the decrease in the thickness between linseeds and the isolation medium. Furthermore, upon increasing the T, the polysaccharide solubilized greatly in the isolation medium, as a result, the diffusion coefficient increased. The greater the value of the diffusion coefficient, the greater content of the mucilage extracted from plant seeds.[28] However, at high T, *i.e*., 110 ºC, the decrease in the yield of LSM was due to the degradation of the polysaccharides of LSM. A similar trend in the yield of LSM related to the temperature has been reported earlier.[29]

Effect of seed-to-water ratio

To evaluate the effects of seed-to-water ratio on the yield of LSM, the r was varied between 1:5-1:35 w/v, and the rest of the parameters were kept constant, *i.e*., pH of 7, T of 70 °C, and t of 6 h. The yield of the LSM was found to increase from 5.12 to 12.54% by varying r from 1:5 to 1:20 w/v. At r of 1:20 w/v, the highest yield of LSM, *i.e*., 12.54%, was obtained. The increase in the yield of LSM with the increase in the r was due to the strengthening of the driving force between the water molecules of the isolation medium. A strong driving force will push the mucilage out of the seed coat greatly and *vice versa*. However, beyond r of 1:20 w/v, the yield of LSM was not significantly changed due to the presence of dynamic equilibrium between LSM and water molecules of isolated medium (Fig. 1(c)).[20]

Effect of seed-to-water contact time

The effect of seed-to-water contact time on the yield of LSM was investigated by altering the t from 1 to 12 h. Other extraction parameters, *i.e*., pH of 7, T of 70 °C, and r of 1:20 w/v were kept constant. The yield of LSM was very low at the beginning, *i.e*., 4.52% after 1 h. Later, the yield of LSM tended to increase with the increase in t and reached to maximum, *i.e*., 12.58% after 6 h. No significant change in the yield of LSM was

observed after 6 h due to the establishment of dynamic equilibrium (Fig. 1(d)). The increased yield of LSM upon increasing the t was due to the presence of high water content. The high water content allowed the water molecules to enter the seed coats of linseeds, the seeds swelled and released a significant amount of LSM.[16,21]

Overall, it was found that the pH, T, r, and t had a significant effect on LSM yield. The highest yield of LSM was obtained at a pH of 7, T of 70 °C, r of 1:20 w/v, t of 6 h. Based on these findings, three different levels, such as low (-1, pH = 6, T = 50 ºC, r = 1:10 w/v, and t = 3 h), moderate (0, pH = 7, T = 70 ºC, r = 1:20 w/v, and t = 6 h), and high levels (+1, pH = 8, T = 90 ºC, r = 1:30 w/v, and t = 9 h), were selected and an RSM-BBD consisting of 29 experimental runs was constructed (Table 2).

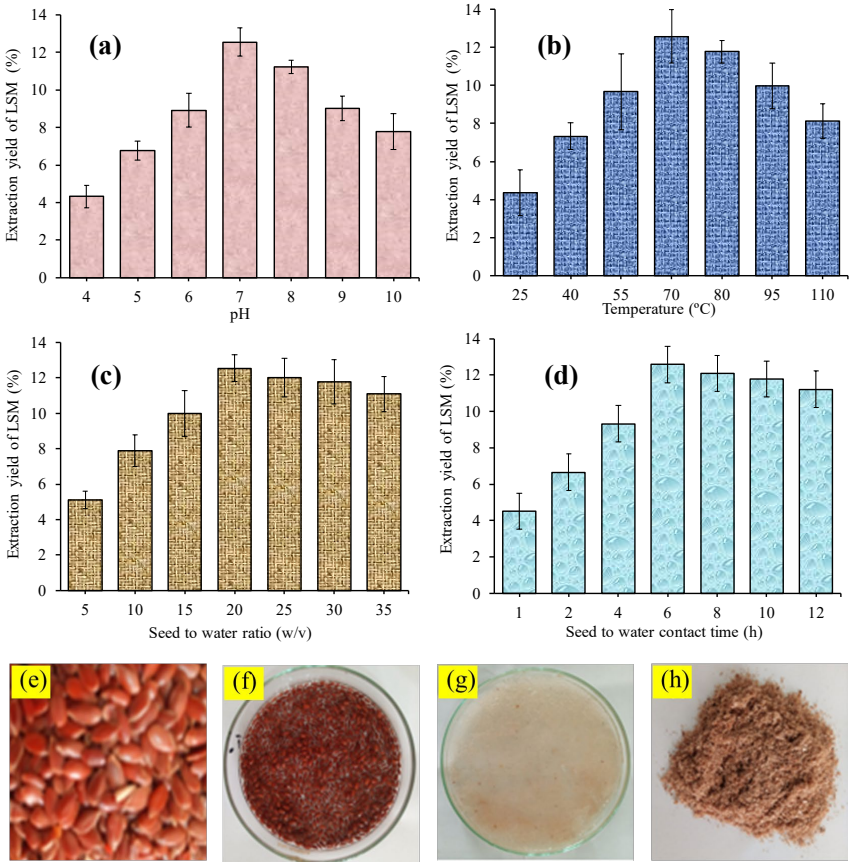

Figure 1: Effects of pH (a), T (b), r (c), and t (f) on the yield of LSM; images of linseeds in dry form (e), swollen linseeds (f), isolated and purified LSM in wet form (g), and dry powder form (h)

Response surface modeling

ANOVA fitting and RSM-BBD desirability

The statistical significance of the experimental results obtained for the optimization of various extraction conditions to achieve the highest yield of LSM from the complete design of the RSM-BBD were evaluated in the form of multiple regression analysis and ANOVA (Table 3). The *p* value of the ANOVA was found less than 0.001 (highly significant), which indicated that the second-order polynomial model equation (Eq. (6)) fitted well to explain the isolation yield data of LSM.

$$\text{Yield of LSM (\%)} = 12.48 + 0.8925pH + 0.51T + 1.0783r + 0.049t - 6.017pH^2 - 2.048T^2 - 0.7183r^2 - 3.0895t^2 + 1.2775pHT - 0.8125pHr - 0.4875pHt - 0.8625Tr + 0.0000Tt + 2.435rt \quad (6)$$

ANOVA (Table 3) showed that the yield of LSM was solely dependent on all of the four tested/independent variables in linear form. The *p* values were found to be less than 0.001 (highly significant) for pH and r, 0.00502 < 0.01 (significant) for T, and 0.7724 > 0.05 (non-significant) for t. It means that the yield of LSM was linearly related to the pH, r, and T and non-linearly related to the t (Table 3).

In the case of squared independent variables, the *p* values were found to be less than 0.001 (highly significant) for pH^2, T^2, and t^2, and 0.00586 < 0.01 (significant) for r^2. It indicated that the yield of LSM was linearly related to all of the tested variables in squared form. While considering the interaction terms, the combined effect of pH *vs.* T, T *vs.* r, and r *vs*. t had a highly significant effect, pH *vs.* r had a significant effect, pH *vs.* t and T *vs.* t had a non-significant effect on the extraction yield of LSM. The overall order is Tt (p = 1.0000) > pHt (p = 0.1155) > pHr (p < 0.0138) > Tr (p = 0.0098) > pHT (p < 0.0001) > rt (p < 0.0001) (Table 3).

The applicability of the RSM-BBD to the yield data of LSM was further determined in terms of R^2, $R^2_{\text{-adjusted}}$, and $R^2_{\text{-predicted}}$. Less than 1% difference between R^2 (0.9860) and $R^2_{\text{-adjusted}}$ (0.9722) and less than 4% difference between $R^2_{\text{-adjusted}}$ (0.9722) and $R^2_{\text{-predicted}}$ (0.9374) were found, which indicated the suitability of this model to explain the extraction yield data of LSM.[21] The value of %CV was found to be 7.63%, which is greater than 10%, and also indicated the suitability of the extraction yield experiments with better precision and adequacy according to the thumb rule.[30]

Moreover, the value of ADP was found to be 25.362, which is greater than the normal desired value, *i.e*., 4.0 (Table 3). It means that the signals-to-noise ratio is less, however, model (RSM-BBD) desirability is greater for the extraction optimization of LSM to acquire its highest yield.[31] The value of lack of fit for ANOVA analysis in this study was found to be 0.5787, which is greater than 0.05 at a 95% confidence level, and hence non-significant. It shows that the RSM-BBD for predicting the extraction yield of LSM was adequately precise.

Table 2
RSM-BBD and experimental *vs.* theoretical yields of LSM in percentage (%)

Run	Independent variables				Yields (%)	
	pH	T (°C)	r (w/v)	t (h)	Experimental Y1	Theoretical Y2
1	7	70	20	6	12.75	12.48
2	8	70	20	9	3.21	3.83
3	8	70	10	6	6.56	6.371
4	7	90	20	9	8.25	7.90
5	8	70	20	3	4.67	4.70
6	8	50	20	6	3.9	3.52
7	7	90	20	3	7.76	7.80
8	7	70	30	3	6.95	7.27
9	6	70	10	6	2.45	2.96
10	6	70	20	9	3.21	3.02
11	7	50	20	9	6.62	6.88
12	7	70	20	6	12.65	12.48
13	7	90	10	6	9.75	10.01
14	7	50	20	3	6.13	6.78
15	7	50	10	6	7.11	7.26
16	6	50	20	6	4.56	4.29
17	7	70	10	9	5.67	5.21
18	7	90	30	6	10.75	10.44
19	7	70	10	3	10.25	9.98
20	7	70	20	6	11.43	12.48
21	7	70	20	6	12.85	12.48
22	8	90	20	6	6.97	7.09
23	8	70	30	6	7.11	6.90
24	7	70	30	9	12.11	12.23
25	6	70	20	3	2.72	1.94
26	6	70	30	6	6.25	6.74
27	7	70	20	6	12.72	12.48
28	7	50	30	6	11.56	11.14
29	6	90	20	6	2.52	2.75

Table 3
Results of ANOVA for optimized extraction of LSM using RSM-BBD

Source	Sum of squares	Degree of freedom	Mean	*F*-value	*p*-value [a,b,c]
Model	331.0594	14	23.6471	70.87865	< 0.0001***
Linear terms					
pH	9.558675	1	9.558675	28.6507	< 0.0001***
T, °C	3.1212	1	3.1212	9.355331	0.00502***
r, w/v	13.95363	1	13.95363	41.82393	< 0.0001***
t, h	0.029008	1	0.029008	0.086948	0.7724[ns]
Quadratic terms					
pH^2	234.8451	1	234.8451	703.9132	< 0.0001***
T^2	27.21515	1	27.21515	81.57335	< 0.0001***
r^2	3.347045	1	3.347045	10.03227	0.00685***
t^2	61.91692	1	61.91692	185.5867	< 0.0001***
Interaction terms					
pHT	6.528025	1	6.528025	19.56678	< 0.0001***
pHr	2.640625	1	2.640625	7.914879	< 0.0138*
pHt	0.950625	1	0.950625	2.849356	0.1155[ns]
Tr	2.975625	1	2.975625	8.918991	0.0098***
Tt	0	1	0	0	1.0000 [ns]
Rt	23.7169	1	23.7169	71.08786	< 0.0001***
Residual	4.670792	14	0.333628		
Lack of fit	3.271992	10	0.327199	0.935653	0.5787 [ns]
Pure error	1.3988	4	0.3497		
Cor. total	335.7302	28			
Standard deviation	0.5776				
Mean	7.5669				
%CV	7.63%				
R^2	0.9860				
$R^2_{\text{-adjusted}}$	0.9722				
$R^2_{\text{-predicted}}$	0.9374				
PRESS	21.0323				
ADP	25.362				

[a]Significant (*$p < 0.05$); [b]Highly significant (**$p < 0.01$); [c]Super significant (***$p < 0.001$); [ns]Non-significant

The 2D-desirability plots are presented in Figure 2. The desirability for each factor was found to be 1, which also witnessed about suitability and desirability of the quadratic and RSM-BBD for the optimization of the highest yield of LSM.

Interpretation of response surface plots

The 3D-RS and 2D-C plots were recorded to study the correlation between the independent and dependent variables by keeping two variables at their constant pre-optimized values. The red regions under circular lines indicated the regions with the highest yield of LSM in the 2D-C plots (Fig. 3) and regions that are bulged out in the 3D-RS plots also indicated the highest yield of LSM (Fig. 4).

It can be seen that with the increase in T and pH, the yield of LSM increased and reached 12.54% at pH of 7.04 and T of 73.22 °C. After that, the yield of LSM decreased to 7.53% at a pH of 7.96 and T of 89.46 °C (Figs. 3(a) and 4(a)). The study of the combined effect of pH and r revealed that the yield of LSM was increased with the increase of pH and r and a maximum yield of 12.79% was obtained at pH of 7.07 and r of 1:23.72 (Figs. 3(b) and 4(b).

Figures 3(c) and 4(c) show that with the increase in the pH and t, the yield of LSM was increased and reached a maximum of 12.50% at a pH of 7.03 and t of 5.98 h. After these threshold points, the yield of LSM decreased to 3.11% at pH of 6 and t of 8.97 h. The combined effect of T and r is presented in Figures 3(d) and 4(d). It can be revealed that at T of 69.74 °C and r of 1:20.19 w/v, the yield of LSM was the highest and equal to 12.49%. As T increased to 89.58 °C and r increased to 1:10.54 w/v, the LSM yield decreased to 10.15%.

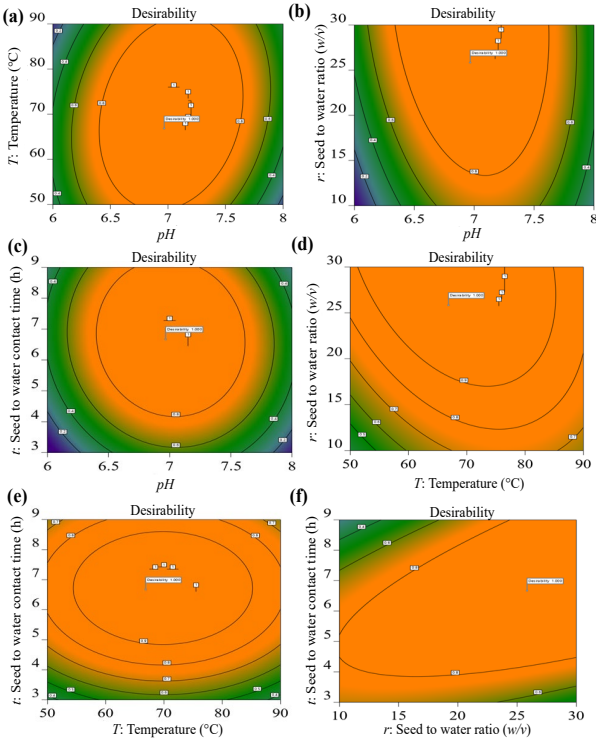

Figure 2: Desirability plots showing correlations between pH and T (a), pH and r (b), pH and t (c), T and r (d), T and t (e), and r and t (f) in response to their effect on the yield (%) of LSM

Different T and t had a significant quadratic effect on the yield of LSM, as can be seen in Figures 3(e) and 4(e). The maximum yield of 12.51% was obtained at T of 72.43 °C and t of 5.98 h. The correlation between r and t in response to their effect on the extraction yield of LSM was also studied and is presented in Figures 3(f) and 4(f). The maximum yield of 12.48% was obtained at r of 1:20.31 w/v and t of 5.78 h.

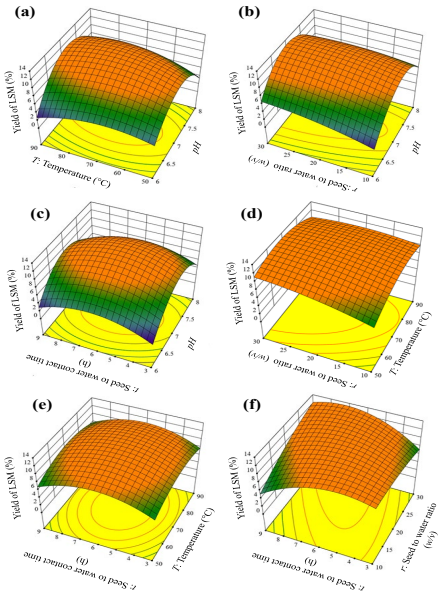

Figure 3: 3D-RS plots showing the correlations between pH and T (a), pH and r (b), pH and t (c), T and r (d), T and t (e), and r and t (f) in response to their effect on the yield (%) of LSM

Comparison of experimental vs. theoretical yields of LSM

Figure 5 shows the scattered plot between experimental and theoretical yields of LSM. In the graph, the straight line represents the experimental yield of LSM, whereas the scattered points on the straight line represent the theoretical yield of LSM. To eliminate the discrepancies between experimental and theoretical yields, the points showing the experimental yield were skipped. The comparison between the experimental and theoretical yields of LSM at each experimental run was done and the values were found comparable with each other without any significant difference (Table 2, Fig. 5). This also showed that the RSM-BBD is an adequate model for the optimization of extraction conditions to get the highest yield of LSM.

Optimum conditions for extraction yield of LSM

The pH of 7.09, T of 71.97 °C, r of 1:24.50 w/v, and t of 6.24 h were ideal conditions to get the highest yield of LSH, *i.e.*, 12.88% according to Design-Expert. All of these conditions are quite similar to the preliminary conditions (Fig. 1; Table 2) as well as conditions obtained from the comparison of experimental and theoretical yields (Fig. 5; Table 2) of LSM and the highest yield of LSM, *i.e.*, 12.85% obtained.

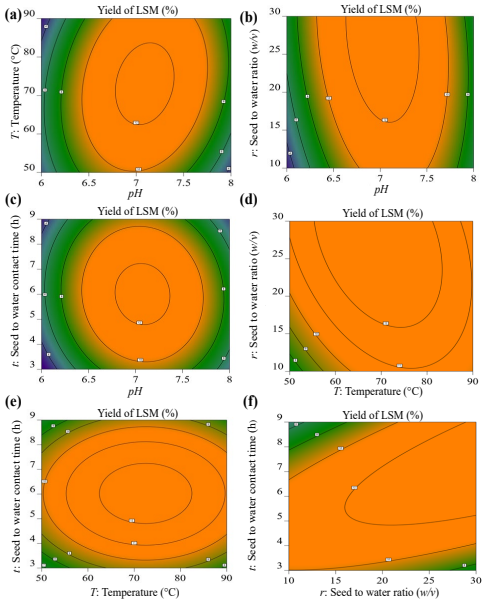

Figure 4: 2D-C plots showing the correlations between pH and T (a), pH and r (b), pH and t (c), T and r (d), T and t (e), and r and t (f) in response to their effect on the yield (%) of LSM

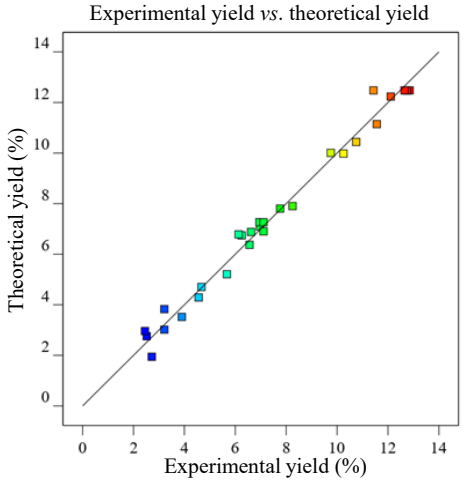

Figure 5: Comparison between experimental *vs.* theoretical yields (%) of LSM

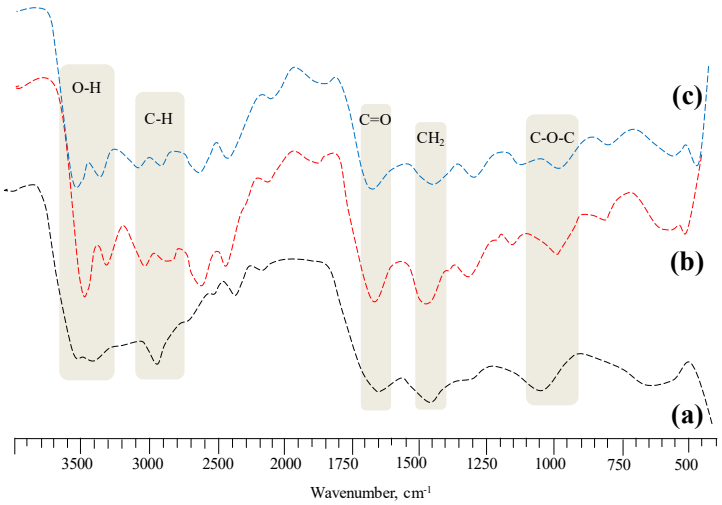

Figure 6: FTIR spectra of LSM (a), ciprofloxacin (b), and M3 (c)

Compatibility studies

The FTIR spectrum of LSM showed the characteristics bands of –OH, C=O, CH_2, and C-O-C at 3390, 1622, 1622, and 1035 cm^{-1}, respectively (Fig. 6(a)). The characteristic bands of ciprofloxacin appeared at 3373 and 3535 cm^{-1}, 1670, 1465, and 987 cm^{-1} for -OH, C=O of carboxylic acid, CH_2, and C-O-C, respectively (Fig. 6(b)).[32] In the FTIR spectrum of the formulation M3 (Fig. 6(c)), all these characteristic bands of ciprofloxacin and LSM are observed at their specific wavenumbers, indicating the absence of any interaction among the ingredients of the formulation.

Evaluation of LSM as pH-responsive material

pH-responsive swelling studies

The swelling studies of LSM-based tablet formulation (M3) were performed at pH 1.2, 6.8, and 7.4. The swelling of M3 was found in the order of pH 1.2 < pH 6.8 < pH 7.4 (Fig. 7(a)). Maximum swelling of M3 in the buffer of pH 6.8 and 7.4 was found to be of 8.6 and 10.1 g/g after 24 h, respectively, whereas minimum swelling of M3 was found to be of 4.2 g/g in the buffer of pH 1.2 after 24 h. At pH 6.8 and 7.4, the carboxylic acid (-COOH) groups of LSM get ionized to carboxylate ions ($-COO^-$) and offer electrostatic anion-anion ($-COO^-$ and $-COO^-$) repulsions among the polymer chains of LSM, which are responsible for the increase of formulation M3 swelling. At pH 1.2, the ionized -COOH groups are not available in the polymeric chains of LSM due to the domination of attractive forces between the polymeric chains of LSM and resulted in nearly off swelling at pH 1.2.[33]

pH-responsive on-off switching studies

The tablet M3 swelled rapidly in the buffer of pH 7.4 and was deswelled after being transferred to the pH 1.2 buffer. At pH 7.4, the -COOH groups of LSM started to ionize, *i.e.*, $-COO^-$, and caused electrostatic repulsion (anion-anion) between the similar $-COO^-$ groups by diminishing hydrogen bonding and hence swelled. At pH 1.2, the carboxylic acid groups ($-COO^-$) get protonated and are converted to -COOH. Therefore, negligible chances of anion-anion interactions exist in this medium, resulting in the deswelling of LSM of M3. Figure 7(b) shows the results of on-off switching of M3 up to 3 consecutive cycles in a reproducible manner.

In vitro drug release studies

At pH 7.4, the sustained release pattern of ciprofloxacin was examined and the overall release was found to be 93.79% after 24 h (Fig. 8(a)). The release data was fitted to different kinetic models and the value of R^2 was found to be 0.9939 for the first-order kinetic model, which was considereed the best-fit model (Fig. 8(b)). The value of n was found to be 0.500 after fitting ciprofloxacin release data to the Korsmeyer-Peppas model. It is known that if the value of *n* is less than 0.45, the mechanism of drug release is considered Fickian diffusion; if the value of *n* lies between 0.45-0.89, the mechanism of drug release is non-Fickian diffusion; if the value of $n = 0.89$, the mechanism of drug release is case-II transport, and if the value of *n* is greater than 0.89, the mechanism of drug release is super case-II transport.[26,27] Hence, the ciprofloxacin release mechanism from M3 followed non-Fickian diffusion (Table 4).

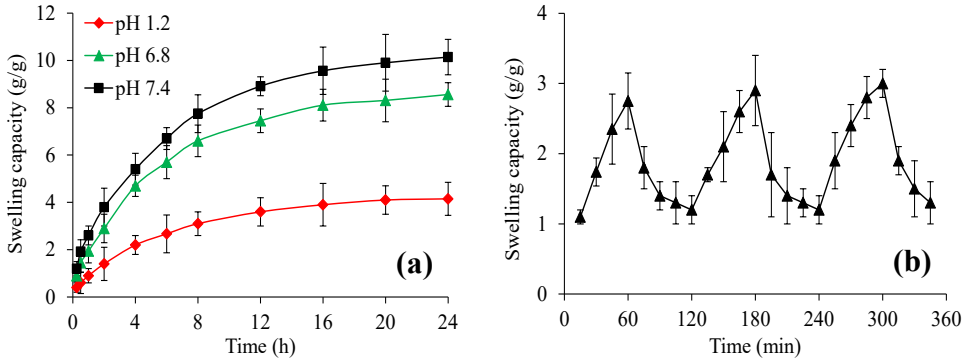

Figure 7: Swelling of M3 at pH 1.2, 6.8, and 7.4 (a), and on-off switching of M3 at pH 7.4 and 1.2 (b)

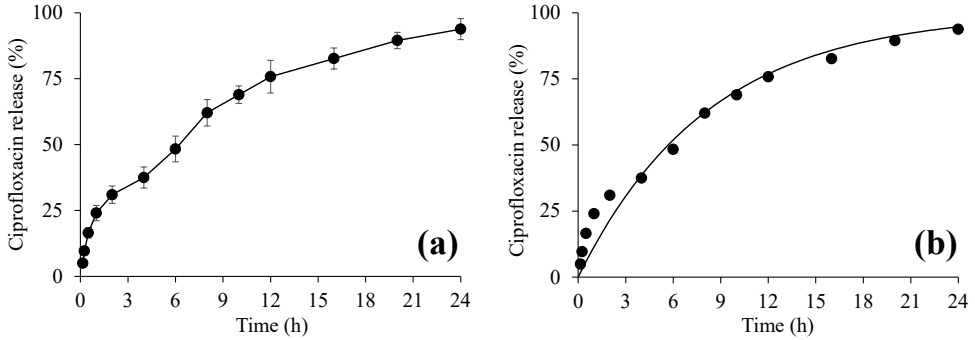

Figure 8: Ciprofloxacin release (a) and first-order kinetics (b) from M3 at pH 7.4

Table 4
Kinetic data for the release of ciprofloxacin from M3

Formulation code	First-order kinetics model		Korsmeyer-Peppas model		
	R^2	K_1	R^2	K_{KP}	n
M3	0.9939	0.1220	0.9887	20.77	0.500

CONCLUSION

RSM-BBD was shown to be an effective statistical approach for maximizing the extraction yield by varying pH, T, r, and t using Design-Expert software. A pH of 7, T of 70 °C, r of 1:20 w/v, and t of 6 h were found optimal conditions. High swelling of LSM-based tablet formulation, *i.e.*, M3, was seen in DW at pH 7.4 and 6.8, while minor swelling was observed at pH 1.2. Ciprofloxacin release from M3 demonstrated the suitability of this polymeric material for developing sustained-release formulations. Current research suggests that LSM can be used as a safe pharmaceutical excipient in the formulation design of oral and topical systems as well as other medicinal and biological applications.

ACKNOWLEDGEMENTS. The authors thank Trillium Pharmaceuticals, Faisalabad, Pakistan for the provision of Ciprofloxacin (99.5%, Fuxin Long Rui Pharmaceutical Company, China) as a gift.

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