EXTRACTION OPTIMIZATION OF A SUPERPOROUS POLYSACCHARIDE-BASED MUCILAGE FROM *SALVIA SPINOSA* L.

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Herein, we optimized eco-friendly extraction parameters to get the maximum yield of a novel polysaccharide-based mucilage (SSH) from seeds of *Salvia spinosa*. The dependency of the extraction yield of SSH on the pH of the extraction medium (pH 6-8), extraction temperature (25-75 °C), seed/water ratio (1:10-1:40 w/v), and seed–water contact time (1-4 h) was evaluated using response surface methodology–Box Behnken design (RSM–BBD). A second-order polynomial equation provided the best fit to the studied response with $p < 0.0001$. The optimum conditions to achieve the maximum yield of SSH (7.35%) were at pH 7, extraction temperature of 50 °C, seed/water ratio of 1:25 w/v, and seed–water contact time of 2.5 h. Scanning electron microscopic analysis of SSH revealed its superporous nature.

Keywords: *Salvia spinosa* mucilage, response surface methodology, extraction optimization, superporous

INTRODUCTION

The demand for novel polysaccharide-based materials obtained from natural sources for their use in the development of different drug delivery systems has been increasing in recent years, mainly due to their easy availability, cost-effectiveness, biodegradability, biocompatibility and non-toxicity, compared to synthetic ones.[1-4] Naturally occurring polysaccharides have shown potential in a wide range of pharmaceutical, biomedical and functional food applications, owing to their unique chemical composition, mimicking the human cellular structure.[5-7]

With the increasing interest in research activities regarding hydrogel/mucilage extraction from plant seeds, there arises a need for robust experimental designs to optimize the experimental conditions to get the maximum yield and save precious time. Response surface methodology (RSM) for designing experiments is receiving considerable attention in dealing with the low yield of mucilage or other constituents, where the extraction process is influenced by multivariable independent factors, *i.e.*, pH of the extraction medium, extraction temperature, seed/water ratio, seed–water contact time, *etc.*[8,9] The RSM is a collection of statistical and mathematical procedures, and provides an authentic relationship between dependent responses and a number of independent factors. It deals with the modeling of one or more than one parameter to optimize the ideal conditions at which the highest extraction yield can be achieved by correlating input and output parameters.[10] Therefore, it is one of the most widely accepted methods used for extraction optimization of mucilage, as well as other bioactive compounds, from various plant seeds.[11]

Salvia spinosa (syn. Kanocha) is one of the most widely distributed plants of *Salvia* genus and is mostly found across the Mediterranean and Saharo-Arabian phytogeographic regions. *S. spinosa* is a perennial short-lived shrub that can grow to 30-60 cm tall, with the flowering season extending from April to June. The seeds of *S. spinosa* are tasteless and contain a pertinacious mucilage layer that imbibes a large amount of water when moistened, hence, producing a thick mucilaginous drink, having a wide range of pharmaceutical applications.[12-14] Due to its mucilaginous nature, the seeds of *S. spinosa* are beneficially utilized in several gastrointestinal disorders, *i.e*., infections, piles, bleeding, and internal inflammation, and as a functional food.[15,16]

In the present study, we aimed to extract the mucilage (SSH) from *S. spinosa* seeds and optimized the extraction yield by studying the effect of four different independent factors, *i.e*., pH of the extraction medium (pH 6-8), extraction temperature (25-75 °C), seed/water ratio (1:10-1:40 w/v), and seed–water contact time (1-4 h) through the response surface methodology–Box Behnken design (RSM–BBD). The surface morphology of the SSH was studied through scanning electron microscopy (SEM).

EXPERIMENTAL

Materials

Seeds of *S. spinosa* were obtained from the indigenous marketplace of District Sargodha, Pakistan, and the taxonomic identification was verified by a botanist, Mr. Hassan Sher, from the Department of Botany, University of Swat, Mingora, Pakistan. The seeds were sieved to remove any dirty material and then kept in an air-tight jar. Analytical grade NaOH and HCl were acquired from Merck Chemicals GmbH, Darmstadt, Germany. *n*-Hexane was provided by Riedel-de Haen, Germany. All other solvents were of analytical grade and used as such, without any further purification. Deionized water (DW) was used during this research work.

Methods

Extraction of SSH and yield calculation

The procedure for the extraction of SSH was followed as described in the literature, with slight modification.[17] Briefly, seeds of *S. spinosa* were soaked in DW (*e.g*., seed/water ratio, 1:25 w/v) for 2.5 h at room temperature and then warmed at 50 °C for 30 min. The mucilage extruded from the seeds was separated by rubbing with a spatula and isolated using a cotton cloth. The isolated mucilage was purified with *n*-hexane (three times) and then with DW to remove non-polar and polar impurities, respectively. After purification, the mucilage was centrifuged at 4000 rpm for 1 h to isolate the sediment paste, *i.e*., *S. spinosa* hydrogel/mucilage (SSH), and then dried in a vacuum oven at 60 °C after spreading on a steel tray. Finally, vacuum-dried SSH was homogenized to fine powder by passing through mesh no. 60 and stored in a desiccator until further use.

The extraction yield of SSH was calculated using Equation 1:[18]

$$Extraction\ yield\ of\ SSH\ (\%) = \frac{weight\ of\ extracted\ SSH\ after\ drying}{weight\ of\ seeds\ taken\ for\ extraction} \times 100 \quad (1)$$

Experimental design and statistical analysis

Before constructing a design, some preliminary studies were performed to check the effect of a single factor on the extraction yield of SSH. For this purpose, the effects of the pH of the extraction medium (pH 1-10), extraction temperature (25-75 °C), seed/water ratio (1:10-1:40 w/v), and seed–water contact time (1-4 h) were studied. Using the results obtained in these studies, three different levels, such as low (-1), moderate (0), and high (+1), were incorporated for each independent variable, and the combined effect of two variables on the extraction yield of SSH was evaluated by applying RSM-BBD. The statistical package, Design-Expert version 11.1.2.1 (Stat-Ease Inc., Minneapolis, USA) was used for regression and graphical analysis of the extraction yield of SSH. In the evaluation of model equations (Eqs. 2 and 3), some composite interactions were also observed, apart from linear and quadratic interactions. Therefore, to predict the response variable (*Y%*) and statistical significance of the model design, the mean values of extraction yield data were fitted into a second-polynomial equation (Eq. 2):

$$Y = \beta_o + \beta_1A + \beta_2B + \beta_3C + \beta_4D + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^2 + \beta_{44}D^2 + \beta_1\beta_2AB + \beta_1\beta_3AC + \beta_1\beta_4AD + \beta_2\beta_3BC + \beta_2\beta_4BD + \beta_3\beta_4CD + E_i \quad (2)$$

where *Y* represents the dependent variable (mucilage extraction yield, *i.e*., extraction response), whereas *A* (pH of the extraction medium), *B* (extraction temperature), *C* (seed/water ratio), and *D* (seed–water contact time) express the different independent variables; the coefficient of regression for the intercept (β_o), linearity (β_1, β_2, β_3, β_4), squared (β_{11}, β_{22}, β_{33}, β_{44}), interaction ($\beta_1\beta_2$, $\beta_1\beta_3$, $\beta_1\beta_4$, $\beta_2\beta_3$, $\beta_2\beta_4$, $\beta_3\beta_4$), and E_i is the error function.

Moreover, the experimental (actual) and theoretical (predicted) yield values were compared and the quality of the fit of the polynomial model for the assessment of the regression coefficient (R^2), adjusted-R^2 (R^2-adj), predicted-R^2 (R^2-pred), coefficient of variance (CV (%)), the predicted error sum of squares (PRESS), adequate precision (ADP), standard error (SE) and lack of fit was determined. The statistical significance of the RSM-BBD, *viz.* analysis of variance (ANOVA), was determined with *p*-value and *F*-value, and through the scattered plot (actual *vs.* predicted). The 3D response surface and 2D contour plots were also drawn to see the relationship between the different independent variables and the response (extraction yield of SSH) and to find the location of optimum experimental conditions and model desirability.

SEM analysis

The surface morphology of SSH was evaluated by recording their scanning electron microscopic images using a SEM (FEI-NOVA, NanoSEM-450), equipped with a low-energy Everhart-Thornley detector (ETD). The dried SSH sample (100 mg) was first swollen in DW and then sonicated for 30 min to remove air bubbles, if any. Later, the sonicated sample of SSH was freeze-dried and cut into transverse and longitudinal cross-sections using sharp blades. These cross-sections were coated with gold using a sputter coater (Denton, Desk V HP), and SEM images were recorded along transverse and longitudinal cross-sections at different magnifications.

RESULTS AND DISCUSSION

Extraction of SSH

The SSH was extracted from seeds of *S. spinosa* using the hot water extraction method. The hydrophilic nature of the polysaccharide-based SSH allowed the water molecules to penetrate the *S. spinosa* seeds through microscopic pores. Consequently, seeds swell and SSH comes out of seed-coats. After centrifugation, the SSH was separated and further purified with *n*-hexane and water. The SSH was dried and found colorless. The preliminary extraction conditions to get the maximum yield (%) of SSH in dry powder form were first optimized by performing some preliminary studies and then further optimized statistically by RSM-BBD.

Preliminary studies for the optimization of extraction conditions

Effect of pH

The influence of pH on the extraction yield of SSH is one of the most important parameters to be studied for the utilization of such materials in pharmaceutical and biomedical applications. The effect of pH on the extraction yield of SSH was evaluated at constant extraction temperature (50 °C), seed/water ratio (1:25 w/v), and seed–water contact time (2.5 h). The results of the effect of pH on the extraction of SSH are depicted in Figure 1a. The pH range selected was 4-10 because at pH < 4 only negligible extraction yield of SSH was obtained, *i.e.*, < 1% (not reported here) and could be visualized from the swollen seeds shown in Figure 1e (red color is due to the staining with a permitted food colorant). However, at pH ranging between 4-6, the yield of SSH was insufficient, *i.e.*, ≤ 3.8%, whereas the extraction yield of SSH increased suddenly to a nearly double value of 7.35% at pH 7 (DW). Nonetheless, beyond pH 7, the extraction yield of SSH decreased again up to 3.1% at pH 10. Similar results have also been reported by Golalikhani *et al.*[19] Owing to the high swelling ability of the SSH at pH 7, the extraction yield of SSH was increased up to pH 7. Whereas, the extraction yield of SSH decreased afterwards, mainly because of the possibility of dissolution of SSH at alkaline pH, *i.e.*, pH 10. Therefore, the extraction yield of SSH was decreased at pH 10. At alkaline pH (pH 10), a relatively low extraction yield of SSH (3.1%) has been observed, as compared to acidic pH (3.8%), which might be caused by the conversion of insoluble constituents to soluble ones after hydrolysis.[20]

Effect of temperature

The effect of extraction temperature on the extraction yield of SSH was studied within the temperature range of 25-75 °C, at a constant seed–water contact time of 2.5 h, seed/water ratio of 1:25 w/v, and pH of 7 (DW) (Fig. 1b). It was observed that, at low extraction temperature, *i.e.*, 25 °C, the extraction yield of SSH was low, *i.e.*, 4.2%. However, with an increase in extraction temperature from 25 to 50 °C, an increase in the extraction yield was recorded from 4.2 to 7.31%. The maximum extraction yield of SSH (7.31%) appeared at 50 °C and, after that, it decreased to 6.02% (Fig. 1b). This might be because, at increasing extraction temperature, the solubility of polysaccharides in the extraction solvent, *i.e.*, aqueous medium (DW), increases, which leads to an increase in the diffusion coefficient of polysaccharides. Consequently, the polysaccharide mass released from plant seeds also increases. Hence, high extraction yield of SSH was observed until 50 °C.[21] Moreover, at high extraction temperature (50 °C), the seeds became less sticky and released a high proportion of mucilage. However, after 50 °C (an optimum temperature for maximum extraction yield of SSH), there might be a chance of polysaccharide degradation, and because of this, the extraction yield of SSH (6.02%) seemed to decrease (at 75 °C).[20,22]

Effect of seed/water ratio

The influence of different seed/water ratios, *i.e.*, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40 w/v, on the extraction yield of SSH, was inspected at 50 °C extraction temperature, 2.5 h seed–water contact time, and pH of 7, and the results obtained were recorded (Fig. 1c). It is obvious that the *S. spinosa* seeds released a large amount of SSH as the seed/water ratio increased from 1:10 to 1:30. A possible reason behind this trend is the driving force exerted by water molecules to push the mucilage out from the seeds. At 1:25 w/v, the highest extraction yield of SSH, *i.e.*, 7.32%, was achieved due to the greater driving force exerted by water molecules. After that, it attained a constant state of dynamic equilibrium.[23] Therefore, for economic considerations, the utilization of more DW for extraction purposes, *i.e.*, seed/water ratio beyond 1:40 w/v, was not studied.

Effect of seed–water contact time

The effect of seed–water contact time on the extraction yield of SSH was evaluated by changing the time from 1 to 4 h. The other extraction conditions were maintained as follows: a pre-optimized extraction temperature of 50 °C, seed/water ratio of 1:25 w/v, and pH of 7. Results indicated that the extraction yield of SSH increased initially with the increase in seed–water contact time, mainly due to more time for water molecules to enter the seed coat and extrude the mucilage/hydrogel completely. The maximum extraction yield of SSH was found to be 7.29% at 2.5 h and, after that time interval, the extraction yield of SSH was maintained at a state of dynamic equilibrium (Fig. 1d).

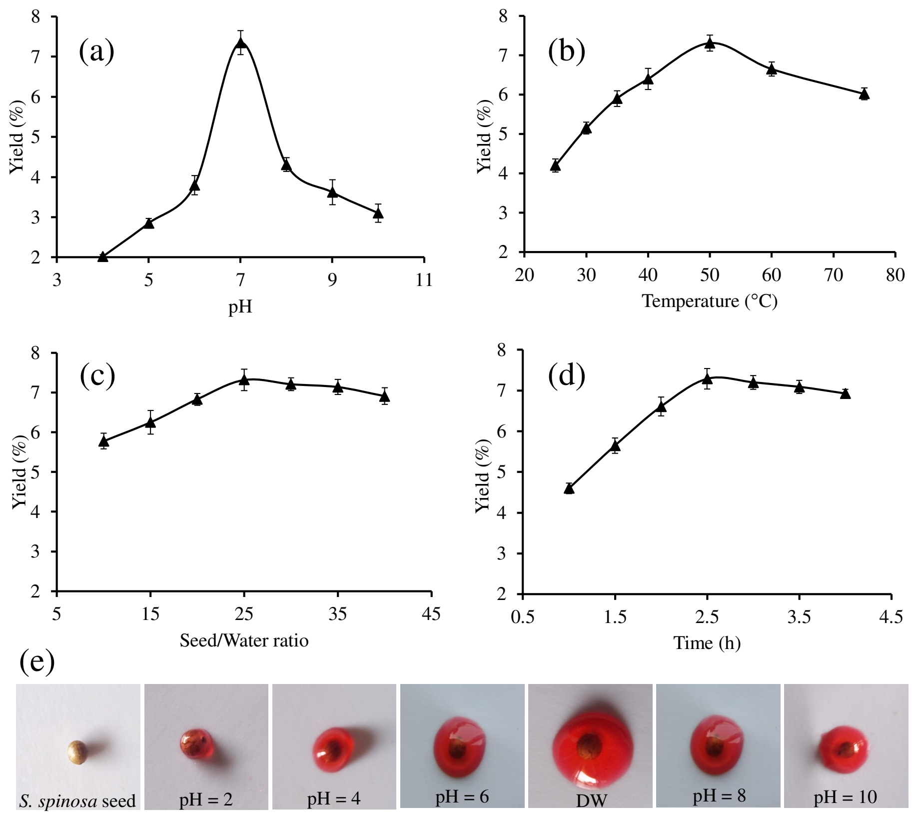

Figure 1: Effect of pH (a), temperature (b), seed/water ratio (c), and seed–water contact time (d) on the extraction yield (%) of SSH; images of the swollen seeds of *S. spinosa* at different pH for 2.5 h (e)

Table 1
Box-Behnken experimental design and actual *vs.* predicted yields (%) of SSH

Run	Independent variables				Yield (%)	
	pH A	Extraction temperature (°C) B	Seed/water ratio (w/v) C	Seed–water contact time (h) B	Actual Y	Predicted Z
1	6	50	25	1	3.13 ± 0.06	2.99
2	7	25	25	4	3.87 ± 0.11	4.02
3	7	50	25	2.5	7.21 ± 0.05	7.27
4	7	75	25	1	4.01 ± 0.9	4.19
5	6	50	10	2.5	3.17 ± 0.88	3.34
6	7	50	40	4	6.13 ± 0.11	6.13
7	8	75	25	2.5	5.64 ± 0.21	5.51
8	7	50	25	2.5	7.35 ± 0.35	7.27
9	7	25	10	2.5	3.04 ± 0.09	3.10
10	6	50	40	2.5	3.97 ±0.10	4.25
11	8	50	25	4	5.03 ± 0.071	5.02
12	8	50	40	2.5	5.17 ± 0.04	5.33
13	7	50	25	2.5	7.35 ± 0.94	7.27
14	7	75	10	2.5	4.25 ± 0.48	4.23
15	7	25	40	2.5	4.12 ± 0.56	4.00
16	8	50	10	2.5	3.51 ± 0.35	3.55
17	7	50	25	2.5	7.33 ± 0.32	7.27
18	7	50	40	1	4.49 ± 0. 91	4.39
19	7	75	40	2.5	6.23 ± 0.77	6.02
20	6	75	25	2.5	4.26 ± 0.25	4.26
21	7	50	25	2.5	7.13 ± 0.004	7.27
22	7	75	25	4	6.33 ± 0.93	6.47
23	7	50	10	4	4.47 ± 0.41	4.40
24	8	25	25	2.5	3.57 ± 0.77	3.39
25	6	50	25	4	5.25 ± 0.21	5.01
26	7	25	25	1	3.32 ± 0.55	3.51
27	7	50	10	1	3.61 ± 0.67	3.35
28	8	50	25	1	4.16 ± 0.22	4.24
29	6	25	25	2.5	3.39 ± 0.61	3.29

As long as the DW lies in contact with the seeds of *S. spinosa*, it penetrates the seeds and acts as a driving force releasing the mucilage from the pericarp of the seeds.[24] Hence, the extraction yield of SSH increases up to 2.5 h due to greater exposure of the seeds to an aqueous medium.[25] After 2.5 h, the maximum penetration of the DW into the seeds of *S. spinosa* was achieved; therefore, the extraction yield of SSH did not increase beyond that seed–water contact time. The aforesaid preliminary investigations revealed that pH, extraction temperature, seed/water ratio, and seed–water contact time have a significant effect on the extraction yield of SSH extruded from seeds of *S. spinosa*. The ideal experimental extraction conditions at which maximum extraction yield of SSH (7.35%, *i.e*., 7.35 g/100 g) was attained were: pH 7, the extraction temperature of 50 °C, seed/water ratio of 1:25 *w/v*, and seed–water contact time of 2.5 h. Therefore, based on these preliminary studies for the optimization of extraction conditions, RSM-BBD was constructed by selecting three different levels of each factor. The levels are low (pH 6.0; extraction temperature of 25 °C; seed/water ratio of 1:10 w/v and seed–water contact time of 1 h), moderate (pH 7; extraction temperature of 50 °C; seed/water ratio of 1:25 w/v and seed–water contact time of 2.5 h) and high (pH 8, extraction temperature of 75 °C; seed/water ratio of 1:40 w/v and seed–water contact time of 4 h)

Response surface modeling

Model fitting

According to RSM-BBD, there were a total of 29 experimental runs (Table 2) for statistical optimization of ideal conditions at which the seeds of *S. spinosa* released the highest amount of SSH. The second-order polynomial model provided the best fit by considering *F*- (large) and

p-values ($p < 0.05$ significant, $p < 0.01$ highly significant, and $p < 0.001$ super significant) of the model designed. The values of the regression coefficients were determined from the obtained second-order polynomial equation (Eq. 3) and fitted to calculate the predicted yield (Fig. 3).

$$\text{SSH extraction yield (\%)} = 7.274 + 0.325833A + 0.784167B + 0.671667C + 0.696667D - 1.69408A^2 - 1.47158B^2 - 1.45533C^2 - 1.25033D^2 + 0.3AB + 0.215AC - 0.3125AD + 0.225BC + 0.4425BD + 0.195CD \quad (3)$$

The result from the ANOVA table (Table 2) indicated that the extraction yield of SSH was purely dependent on four independent parameters of extraction, *i.e.*, pH of extraction medium, extraction temperature, seed/water ratio, and seed–water contact time on account of p-valuesGenerally, the smaller the p-values, the higher will be the level of confidence in the corresponding coefficients. By considering p-values, *i.e.*, $p < 0.0001$, it was found that the extraction yield of SSH related non-linearly with the pH of the extraction medium, extraction temperature, seed/water ratio, and seed–water contact time. Linearity between the dependent and independent variables has been validated through the correlation measurements depicted in Figure 2. A weak positive correlation of 0.147 was found between the pH of the extraction medium and the extraction yield of SSH, showing a non-linear relationship between the pH of the extraction medium and the extraction yield of SSH. Similarly, the increase in the extraction yield of SSH by increasing the pH was observed and reached a maximum at pH 7. Beyond pH 7, the extraction yield of SSH tends to decrease. A nearly similar trend was also achieved for the other three factors, with correlation values of 0.354, 0.303 and 0.314, for extraction temperature, seed/water ratio and seed–water contact time, respectively.

The quadratic effect (squared) of all of the underlying parameters also has a pronounced effect and appeared non-linearly, as well by considering p-values (Table 2). From the interaction terms, the effect of interaction between pH *vs.* seed/water ratio (AC) and seed/water ratio *vs.* seed–water contact time (CD) were non-significant; pH *vs.* extraction temperature (AB) and extraction temperature *vs.* seed/water ratio (BC) were less significant, and pH *vs.* seed–water contact time (AD) and extraction temperature *vs.* seed–water contact time (BD) were found highly significant on account of p-values (Table 2). The overall order in the case of the effect of interaction on the extraction yield of SSH appeared as: BD (p = 0.000705) > AD (p = 0.008628) > AB (p = 0.010989) > BC (p = 0.045363) > AC (p = 0.054407) > CD (p = 0.077671). The model adequacy was further estimated by valuing the standard deviation of the model (0.3689) and comparing the corresponding R^2 (0.990045), R^2-adj (0.980089), and R^2-pred (0.945468). A difference of less than 1.0% and 4.0% between R^2 and R^2-adj, and R^2-adj and R^2-pred, respectively, indicated a good agreement between them and evidenced the fitness of the RSM-BBD.

CV (%) is a valuable tool to measure the fitness level of the model and result reproducibility. Its value is used to express the ±SD in terms of the percentage of the mean. The cases where CV (%) < 10% indicate insignificant variation in the mean value and hence show the development of the response model with great satisfaction and reproducibility of the results. However, the cases where CV (%) > 10% indicate significant variation in the mean value and do not favor the development of a satisfactory response model.[26] In the present study, for the extraction of SSH from *S. spinosa* seeds, the CV (%) value was 4.228182%, which witnessed a good agreement between the actual and predicted values.

The very low value of pure error, *i.e.*, 0.03952, also indicated the good reproducibility of the extraction yield data of SSH. The ADP value of the model was calculated to find the signal–noise ratio and model desirability. It was found to be 29.0493, which is greater than a normal desired value, *i.e.*, 4.0. Hence, this proved that the model is desirable for optimizing the extraction yield of *S. spinosa*.[18]

The lacks of fit and pure errors are important parameters to indicate the successful fitness or failure of a model to the experimental data. In those cases, where the value of lack of fit is significant, the model cannot be applied to experimental data and hence the response predictor should be discarded. Contrarily, if the value of lack of fit is non-significant, then the model can be successfully applied to the experimental data and the response predictor should not be discarded. In this study, ANOVA showed a non-significant lack of fit at 95% confidence interval, along with a pure error of 0.00988.

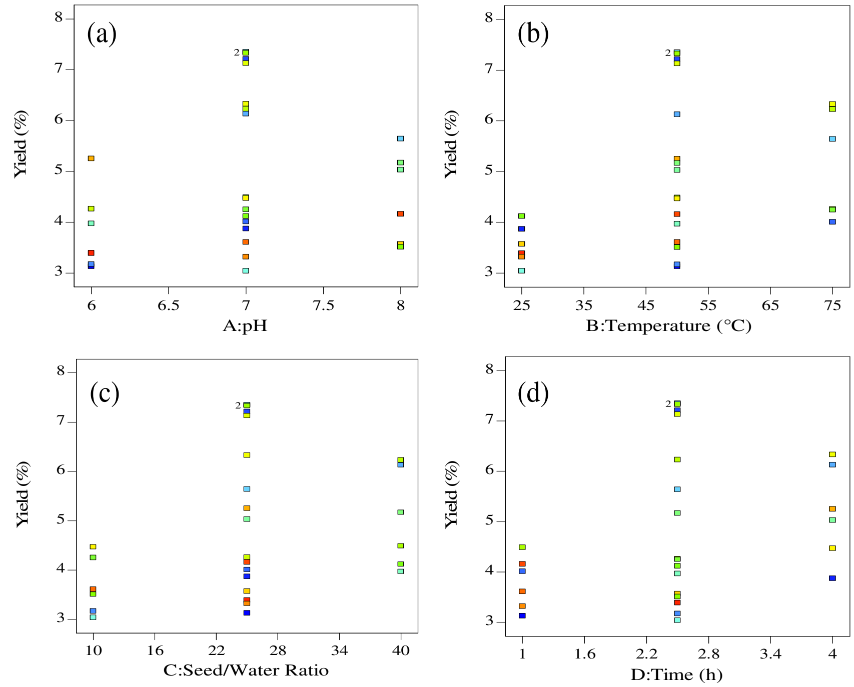

Figure 2: Effect of pH (a), temperature (b), seed/water ratio (c), and seed–water contact time (d) on the extraction yield (%) of SSH obtained from *S. spinosa* seeds

Table 2
ANOVA for the experimental results of the RSM-BBD for the extraction yield (%) of SSH

Source	Sum of squares	DF[a]	Mean	*F*-value	*p*-value[b,c,d]
Model	58.41472	14	4.17248	99.44709	< 0.0001***
Linear					
A - pH	1.274008	1	1.274008	30.36477	< 0.0001***
B - Temperature (°C)	7.379008	1	7.379008	175.8716	< 0.0001***
C - Seed/water ratio (w/v)	5.413633	1	5.413633	129.0288	< 0.0001***
D - Seed–water contact time (h)	5.824133	1	5.824133	138.8127	< 0.0001***
Quadratic					
A^2	18.61569	1	18.61569	443.6871	< 0.0001***
B^2	14.04686	1	14.04686	334.7935	< 0.0001***
C^2	13.73835	1	13.73835	327.4404	< 0.0001***
D^2	10.14054	1	10.14054	241.6901	< 0.0001***
Interaction					
AB	0.36	1	0.36	8.580257	0.010989*
AC	0.1849	1	0.1849	4.406915	ns
AD	0.390625	1	0.390625	9.310175	0.008628***
BC	0.2025	1	0.2025	4.826395	0.045363*
BD	0.783225	1	0.783225	18.66742	0.000705***
CD	0.1521	1	0.1521	3.625159	ns
Residual	0.587395	14	0.041957		
Lack of Fit	0.547875	10	0.054788	5.545294	ns
Pure Error	0.03952	4	0.00988		
Cor. Total	59.00212	28			

SD = 0.204834; Mean = 4.844483; R^2 = 0.990045; Adjusted-R^2 = 0.980089; Predicted-R^2 = 0.945468; CV (%) = 4.228182%; PRESS = 3.22; Adequate precision (ADP) = 29.0493

[a] DF: Degree of freedom, [b] Significant (*$p < 0.05$), [c] Highly significant (**$p < 0.01$), [d] Super significant (***$p < 0.001$), [ns] Non-significant

Hence, from the aforesaid discussion, it can be concluded that the constructed quadratic model was well suited for this experimental setup for the extraction of SSH from *S. spinosa* seeds. Moreover, this investigation also led to the conclusion that the RSM-BBD is suitable for the experimental extraction yield data of SSH and could be applied to deduce the design space

Checking of model adequacy and desirability

For checking model adequacy, a graph between experimental (actual) and theoretical (predicted) yields of SSH was plotted (Fig. 3). In the graph, the straight line shows the actual yield of SSH, whereas the scattered points displayed randomly on the straight line represent the predicted yield of SSH. The points showing the actual yield of SSH were skipped to avoid conflict between actual and predicted yields of SSH, because an inadequate model may mislead toward the wrong investigation of extraction yield. As the predicted plots were found in close touch with the actual ones, it could be concluded that the designed quadratic model satisfactorily described the extraction yield of SSH by RSM-BBD. Moreover, the desirability for the aforesaid optimized formulation was 0.904 in each case (Fig. 4).

Interpretation of response surface plots and optimization of extraction yield

Response surface 3D and contour 2D plots are the graphical representation of the quadratic equation. These plots were obtained by applying RSM-BBD onto the experimental yield data of SSH, using Design-Expert, to interpret the quadratic effect of tested parameters on the extraction yield of SSH. Plots were generated by varying two parameters within the experimental range at the central value of the testing ranges for the recipient two factors.

The extraction yield of SSH was studied as a function of different pH values of the extraction medium and various extraction temperatures, and the results were recorded in terms of 3D response surface (Fig. 5a) and 2D contour plots (Fig. 6a). The seed/water ratio and seed–water contact time were kept constant at 1:25 w/v and 2.5 h, respectively. The results revealed that, once the pH of the extraction medium and extraction temperature were increased, the extraction yield of SSH was also increased linearly until it reached maximum spot hits at 7.36%, showing the highest extraction yield of SSH. The pH and extraction temperature at that point were 7.09 and 52.81 °C, respectively. After these threshold levels, a significant decrease in the extraction yield of SSH, *i.e.*, 3.35% was recorded.

The 3D response surface and 2D contour plots showing the effect of independent variables pH and seed/water ratio at constant extraction temperature (50 °C) and extraction time (2.5 h) are presented in Figures 5b and 6b. It can be seen that a significant increase in the extraction yield of SSH was achieved with the increasing pH of the extraction medium and seed/water ratio. A maximum yield of 7.36% of SSH was obtained at a pH of 7.04 and seed/water ratio of 1:26.72 w/v.

In Figures 5c and 6c, the combined effect of pH of the extraction medium and seed–water contact time at constant extraction temperature (50 °C) and seed/water ratio (1:25 w/v) is shown. It is obvious that, at a pH of 6.96 and seed–water contact time of 2.63 h, the extraction yield of SSH was quite insignificant.

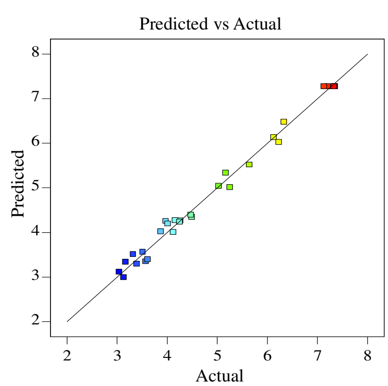

Figure 3: Comparison between predicted and actual yields (%) of SSH from *S. spinosa* seeds

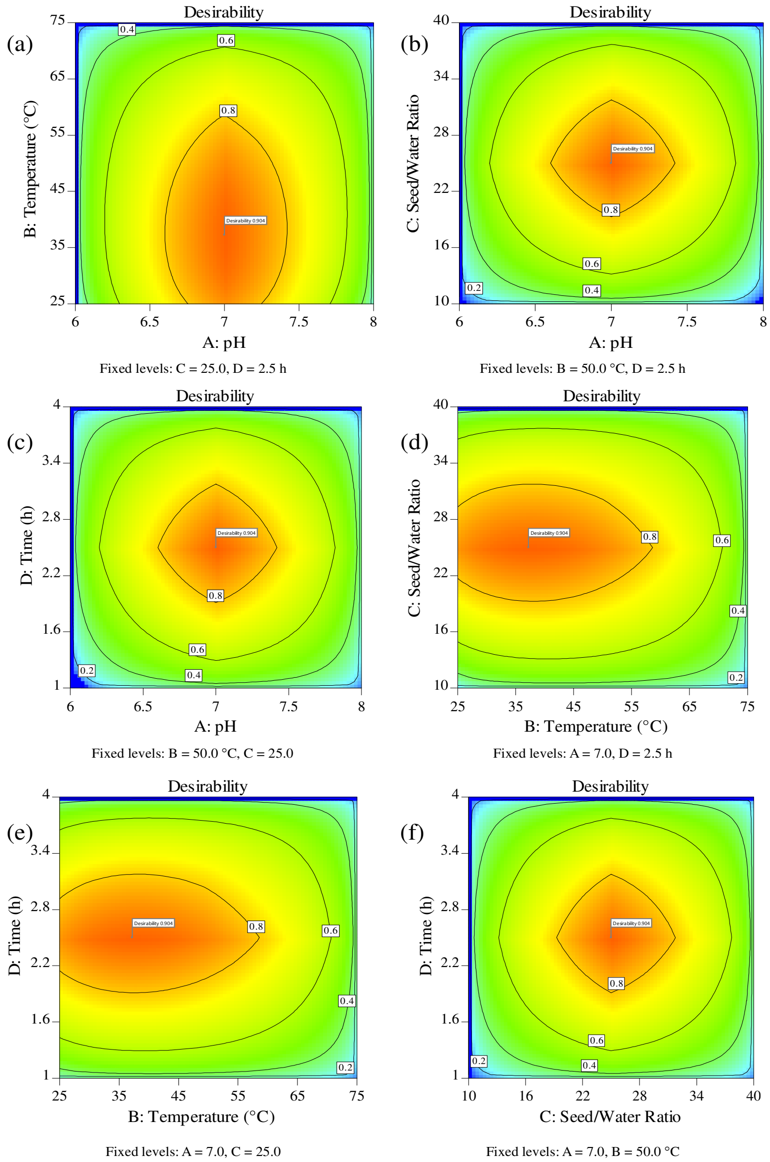

Figure 4: Desirability plots for pH *vs.* temperature (a), pH *vs.* seed/water ratio (b), pH *vs.* seed–water contact time (c), temperature *vs.* seed/water ratio (d), temperature *vs.* seed–water contact time (e), and seed/water ratio *vs.* seed–water contact time (f), showing significant interaction effects on SSH extraction yield (%) from *S. spinosa* seeds

However, with the increase in extraction medium pH and seed–water contact time, a significant increase in the extraction yield (7.35%) of SSH was seen up to a point (pH 7 and seed–water extraction time – 2.5 h). Afterward, the extraction yield of SSH tended to decrease and achieved a minimal point at a pH of 7.9 and seed–water contact time of 3.93 h. The effect of different extraction temperatures and seed/water ratio also had a prominent impact on the extraction yield of SSH and was found nearly similar to the effect of different extraction temperatures and seed–water contact time subjected to the constant conditions of pH 7 and seed–water contact time of 2.5 h (Figs. 5d and 6d). The maximum extraction yield of SSH, *i.e.*, 7.35%, appeared around an extraction temperature of 62.51 °C and seed/water ratio of 1:31.76 w/v. After that, once the extraction temperature hits the point of 75 °C and the seed/water ratio reached 1:40 w/v, the extraction yield of SSH decreased to 6.23%.

Figures 5e and 6e show the quadratic effect of different extraction temperatures and seed–water contact time at a constant pH of 7 and seed/water ratio of 1:25 w/v on the extraction yield of SSH. It can be seen that both variables had a pronounced effect on the extraction yield of SSH. The extraction yield of SSH was found to be increased upon increasing extraction temperature from 25 to 55 °C, and seed–water contact time from 1 to 2.5 h. However, at 55 °C and 2.5 h, it reached a plateau region and maximized the extraction yield of SSH to 7.35%.

At fixed extraction temperature (50 °C) and extraction medium pH (pH 7), the 3D response surface and the 2D contour plots were recorded to assess the dependency of SSH extraction yield on different seed/water ratios and different seed–water contact time. The results incorporated in Figures 5f and 6f indicate that, at seed/water ratio (1:11.88 w/v) and seed–water contact time (1.14 h), the extraction yield of SSH was low, *i.e.*, 4.0%. However, beyond these threshold levels, linearity was observed between the extractions yields of SSH *vs.* seed/water ratio and seed–water contact time. Nearly 6.0% of SSH extracted from *S. spinosa* seeds was observed at a seed/water ratio of 1:18 w/v and seed–water contact time of 1.8 h.

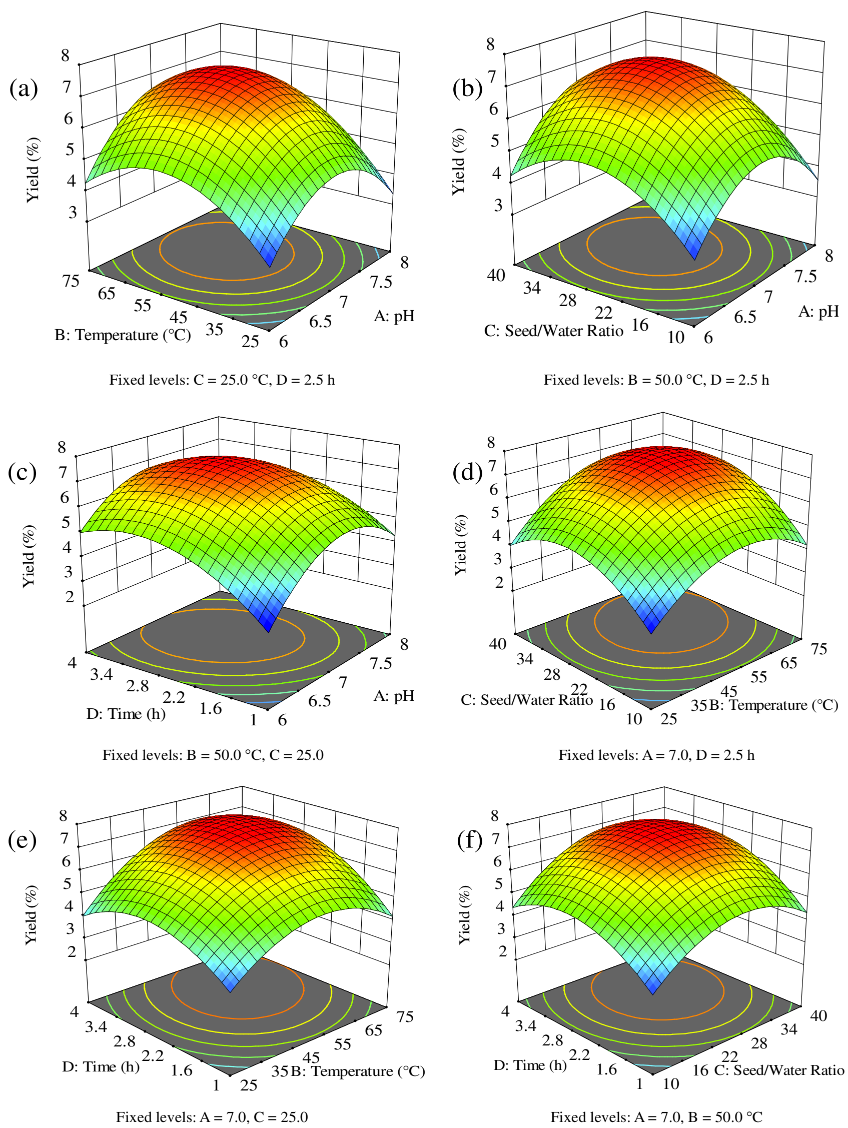

Figure 5: 3D response surface plots for pH *vs.* temperature (a), pH *vs.* seed/water ratio (b), pH *vs.* seed–water contact time (c), temperature *vs.* seed/water ratio (d), temperature *vs.* seed–water contact time (e), and seed/water ratio *vs.* seed–water contact time (f), showing significant interaction effects on SSH extraction yield (%) from *S. spinosa* seeds

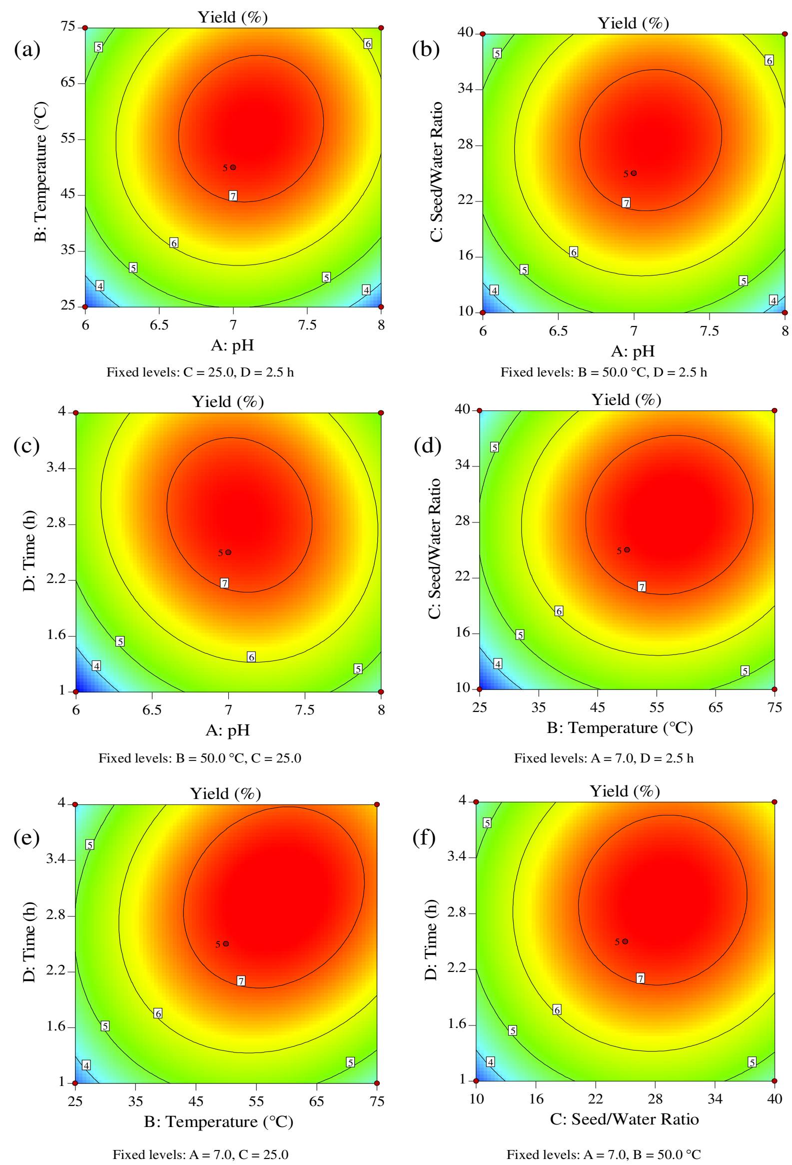

Figure 6: 2D contour plots for pH *vs.* temperature (a), pH *vs.* seed/water ratio (b), pH *vs.* seed–water contact time (c), temperature *vs.* seed/water ratio (d), temperature *vs.* seed–water contact time (e), and seed/water ratio *vs.* seed–water contact time (f), showing significant interaction effects on SSH extraction yield (%) from *S. spinosa* seeds

In the 3D response surface plots, it can be seen that the optimal areas were bulged out at a yield of around 7.0% (Fig. 5). In the 2D contour plots, the continuous red areas demarcated by the clear circular lines indicated the optimal regions for the maximum extraction yield of SSH. These numbers were mid-values for each independent variable and are nearly around 7.0% (Fig. 6).

Comparison of extraction yield of SSH with already reported hydrogels

According to Design-Expert, the optimal conditions at which the highest yield of SSH, *i.e.*, 7.27%, could be obtained were found to be the following: extraction medium pH 7, extraction temperature of 50 °C, seed/water ratio of 1:25 w/v, and extraction time of 2.5 h. These conditions showed closeness between the maximum yield calculated experimentally, *i.e.*, 7.35%, at pH 7, extraction temperature of 50 °C, seed/water ratio of 1:25 w/v, and seed–water contact time of 2.5 h (Table 1, run 13). The same evidence was also achieved from the scattered plot between actual and predicted yields of SSH (Fig. 3). Moreover, these conditions and obtained SSH yield by RSM-BBD (7.27%) also agreed with preliminary optimized conditions and yield. Therefore, we can conclude that the extraction conditions at run 13 (Table 1) are the optimized ones to get the highest yield of SSH.

A comparison between the extraction yields of SSH obtained from *S. spinosa* seeds with mucilage extracted from seeds of some commercially and pharmaceutically important plants showed that the SSH released from seeds of *S. spinosa* has a decent place among them. The extraction yield of SSH was found at 7.27% (actual) and 7.35% (predicted), which is greater than the extraction yield of *Durio zibethinus* (1.2%),[27] *Tiliacora triandra* (4.54%),[28] *Salvia hispanica* (4.95%),[29] and *Lepidium perfoliatum* (6.46%).[30]

SEM analysis of SSH

SEM analysis of swollen and then freeze-dried SSH was conducted to obtain an insight into the surface morphology, texture, arrangement of internal porous structure, and microporous channeling present in the internal structure, by observing transverse and longitudinal cross-sections. Figure 7 presents the SEM images along with their histograms.

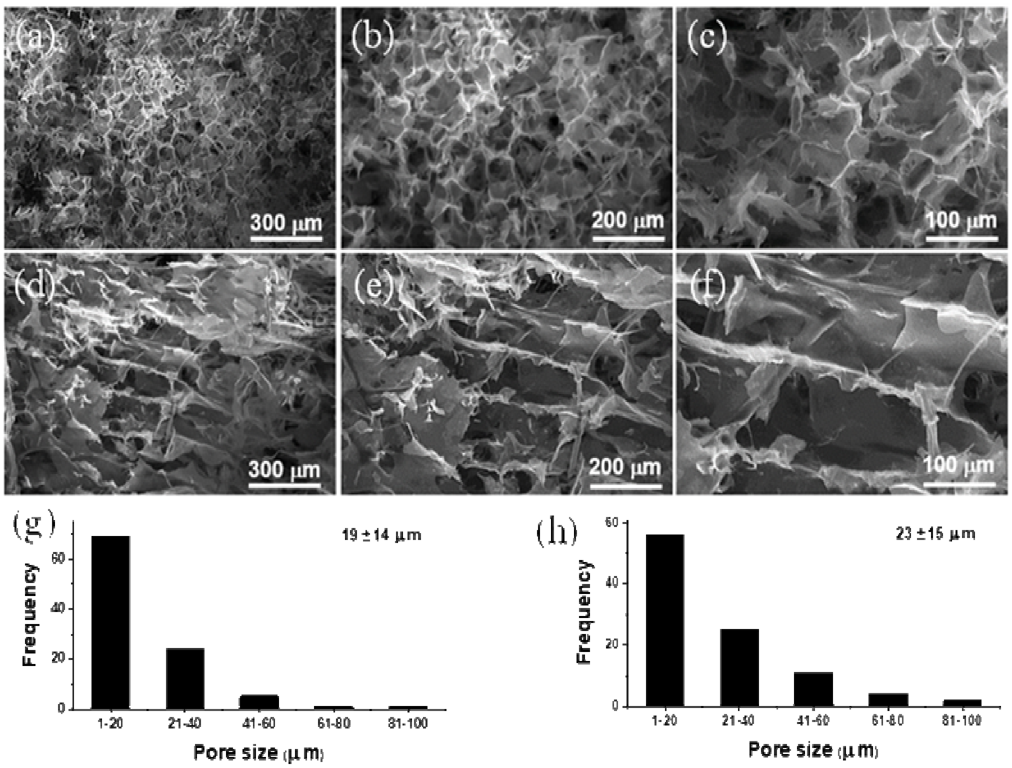

Figure 7: SEM images of transverse (a-c) and longitudinal (d-f) cross-sections of swollen and freeze-dried SSH (average pore size 19 ± 14 µm in transverse and 23 ± 15 µm in longitudinal cross-sections); Histograms showing the size distribution of micropores of transverse (g) and longitudinal (h) cross-sections

It is clear from the SEM images that there is uniform distribution of the microporous and interconnected channels in the structure of SSH, with an average pore size of 19 ± 14 µm in transverse and 23 ± 15 µm in longitudinal cross-sections. Therefore, the presence of this kind of channels allows SSH to absorb water and other biological fluids. Hence, SSH has an excellent swelling capacity and can be used for the development of conventional, as well as sustained or targeted drug delivery systems (DDS).

CONCLUSION

RSM-BBD has been proven an effective statistical tool to optimize the extraction parameters, *i.e.*, pH of the medium, extraction temperature, seed/water ratio, and seed–water contact time, to get the maximum yield, *i.e.*, 7.27%, by Design-Expert software. The optimum conditions to achieve the maximum yield of SSH were observed to be the following: pH 7, extraction temperature of 50 °C, seed/water ratio of 1:25 w/v, and seed–water contact time of 2.5 h.

Moreover, the second-order polynomial equation provided the best fit for the studied response. The SSH appeared as a porous material, as demonstrated in SEM analysis, since it showed elongated channels upon swelling and then freeze-drying. Such materials are promising in the development of intelligent drug delivery systems due to their smart nature against various stimuli.

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