RESEARCH PAPER

Box-Behnken Design Assisted Formulation and Evaluation of Alginate Nanocarriers As an Efficient Drug Delivery System for Nigella Sativa Extract: An In-Vitro Antidiabetic Testing

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ABSTRACT
Nigella sativa is a plant well-known for its many medicinal properties and therapeutic activities. This study uses sodium alginate, a natural biodegradable polymer to formulate and optimize a nanoparticle formula for Nigella sativa extract. The hydroethanolic extract was loaded into sodium alginate nanoparticles by ionic gelation technique and optimized by Box-Behnken design. Use of chitosan was found to have a positive impact on entrapment efficiency while polysorbate 80 reduced the entrapment of the extract. Polymer concentration, crosslinking agent and homogenization were considered as independent variables. The optimized formulation has an average entrapment efficiency of 40.58%, average particle size of 221.2 nm and a polydispersity index of 0.372. Release kinetics study found that the formulation adheres to Baker Lonsdale Model indicating a spherical matrix with diffusion mechanism. Sodium alginate nanoparticles are a suitable drug delivery system for the Nigella sativa extract and impacts the drug efficiency significantly.

Keywords: Nigella sativa, antidiabetic activity, Box-Behnken design, sodium alginate nanoparticles, Traditional knowledge

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INTRODUCTION

Globally, 1 in 9 people have diabetes and 7 out 10 diabetes patients are of working age. International Diabetes Federation (IDF) has estimated the number of diabetes patients across the world to be 589 million adults, of which 90% of cases are type 2 diabetes. [1] *Nigella sativa* has shown promising antidiabetic effects by improving glucose regulation, insulin sensitivity and reducing oxidative stress. [2,3]

Sodium alginate is a natural polymer and an important food adjuvant.[4] Alginate swells and dissolves slowly under alkaline environment, but it remains stable in acidic conditions. Hence alginate can encapsulate the phytoconstituents and release it in a controlled manner in the alkaline environment of small intestine. Alginate has been considered as nanocarrier due to its biodegradability and biocompatibility. [5]

Encapsulating *Nigella sativa* extract in alginate nanoparticles can enhance its stability in the gastric environment and its bioavailability. It also ensures slow absorption after oral administration, thereby lowering the dose required for the therapeutic benefit. This study aims to formulate *Nigella sativa* extract loaded alginate nanoparticles for oral delivery. Ionic gelation method was used for the formulation of sodium alginate nanoparticles with calcium chloride as crosslinking agent. Chitosan was used as polyelectrolyte in addition to calcium chloride where the carboxylate group of alginate interacts with ammonium group of chitosan. [6–8]

MATERIALS AND METHODS

Materials

Chitosan and sodium alginate were purchased from Hi Media, Calcium chloride from Loba Chemie, Polysorbate 80 from Bangalore Fine Chemicals, Nigella sativa from

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MM Enterprises, alpha-amylase enzyme from Sigma Aldrich and 3,5-dinitrosalicylic acid from SD fine Chem

Plant authentication:
Seeds of *Nigella sativa* was purchased and authenticated at Agharkar Research Institute, Pune

Extraction and phytochemical analysis of *Nigella sativa* seeds
Nigella sativa seeds were cleaned, washed, dried under shade and used for extraction. Hydroethanolic (70:30) solvent was used for the extraction. The plant material was macerated for 48 hours with periodic mixing. The extract was filtered and the solvent was allowed to evaporate under stirring and was further dried using freeze dryer. [9,10] The seed extract was subjected to phytochemical tests for flavonoids, phenols, tannins, saponins and alkaloids. [11]

Total Flavonoid and Phenolic Content (TFC & TPC)

TFC was determined spectrophotometrically by aluminium chloride reagent method using quercetin as the standard control. For the determination of TPC, Folin-Ciocalteu reagent method was used with gallic acid as standard control. [12–14]

Formulation of Sodium alginate nanoparticles
Sodium alginate was dissolved in water, incubated for 3 hours and mixed to uniformity. Aqueous solution of extract was added and mixed for 15 minutes. Calcium chloride is added dropwise under magnetic stirring at 1200 RPM for 30 minutes. Chitosan was added dropwise as polyelectrolyte and stirring continued for 30 minutes. The obtained nanoparticles were homogenized at high speed for 15 minutes at different rpm. After a 30-minute centrifugation at 12000 RPM, entrapment efficiency (EE), particle size, zeta potential (ZP), polydispersity index (PDI) were evaluated. [5,15–23]. Initial study was conducted by varying factors one-at-time. The impact of critical process variables on the formulation of nanoparticles were studied using Box-Behnken design. (as depicted in table 1 and 2).

Table1: Initial study of impact of chitosan and polysorbate 80

Formulation code	Sodium alginate 3 mg/ml	Calcium chloride 2mg/ml	Chitosan 0.6 mg/ml	Polysorbate 80	EE %	Particle size	PDI
FC1	20 ml	4 ml	4 ml	Nil	40	234.8	0.389
FC2	20 ml	4 ml	4 ml	1%	25.2	212.4	0.451
FC3	20 ml	4 ml	8 ml	1%	4.7	280.9	0.411

Table 2: Formulation Composition of alginate nanoparticles in Box-Behnken design

Formu-lation code	RUN	Factor			Response		
		A	B	C	Y1	Y2	Y3
		Sodium Alginate (mg/ml)	Calcium chloride (mg/ml)	Homogeni-zation time (rpm)	EE (%)	Average particle size (nm)	PDI
FA1	1	1	1	12500	31.62	232.1	0.437
FA2	2	2	1	15000	35.8	239.2	0.387
FA3	3	2	2	12500	30.96	212.5	0.534
FA4	4	1	3	12500	28.8	258.7	0.394
FA5	5	3	2	10000	38.27	194.1	0.375
FA6	6	2	2	12500	32.1	199.1	0.547
FA7	7	2	2	12500	33.95	201.4	0.557
FA8	8	2	3	15000	31.1	259.1	0.422
FA9	9	3	3	12500	36.98	208.9	0.495
FA10	10	2	3	10000	33.07	264.9	0.32
FA11	11	3	1	12500	40.05	253.7	0.388
FA12	12	2	2	12500	32.58	208.4	0.482
FA13	13	1	2	10000	31.22	210.5	0.399
FA14	14	2	1	10000	34.78	259.5	0.296
FA15	15	3	2	15000	37.2	190.8	0.488
FA16	16	2	2	12500	29.48	218.3	0.491
FA17	17	1	2	15000	30.08	201.5	0.449

Evaluation of Nanoparticles

1) Determination of Particle size, PDI and Zeta potential

These were determined using dynamic light scattering using Horiba Scientific nano Partica Nano Particle Analyzer SZ-100. [24]

2) FTIR Study

Fourier transform infrared spectrum (FTIR) was recorded using Shimadzu Infrared Spectrophotometer FTIR 8400S. [24]

3) Determination of Drug EE

The formulated nanoparticles were centrifuged for 1 hour at 12000 RPM. The supernatant was analysed for the concentration of free extract. The amount of drug (extract) in the supernatant is the residual amount. [25] The percentage of encapsulation is calculated as:

%EE = [(initial quantity of extract – residual quantity of extract)/initial quantity of extract] x 100

4) Determination of Process Yield and Loading Capacity

The loading capacity of the optimized formulation was found using the same method as of drug EE. The formulation was lyophilized and weighed.

% Yield of nanoparticles = [weight of nanoparticles/weight of raw materials added] x 100

The percentage of drug loading is calculated as:

%LC = [(initial amount of extract – residual amount of extract)/weight of nanoparticles] x 100

All quantities were recorded in microgram for the calculation. [24]

5) In-vitro extract release and kinetic studies

Dialysis bag method was used to study the *in-vitro* drug (extract) release from the optimized formulation. Formulated nanoparticles (250 mg) was suspended in 5 ml of purified water and transferred into the dialysis bag. The bag was immersed in 30ml of purified water in a beaker under stirring at 150 rpm to remain homogeneous. Samples were collected and studied spectrophotometrically to quantify the drug concentration in the media. [26–28]

The cumulative percentage of released drug was plotted as a function of time and kinetic models were applied. An adequate fit of the release data to the kinetic models suggests the best model to explain the pattern of drug release. [29]

6) In-vitro antidiabetic activity

α-amylase inhibition assay was performed on the plant extract and formulation using the DNSA method using 3,5-dinitrosalicylic acid reagent. Test samples were evaluated at concentrations of 3.125, 6.25, 12.5, 25, 50, 100 and 200µg/ml. From each working solution, 2µl of the test samples were mixed with 198 µl of 1 U/mL α-amylase enzyme solution (in 0.02M phosphate buffer). For 10 minutes, this mix was incubated at 37°C, after which soluble starch solution (200 µL of 1% w/v) was added as substrate, and incubated for 10 minutes at 37°C. DNSA was added and heated for 5 minutes to allow the color to develop. The sample is cooled, centrifuged and 200µl of supernatant is transferred to a microplate reader. The absorbance was measured at 540 nm. Decrease in absorbance compared to the vehicle control (without inhibitor) indicated inhibition of α-amylase activity. [30–32]

% Inhibition = $[(A_{control} - A_{sample}) / A_{control}] * 100$

3. RESULTS AND DISCUSSION

Phytochemical analysis of *Nigella sativa* seed extract

The extract was subjected to qualitative phytochemical testing and found to contain flavonoids, phenols, tannins, saponins and alkaloids. The TPC was found to be 17mg Gallic acid equivalent/g extract. The TFC was found to be 71mg Quercetin equivalent/g extract. [12,13]

Impact of formulation variables:

Alginate is an anionic unbranched polysaccharide copolymer of guluronic acid and mannuronic acid which are linked by 1,4-glycosidic linkages. In ionic gelation method, cations like calcium ions can form the egg-box complexes with the guluronic acid blocks resulting in an alginate hydrogel by the phenomenon of gelation. Alginate nanoparticles are used as carriers to increase bioavailability of drugs. Complexation of alginate using crosslinking agents like divalent cations in aqueous medium results in nanoaggregates. Polycationic polymers like chitosan can be used further to form a polyelectrolyte complex coated alginate nanoparticles. Alginate concentration can influence the encapsulation efficiency and size of nanoparticles prepared by this method. It also depends on the interaction between the alginate functional groups and calcium ions. Calcium ions diffusion increases as crosslinking time increases. This leads to increase in viscosity and porosity of alginate resulting in movement of drug molecules from alginate droplets to aqueous medium.[33] Crosslinking time of 30 minutes after addition of calcium chloride as well as chitosan was set for this study. [5,21]

Impact of Calcium chloride concentration:

Concentration of calcium chloride can impact particle size and EE of alginate nanoparticles. During the crosslinking process, the carboxylic group in alginate interact with calcium ions to form a stable three-dimensional network. Higher crosslinking causes more density in the gel matrix which can impact the particle size and EE. The increase in calcium ions can alter the zeta potential to a less negative value.[33]

To optimize the nanoparticle preparation, Design of Experiments were adapted using Design Expert 13 software. Along with Box Behnken design, a few experiments were run on 1 factor at a time analysis to ascertain the critical independent factors. Initial formulations were prepared with 20 ml of 3 levels of sodium alginate (0.6, 1 and 3 mg/ml) and 4 ml of 2 levels of calcium chloride (1 and 2 mg/ml). From this study, 3mg/ml sodium alginate level and 2mg/ml calcium chloride level were selected to further understand the impact of polyelectrolyte - chitosan and surfactant - polysorbate 80. The evaluation results of these formulations are given in table1. [33]

Use of surfactant:

Polysorbate 80 has been used in this study as a surfactant to understand its effect on encapsulation and lower particle

size. A reduction in entrapment was observed in *Nigella sativa* loaded alginate nanoparticles in presence of surfactant Polysorbate 80 (1%) as seen in formulations FC1 and FC2 in table 1.

The hydrophilic nature of the hydroethanolic extract of *Nigella sativa* can contribute to increased loss of drug in aqueous layer during ionic gelation process. The drug in the aqueous layer is discarded as supernatant after centrifugation and does not get encapsulated in the alginate nanoparticles.

Alginate: Chitosan Mass Ratio

Chitosan can impact the drug entrapment based on its concentration. Increase in chitosan concentration has lead to an increase in particle size and influenced entrapment of the drug. Amino groups in chitosan have higher affinity for the mannuronic acid group of alginate. Cations (like calcium) complex with guluronic acid block of alginate. If the concentration of chitosan is high; that is, a reduced alginate: chitosan ratio, it will result in competition between chitosan and calcium ions for guluronic acid blocks after the saturation of mannuronic acid blocks. [33] This can lead to particle aggregation and impact the drug loading negatively. Hence the levels of chitosan have to be at the minimum. In this study, 4ml of 0.6mg/ml chitosan was chosen based on the evaluation parameters in table 1.

Box-Behnken Experimental Design

The independent variables and their levels used in the 17 formulations of Box-Behnken design are enumerated in table 2 along with the dependent variables -EE, particle size and PDI. [34–37]

All of the responded data were fitted into suitable models and the best fit for the dependent variables was determined to be the one with a higher regression coefficient.

Table 3 presents the outcomes of the ANOVA of best-fit model and significant responses with P value less than 0.05 were recorded. All statistical parameters recorded are in table 3 and 4 and p-values less than 0.0500 indicate model terms are significant.

Table 3: Statistical analysis results of ANOVA of the fitted quadratic polynomial model for EE, Particle size and PDI

Response	Source	Sum of Squares	DF	Mean square	F-value	p-value	Significance
EE	Model	138.59	3	46.2	17.79	< 0.0001	Significant
	A	118.43	1	118.43	45.6	< 0.0001	Significant
	B	18.91	1	18.91	7.28	0.0182	Significant
	Lack of Fit	22.35	9	2.48	0.8708	0.6059	Not significant
Particle size	Model	10488.49	9	1165.39	16	0.0007	Significant
	AB	1274.49	1	1274.49	17.5	0.0041	Significant
	A^2	713.77	1	713.77	9.8	0.0166	Significant
	B^2	7941.75	1	7941.75	109.06	< 0.0001	Significant
	Lack of Fit	260.5	3	86.8	1.39	0.3668	Not significant
PDI	Model	0.0879	9	0.0098	14.12	0.001	Significant
	C	0.0158	1	0.0158	22.9	0.002	Significant
	AB	0.0056	1	0.0056	8.13	0.0246	Significant
	B^2	0.0287	1	0.0287	41.53	0.0004	Significant
	C^2	0.0293	1	0.0293	42.29	0.0003	Significant
	Lack of Fit	0.0003	3	0.0001	0.0839	0.9652	Not significant

Table 4: Regression coefficient of model that was used and recommended by Design Expert software

Model	R^2	Adjusted R^2	Predicted R^2	Standard deviation	% CV	Adequate precision	Comment
Entrapment efficiency (EE)							
Linear	**0.8041**	**0.7589**	**0.7254**	**1.61**	**4.82**		Suggested
2FI	0.8172	0.7075	0.6239	1.78			-
Quadratic	0.9281	0.8358	0.8059	1.33		**13.7782**	-
Particle size							
Linear	0.0521	-0.1667	-0.8454	28.32			-
2FI	0.1735	-0.3224	-2.6692	30.15			-
Quadratic	**0.9537**	**0.8941**	**0.5856**	**8.53**	**3.8**	**11.4689**	Suggested
PDI							
Linear	0.1973	0.012	-0.272	0.0757			-
2FI	0.269	-0.1697	-1.0585	0.0823			-
Quadratic	**0.9478**	**0.8807**	**0.8738**	**0.0263**	**5.99**	**11.0594**	Suggested

Non-significant lack of fit directs to an acceptable model. Adequate Precision above 4 is desirable. High Coefficient of variation (CV) suggests that the data points are widely spread around the average and there is large variability in the data set. The low CV, as seen in table 4, indicates that

the data points are clustering around the average with less variability.

Entrapment efficiency (EE)

For EE, A, B are significant model terms. The high level factor is coded as (+1) and the low level is coded as (-1). By comparing the factor coefficients, the coded equation can be used to identify the relative impact of the factors. Regression equation for EE is:

EE (Y1) = 33.41 + 3.85 A - 1.54 B - 0.395 C

Predicted R^2(0.7254) and adjusted R^2(0.7589) is in agreement as the variance is less than 0.2. The ratio of 13.778 indicates an adequate signal and this model can be used to navigate the design space. Low CV (4.82%) shows a better precision of the experiments.

The EE of the formulations varied from 28.8% to 40.05%. EE followed a linear pattern where it increased with the levels of sodium alginate and decreased with levels of calcium chloride (figure 1).

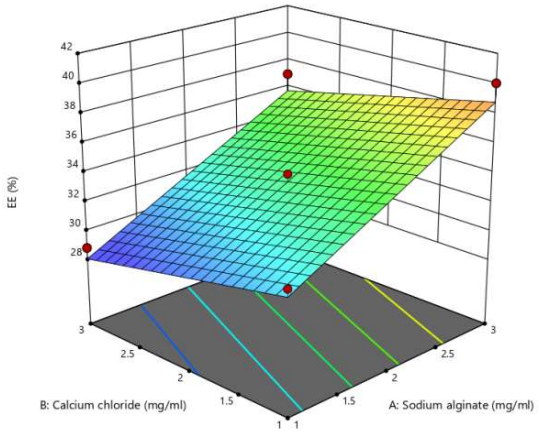

Figure 1: Response Surface Plot of EE

Particle size

For particle size, AB, A^2 and B^2 are significant model terms. Regression equation of the coded value for the particle size is

Particle size (Y2) = 207.94 -6.91 A + 0.8875B -4.8 C - 17.85 AB + 1.43 AC + 3.63 BC - 13.02 A^2 + 43.43 B^2 + 4.3C^2.

The selected variables are able to produce particle size in the range of 190.8nm to 264.9nm. The operational range of the 3 variables are suitable to produce nanoparticles in this size range. Adequate precision of 11.469 indicate an adequate signal. The low CV (3.8%) shows a good precision of the experiments and points to less variability in average particle size while using the 3 variable factors in these set of experiments.

Average particle size aligned towards a quadratic model where the average size increased and then decreased with the levels of sodium alginate and calcium chloride. Increasing homogenization speed showed slight decrease in particle size but does not significantly impact the formulation (figure 2).

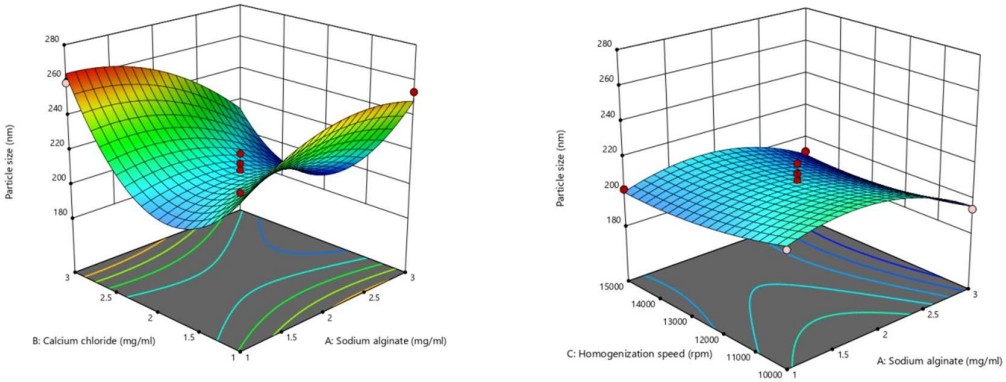

Figure 2: Response Surface Plots of particle size

Polydispersity Index

For PDI, C, AB, B^2 and C^2 are significant model terms. Regression equation representing the coded value for the PDI is

PDI (Y3) = 0.5222 +0.0084A + 0.0154B + 0.0445C + 0.0375AB + 0.0158AC + 0.0027BC - 0.0111A^2 - 0.0928B^2 - 0.0834C^2

The Predicted R^2(0.8738) and adjusted R^2(0.8807) is in agreement as the variance is less than 0.2. Adequate precision of 11.059 indicates an adequate signal. The low CV (5.99%) shows good precision of the experiments.

The PDI ranged from 0.296 to 0.557. PDI decreased with increasing concentration of sodium alginate. PDI increased and increased with increasing concentration of calcium chloride and speed of homogenization (figure 3).

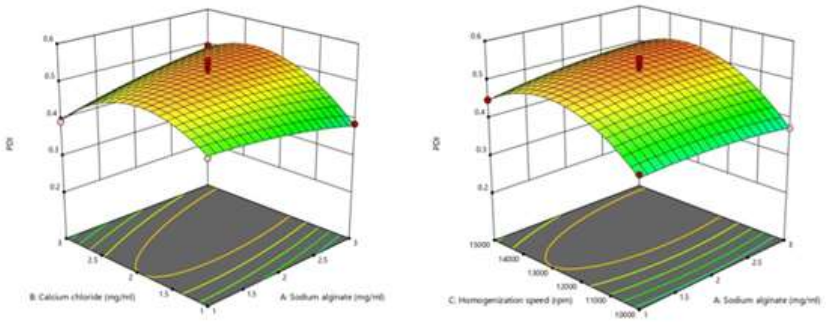

Figure 3: Response Surface Plots of PDI

Optimization of formulation

The criterion for alginate nanoparticles size was set below 210 nm with minimum PDI and maximum EE. The variables sodium alginate level, calcium chloride level and homogenization speed were set at 2.7mg/ml, 1.6mg/ml and 10000 RPM respectively. [34–37]

Evaluation parameters of optimized formulation

The nanoparticle size and PDI of optimized formulation is 221.3 nm and 0.372 respectively (figure 4a).

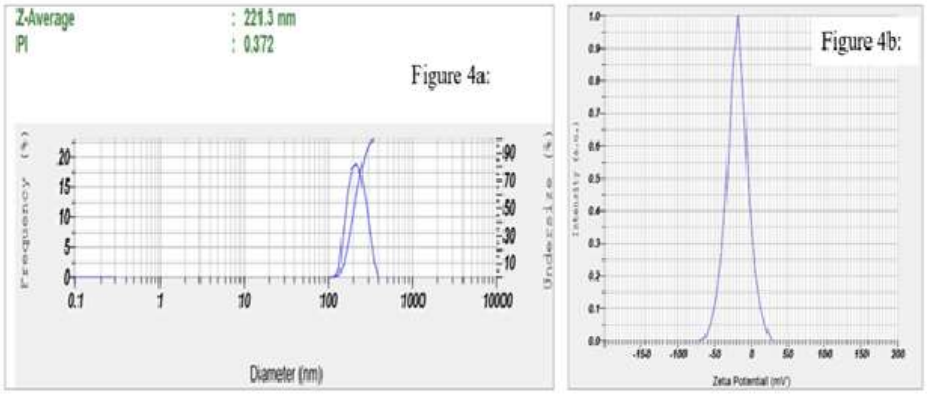

Figure 4a: Particle size of optimized nanoparticles; Figure 4b: zeta potential depicting stable nanoparticle formulation.

A lower PDI value (closer to zero) indicates homogeneity in the prepared nanoparticles. The negative zeta potential value of -18.7 is sufficient to stabilize the formulation in liquid state for short duration (figure 4b). The preparation is lyophilized to dry form to improve stability. The average EE of 40.58% ±3.52 also matches with the design of expert predicted value as seen in table 5. The loading capacity and percentage yield of the best formulation was found to be 53.2% and 83% respectively.

Table 5: Point prediction and observed values of optimized formulation

Material & parameters	Goal	Lower	Upper	Importance	Predicted Value	Observed Value	% Prediction Error
Sodium alginate (mg/ml)	minimize	1	3	3	2.65	2.7	-
Calcium chloride	minimize	1	3	3	1.64	1.6	-

Material & parameters	Goal	Lower	Upper	Import ance	Predicted Value	Observed Value	% Prediction Error
(mg/ml)							
Homogenization (RPM)	minimize	10000	15000	3	10000	10000	-
EE (%)	maximize	30	60	3	36.1258	40.58 ±3.52	1.98
particle size (nm)	minimize	190	230	3	216.7	221.3	-2.12
PDI	minimize	0.3	0.4	3	0.360927	0.372	-3.07

FTIR Study of optimized formulation

The FTIR spectra of the dried extract of Nigella sativa and extract loaded sodium alginate nanoparticles (figure 5a & 5b) indicate the presence of functional groups. Increased hydrogen bonds between the polymer and drug (extract) could lead to positional changes in the peaks, indicating physical entrapment of extract in the polymer matrix. [27]

The characteristic band of absorption at 3421.83cm^{-1} is the OH group of the aromatic ring of carvacrol and thymol. At 3012.91cm^{-1} is the C-H absorption band of aromatic rings and at 2926.11cm^{-1} is the tertiary C-H stretch in thymoquinone, carvacrol and thymol. Absorption at 2854.74cm^{-1} is due to symmetric stretching modes of three methyl groups.(10,38) . At 1629.9 cm^{-1} absorption is the C=C stretching. In the formulation spectrum, the characteristic peak at 1026.16cm^{-1} is C-O-C stretching vibration. The cross-linking of sodium alginate with calcium lead to absorption peak of O-H stretching at 3341.12 cm^{-1}. [27]

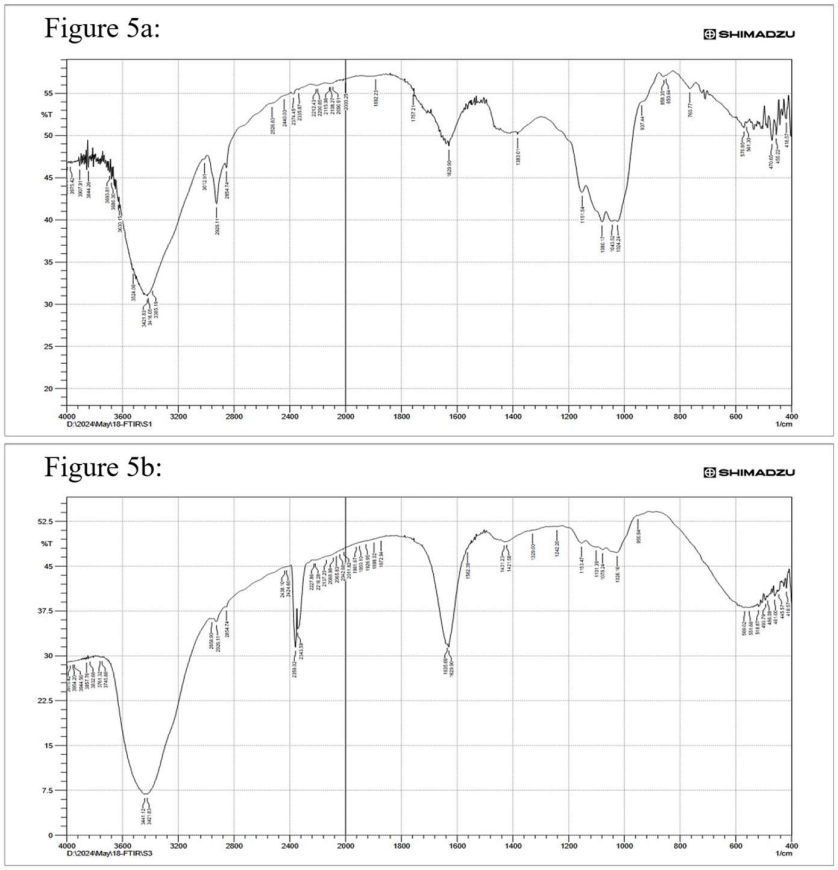

Figure 5a: FTIR Spectra of hydroethanolic extract of *Nigella sativa,* Figure 5b: FTIR Spectra of extract loaded sodium alginate nanoparticle formulation

***In-vitro* drug release study of optimized formulation**

Dialysis bag method was utilized to study the drug release from the optimized sodium alginate nanoparticle loaded with hydroethanolic extract of *Nigella sativa*. Around 60% of drug release was observed in the initial 6 hours of the dissolution study and a nearly complete release of the drug with 24 hours. The pattern of drug release is depicted in Figure 6.

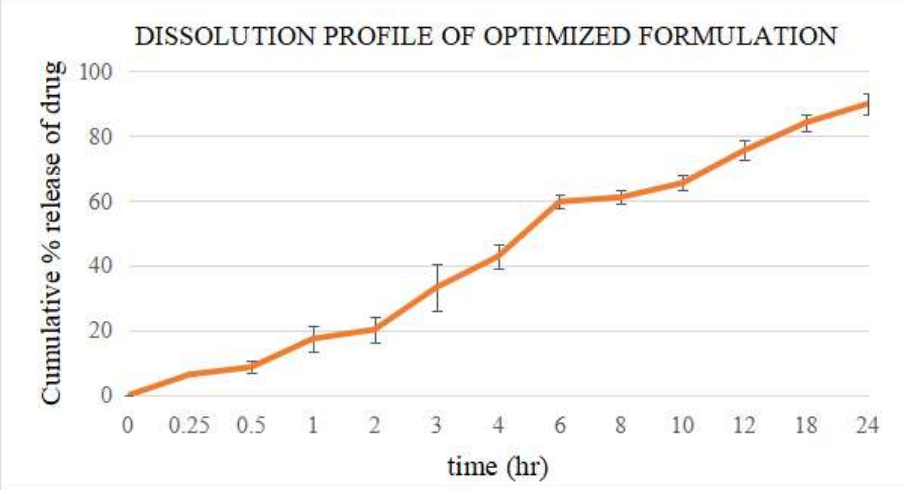

Figure 6: Dissolution Profile of optimized formulation

Drug release kinetics

The extract release of the optimized formulation was fitted into various models of release kinetics. The R^2 values were calculated using MS Excel and best fit (Table 6) was found to be in the Baker Lonsdale Model; developed to describe drug release from a matrix of spherical shape. According to this model, if a plot of $3/2[1-(1-Q)^{2/3}]-Q$ vs time will result in a straight line, diffusion mechanism is the basis for drug release from the spherical matrix. 'Q' stands for fraction of drug released. This model is more appropriate to describe the release of drug from submicron or fine particles.

Table 6: Release kinetic Data

Release kinetics model	R^2
Zero-order	0.8386
First-order	0.9812
Weibull Model	0.9872
Baker Lonsdale Model	0.9889
Hixson Crowell	0.9456
Korsmeyer-Peppas	0.9812
Higuchi model	0.9693

The Weibull model showed an R^2 value of 0.9872 with a slope of 0.81 (figure 7). When the slope of Weibull model is less than 1, it indicates that the release pattern of the formulation is parabolic and is capable of biphasic release. The Korsmeyer-peppas and first order release has shown same R^2 of 0.9812. Water soluble drugs dispersed in porous matrix tend to show first order kinetics. *Nigella sativa* extract and sodium alginate are water soluble. This value also indicates that the sodium alginate nanoparticles have a porous nature. The value of 'n' derived from the Korsmeyer-peppas model is 0.685, indicating a non-fickian drug release from a non-swellable spherical sample. As the n value is less than 1, drug release mechanism includes diffusion through the polymer as well as erosion of the polymer. [27,29]

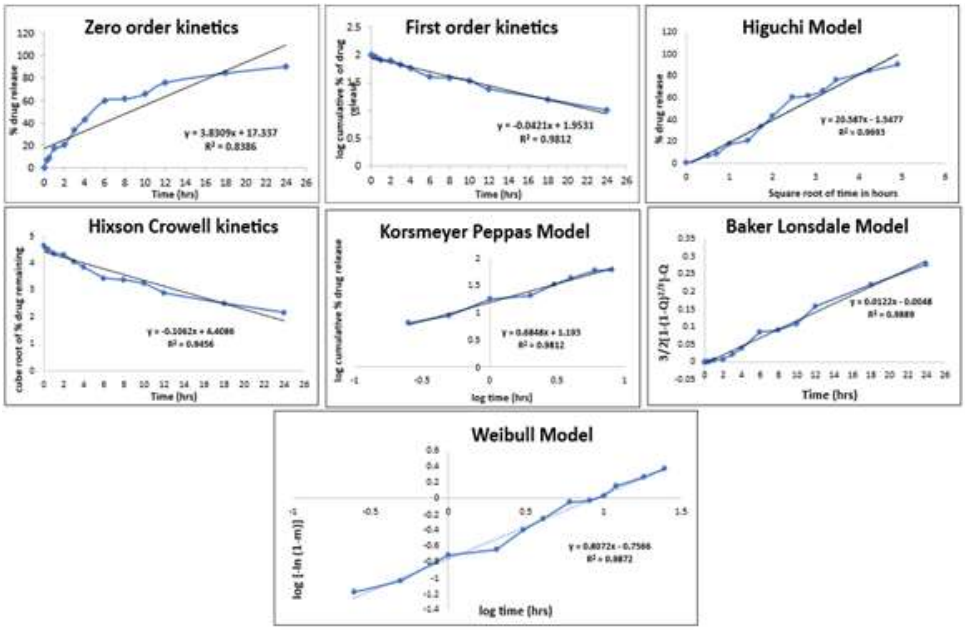

Figure 7: Graphical representation of Release kinetic models

***In-vitro* antidiabetic activity**

The nanoparticle formulation has exhibited good amylase inhibitory activity which reflects on its effectiveness to reduce the starch breakdown.[38] The inhibition of a-amylase was in a dose-dependent manner (figure 8). This test reveals a very significant potentiation of drug (extract of *Nigella sativa*) in the formulation. The entrapment efficiency of the formulation under study is 40.58%. The amylase inhibitory activity of loaded sodium alginate nanoparticles demonstrated IC_{50} value of 192.75 ± 6.531µg/ml; while the IC_{50} value of extract is 62.95 ± 4.055µg/ml. The IC_{50} value of formulation is lower than that of *Nigella sativa* extract because the excipients contribute to 60% of the formulation. The graph below shows a stratified comparison between the inhibitory action of extract vs formulation. The higher IC_{50} value of the formulation is an indicator of a controlled release mechanism.

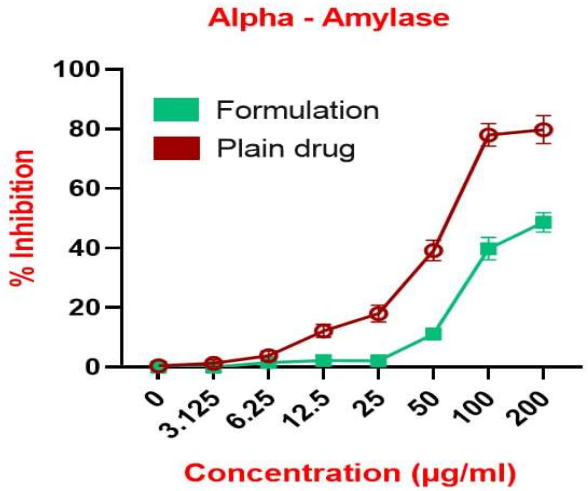

Figure 8: α-amylase activity of *Nigella sativa* extract and optimized formulation

CONCLUSION

The *Nigella sativa* extract loaded sodium alginate nanoparticles were formulated by ionic gelation-homogenization technique. This combined method enabled the synthesis of nanoparticles with size range from 190.8 nm to 264.9 nm. The nanoparticles were prepared in accordance with the Box-Behnken design and subjected to characterization. Based on EE, particle size and PDI, the optimized nanoparticles were formulated and evaluated. The evaluated parameters and predicted values (by design expert software) were close. The lower particle size achieved through this design enables better absorption and

efficiency of the formulation. The FTIR spectra showing the characteristic peaks of *Nigella sativa* in the formulation indicates a physical entrapment rather than a chemical reaction. The optimized formulation was subjected to *in-vitro* dissolution study and release kinetics. The dissolution study demonstrates a slow and gradual release of the drug from the formulation. It could indicate an electrostatic interaction between polymer and drug, responsible for the slower release. Dissolution profile adheres to the Baker Lonsdale Model indicating a spherical matrix with diffusion mechanism. The Weibull model, Korsmeyer Peppas model and first order kinetics also can explain the release pattern from this formulation, which is a non-fickian diffusion and erosion from a non-swelling porous spherical matrix. α-amylase inhibition of optimized sodium alginate formulation proved the *in-vitro* anti-diabetic activity of nanoparticles. The alginate nanoparticles were able to inhibit the α-amylase enzyme at IC_{50} value of 192.75 ± 6.531µg/ml. Extract is released quickly into the medium, whereas the nanoparticle matrix is a spherical matrix with a diffusion-controlled release mechanism. The non-Fickian drug release mechanism, supported by our Korsmeyer-Peppas model data confirms that the formulation provides a stable, porous matrix that facilitates both drug diffusion and polymer erosion. Alginate based nanoformulations are promising carriers for herbal drug delivery systems.

REFERENCES:

1. Home | International Diabetes Federation [Internet]. [cited 2025 Dec 14]. Available from: https://idf.org/
2. Shaukat A, Zaidi A, Anwar H, Kizilbash N. Mechanism of the antidiabetic action of Nigella sativa and Thymoquinone: a review. Frontiers of Nutrition. 2023; 10: :1126272. doi:10.3389/fnut.2023.1126272
3. Hamdan A, Idrus RH, Mokhtar MH. Effects of nigella sativa on type-2 diabetes mellitus: A systematic review. International Journal of Environmental Research and Public Health. 2019; 16(24):4911. doi:10.3390/ijerph16244911
4. Govindaraju R, Karki R, Chandrashekarappa J, Santhanam M, Shankar AKK, Joshi HK, et al. Enhanced Water Dispersibility of Curcumin Encapsulated in Alginate-Polysorbate 80 Nano Particles and Bioavailability in Healthy Human Volunteers. Pharm Nanotechnol. 2019; 7(1):39–56. doi:10.2174/2211738507666190122121242
5. Sorasitthiyanukarn FN, Ratnatilaka Na Bhuket P, Muangnoi C, Rojsitthisak P, Rojsitthisak P. Chitosan/alginate nanoparticles as a promising carrier of novel curcumin diethyl diglutarate. Int J Biol Macromol. 2019; 131:1125–36. doi:10.1016/j.ijbiomac.2019.03.120
6. Nagarwal RC, Kumar R, Pandit JK. Chitosan coated sodium alginate–chitosan nanoparticles loaded with 5-FU for ocular delivery: In vitro characterization and in vivo study in rabbit eye. European Journal of Pharmaceutical Sciences. 2012; 47(4):678–85. doi:10.1016/J.EJPS.2012.08.008
7. Severino P, da Silva CF, Andrade LN, de Lima Oliveira D, Campos J, Souto EB. Alginate Nanoparticles for Drug Delivery and Targeting. Curr Pharm Des. 2019; 25(11):1312–34. doi:10.2174/1381612825666190425163424
8. Jana S, Sen KK, Gandhi A. Alginate Based Nanocarriers for Drug Delivery Applications. Curr Pharm Des. 2016; 22(22):3399-410. doi: 10.2174/1381612822666160510125718.
9. Farouk A, Elbehery H, Embaby H, Abdel-aziz NF, Abd El-wahab T, Abouamer W, Hussein H. Phenolics from Nigella sativa L. straw: Characterization and insecticidal activity against Agrotis ipsilon (Hüfnagel). Heliyon. 2023; 9(12):e22995. doi:10.1016/j.heliyon.2023.e22995
10. Meshksar S, Hadipour Jahromy M, Qomi M, Sami N, Faali F. Formulation and evaluation of the effects of ophthalmic nanoemulsion of Nigella sativa seed extract on atropine-induced dry eye in mice. Phytomedicine Plus. 2024; 4(2): 100541. doi:10.1016/j.phyplu.2024.100541
11. Girme AS, Gaikar NV, Saste GB, Kunkulol RR. Chemical studies on antidiabetic botanical drug: Cassia auriculata. Journal of Pharmacognosy and Phytochemistry. 2018; 7(5):3417–24.
12. Goga A, Hasic S, Becirovic S, Cavar S. Phenolic Compounds and Antioxidant Activity of Extracts of Nigella sativa L. Bulletin of the Chemists and Technologists of Bosnia and Herzegovina. 2012; 39:15–9.
13. Ambreen, Khan MA, Raza A, Yousaf R, Ali H, Darwish H. Impact of zinc oxide nanoparticles on biosynthesis of thymoquinone in cell cultures of Nigella sativa. Plant Nano Biology. 2024; 10: 100109. doi:10.1016/j.plana.2024.100109
14. Prasathkumar M, Raja K, Vasanth K, Khusro A, Sadhasivam S, Sahibzada MUK, Gawwad MRA, Al Farraj DA, Elshikh MS. Phytochemical screening and in vitro antibacterial, antioxidant, anti-inflammatory, anti-diabetic, and wound healing attributes of Senna auriculata (L.) Roxb. leaves. Arabian Journal of Chemistry. 2021; 14:103345. doi:10.1016/j.arabjc.2021.103345
15. Zahoor A, Sharma S, Khuller GK. Inhalable alginate nanoparticles as antitubercular drug carriers against experimental tuberculosis. Int J Antimicrob Agents. 2005; 26(4):298–303. doi:10.1016/j.ijantimicag.2005.07.012
16. El-Houssiny AS, Ward AA, Mostafa DM, Abd-El-Messieh SL, Abdel-Nour KN, Darwish MM, Khalil WA. Drug-polymer interaction between glucosamine

sulfate and alginate nanoparticles: FTIR, DSC and dielectric spectroscopy studies. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2016; 7(2):025014. doi:10.1088/2043-6262/7/2/025014

17. Costa JR, Xavier M, Amado IR, Gonçalves C, Castro PM, Tonon R V, Cabral LMC, Pastrana L, Pintado ME. Polymeric nanoparticles as oral delivery systems for a grape pomace extract towards the improvement of biological activities. Materials Science and Engineering C. 2021; 119:111551. doi:10.1016/j.msec.2020.111551.

18. Sarei F, Dounighi NM, Zolfagharian H, Khaki P, Moradi Bidhendi AS. Alginate Nanoparticles as a Promising Adjuvant and Vaccine Delivery System, Indian J Pharm Sci. 2013; 75(4):442-449. doi: 10.4103/0250-474X.119829

19. Lertsutthiwong P, Rojsitthisak P, Nimmannit U. Preparation of turmeric oil-loaded chitosan-alginate biopolymeric nanocapsules. Materials Science and Engineering C. 2009 Apr 30; 29(3):856–860. doi:10.1016/j.msec.2008.08.004

20. Lertsutthiwong P, Noomun K, Jongaroonngamsang N, Rojsitthisak P, Nimmannit U. Preparation of alginate nanocapsules containing turmeric oil. Carbohydr Polym. 2008; 74(2):209–214. doi:10.1016/j.carbpol.2008.02.009

21. Rami A, Kazemi-Lomedasht F, Mirjalili A, Noofeli M, Shahcheraghi F, Dounighi NM. Outer Membrane Vesicles of Bordetella pertussis Encapsulated into Sodium Alginate Nanoparticles as Novel Vaccine Delivery System. Curr Pharm Des. 2021; 27(42):4341–4354. doi:10.2174/1381612827666210907154715

22. Liu J, Xiao J, Li F, Shi Y, Li D, Huang Q. Chitosan-sodium alginate nanoparticle as a delivery system for ε-polylysine: Preparation, characterization and antimicrobial activity. Food Control. 2018; 91:302–310. doi:10.1016/j.foodcont.2018.04.020

23. Abnoos M, Mohseni M, Mousavi SAJ, Ashtari K, Ilka R, Mehravi B. Chitosan-alginate nano-carrier for transdermal delivery of pirfenidone in idiopathic pulmonary fibrosis. Int J Biol Macromol. 2018; 118:1319–1325. doi:10.1016/j.ijbiomac.2018.04.147.

24. Alqahtani MS, Al-Yousef HM, Alqahtani AS, Tabish Rehman M, AlAjmi MF, Almarfidi O, Amina M, Alshememry A. Preparation, characterization, and in vitro-in silico biological activities of Jatropha pelargoniifolia extract loaded chitosan nanoparticles. Int J Pharm. 2021; 606:120867. doi:10.1016/j.ijpharm.2021.120867

25. Khshemat V, Homayouni-Tabrizi M, Neamati A, Khadem F, Irani M. Fabrication, Characterisation, and Biological Properties of Chitosan Nanoparticles Containing Rapeseed Pollen Extract (RPE) on the MCF-7 Cell Line. Materials Technology. 2022; 37(9):1075–1085. doi:10.1080/10667857.2021.1921099

26. Ahmady AR, Razmjooee K, Nazar V, Saber-Samandari S. Alginate carrier as a controlled thymol delivery system: Effect of particle size. Mater Chem Phys. 2023; 294:126982. doi:10.1016/j.matchemphys.2022.126982

27. De Silva ND, Attanayake AP, Karunaratne DN, Arawwawala LDAM, Pamunuwa GK. Synthesis and bioactivity assessment of Coccinia grandis L. extract encapsulated alginate nanoparticles as an antidiabetic drug lead. J Microencapsul. 2024; 41(1):1–17. doi:10.1080/02652048.2023.2282964

28. Rahaiee S, Shojaosadati SA, Hashemi M. An efficient ionic gelation based nano-delivery system to improve the stability and controlled release of saffron extracts. Biocatal Agric Biotechnol. 2023; 52:102831. doi:10.1016/j.bcab.2023.102831

29. Paarakh MP, Jose PA, Setty CM, Christoper GVP. Release Kinetics-Concepts and Applications. International Journal of Pharmacy Research & Technology.2018; 8(1), 12-20. https://doi.org/10.31838/ijprt/08.01.02

30. Veeramani S, Narayanan AP, Yuvaraj K, Sivaramakrishnan R, Pugazhendhi A, Rishivarathan I, et al. Nigella sativa flavonoids surface coated gold NPs (Au-NPs) enhancing antioxidant and anti-diabetic activity. Process Biochemistry. 2022; 114:193–202. doi:10.1016/j.procbio.2021.01.004

31. Dalli M, Daoudi NE, Abrigach F, Azizi S eddine, Bnouham M, Kim B, Gseyra N. In vitro α-amylase and hemoglobin glycation inhibitory potential of Nigella sativa essential oil, and molecular docking studies of its principal components. Front Pharmacol. 2022; 13:1036129. doi:10.3389/fphar.2022.1036129

32. Prasathkumar M, Sakthivel C, Becky R, Dhrisya C, Prabha I, Sadhasivam S. Phytofabrication of cost-effective selenium nanoparticles from edible and non-edible plant materials of Senna auriculata: Characterization, antioxidant, antidiabetic, antimicrobial, biocompatibility, and wound healing. J Mol Liq. 2022; 367: 120337. doi:10.1016/j.molliq.2022.120337

33. Choukaife H, Doolaanea AA, Alfatama M. Alginate nanoformulation: Influence of process and selected variables. Pharmaceuticals. 2020; 13(335):1–34. doi:10.3390/ph13110335

34. Khuroo T, Khuroo A, Hussain A, Mirza MA, Panda AK, Wani J, Iqbal Z. Qbd based and Box-Behnken design assisted Oral delivery of stable lactone (active) form of Topotecan as PLGA nanoformulation: Cytotoxicity, pharmacokinetic, in vitro, and ex vivo gut permeation studies. J Drug

Deliv Sci Technol. 2022; 77:103850. doi:10.1016/j.jddst.2022.103850

35. Almeida KB, Ramos AS, Nunes JBB, Silva BO, Ferraz ERA, Fernandes AS, Felzenszwalb I, Amaral ACF, Roullin G, Falcao DQ. PLGA nanoparticles optimized by Box-Behnken for efficient encapsulation of therapeutic Cymbopogon citratus essential oil. Colloids Surf B Biointerfaces. 2019; 181:935–942. doi:10.1016/j.colsurfb.2019.06.010 PubMed PMID: 31382343.

36. Manikandan S, Jose PA, Karuppaiah A, Rahman H. The effect of physical stability and modified gastrointestinal tract behaviour of resveratrol-loaded NLCs encapsulated alginate beads. Naunyn Schmiedebergs Arch Pharmacol. 2024; 397(11):9007-9021. doi:10.1007/s00210-024-03223-3

37. Ruiz E, Orozco VH, Hoyos LM, Giraldo LF. Study of sonication parameters on PLA nanoparticles preparation by simple emulsion-evaporation solvent technique. Eur Polym J. 2022;173:111307. doi:10.1016/j.eurpolymj.2022.111307

38. Rani R, Dahiya S, Dhingra D, Dilbaghi N, Kim KH, Kumar S. Improvement of antihyperglycemic activity of nano-thymoquinone in rat model of type-2 diabetes. Chem Biol Interact. 2018; 295:119–132. doi:10.1016/j.cbi.2018.02.006