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Research Article

Formulation Development of Flaxseed Oil Beads Containing ω-Fatty Acid

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Abstract

Background: Nutraceuticals are in high demand as dietary supplements on the international market since decades. Nutritional and therapeutic supplements called nutraceuticals are widely accessible. Alpha linolenic acid (ALA), fibre, proteins, and vital omega-3 fatty acids are all present in high concentrations in flaxseed, one of the oldest and most widely utilised food supplements. The present work was aimed to formulate and develop flaxseed oil beads containing omega fatty acids as a replacement supplement for marine source. Flaxseed oil beads were formulated by ionic gelation method. Minitab 21.1.0 was used to screen and optimise the process and formulation parameters. Plackett Burman design was used for the initial screening. Twelve batches were made ready for screening, and each batch was evaluated for optimization based on the percentage of drug release and the percentage of drug encapsulation efficiency. RSM was used to carry out the optimization. After optimization and validation, the batches showed the satisfactory results complying with IP specifications.

Results: Twelve batches were formulated and evaluated for percentage encapsulation efficiency and percentage drug release. The formulated batches F4, F6 and F10 shown the optimal results.

Conclusion: Polyunsaturated fatty acids found in the flaxseed oil were confirmed with hexabromide test. Several nutritional advantages of the extracted flaxseed oil's omega fatty acid were incorporated in beads utilising the ionic gelation process.

Keywords: Nutraceutical, Flaxseed, Beads, QbD, Omega fatty acid, Alpha linolenic acid.

1. INTRODUCTION

Nowadays "Nutraceutical" movement has created a huge need for the creation of novel dietary supplement formulations. These are the supplements which are used as medication in addition to nutrition to benefit health, prevent chronic diseases, modulate the immune system, lengthen life expectancy, and many other things. The idea of essential fatty acids derives from the fact that they are necessary lipids for the human body because of their cellular roles linked to inflammatory and immune responses. They are also endogenously incapable of synthesis, necessitating their consumption[1].

Omega-3 essential fatty acids are a class of nutraceuticals under the study for food enrichment due to their functional properties. The main classes of essential fatty acids are omega-3 and omega-6, represented by the major compounds in foods: Alpha-Linolenic Acid (ALA, C18:3) and Linoleic Acid (LA, C17:2), respectively[2,3].

Long-chain polyunsaturated fatty acids in the omega-3 family, such as docosahexaenoic acids (DHA, C20:5) and eicosapentaenoic acids (EPA, C22:6, n- 3)., have drawn interest because of their potential to protect cardiovascular illnesses and to regulate body homeostasis Omega-3 fatty acids, which have important cardioprotective qualities, also have anti-inflammatory, antiarrhythmic, vasodilatory, and active effects on dyslipidaemia, diabetes mellitus, and obesity [4,5]. LA and ALA are essential fatty acids since neither humans nor other higher animals are able to manufacture them. Eicosanoids, which are produced from these Fatty Acids, are also referred to as locally acting bioactive signalling lipids. EPA and DHA create anti-inflammatory eicosanoids, whereas arachidonic acid (ARA) produces pro-inflammatory eicosanoids[6,7].

α-Linolenic acid makes a notable contribution to the fatty acids within green leafy tissues of plants, typically comprising over 50 % of the fatty acids present. This is because α-linolenic acid is an essential component of the membranes of thylakoids within chloroplasts. α-Linolenic acid is found in significant amounts in several seeds, seed oils and nuts. Linseeds (popularly known as flaxseeds) and their oil typically consists of 45–55 % of fatty acids as α- linolenic acid. In contrast, soyabean oil, rapeseed oil and walnuts contain 5–10 % of fatty acids as α- linolenic acid[8].

The oils are more prone to oxidation on exposure to different environmental conditions. The oil containing omega fatty acid to encapsulate in beads was one of the difficult approaches to encapsulate in bead formulation. The aim of current research work was aimed to design and develop ω -fatty acid containing pectin beads using QBD.

2. MATERIALS AND METHOD

2.1. Materials

Flaxseeds were obtained from Wagh Brothers Pvt. Ltd. (Nagpur, India). Pectin, Chitosan and Calcium Chloride were purchased from Himedia Laboratories Pvt. Ltd. Acetic acid and

Tween 20 was obtained from Merck Specialities Pvt. Ltd. and SD fine-chem Ltd. Fish oil was purchased from Triveni Chemicals, Gujrat.

2.2. Experimental method

2.2.1. Risk Assessment

The fish-bone diagram was considered to identify the potential risk factors (CQAs) of formulation and development, which affects % encapsulation efficiency and % drug release. Based on literature review and prior knowledge, the failure mode and effect analysis (FMEA) method were further applied in the initial risk analysis parameters of the beads. The RPN limit was set at 36 and 38. Any procedure parameter with an RPN 12 was viewed as a potential basic factor based on terms of severity (S), detectability (D) and probability (P)[9,10].

2.2.2. Phytochemical Extraction

Extraction of omega fatty acid oil containing Seeds: 100gm flaxseeds were cold macerated in a 1:1 ratio with petroleum ether and n-hexane. The supernatant solvent is distilled at 50-60°C after 10-12 days and the oil is collected[11].

2.2.3. Phytochemical evaluation

The different phytochemical components were evaluated for the collected oil such as alkaloids, steroids, terpenoids, flavonoids, saponins, tannins, phenolic compounds, cardiac glycoside, proteins, and carbohydrates[12,13].

2.2.4. Thin Layer Chromatography:

Sample preparation	Few ml of oil containing 40 - 50% of ω-3 Fatty acid was dissolved in 10ml chloroform and sonicated as required for spotting in TLC. As a standard, fish oil was used[14].
	Glass plates were coated with (0.2-0.3 mm) silica gel GF 254 (30 g/60 ml distilled water) and dried at room temperature. Prior to sample application, the coated plates were activated in an oven at 100°C - 120°C for 30 minutes and cooled. Samples were placed with a minimum of 1cm between two adjacent spots (one spot for standard and another for extracted sample). The RF value of the isolated spots were determined by using the following formula. $$\text{Rf Value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$
Development of Solvent System	A variety of solvent systems were tried, with various solvents in different ratios, but the satisfactory resolution was obtained in a mixture of Benzene: Chloroform (80:20)
Identification of Spots	To identify the separated spots, the spots were examined under UV light (254nm) and in an iodine chamber.

2.2.5. Test for Determination of Polyunsaturated Fatty Acid

Hexabromide test: Pipette one ml of oil into a boiling tube (wide-mouthed 100 ml capacity). Drop-wise add 5 mL of chloroform and about 1 mL of bromine until the mixture turns a deep red colour, then cool the test-tube in an ice water bath. Add 1.5 mL of rectified spirit drop-wise while shaking the mixture until the precipitate that formed dissolves, and then add 10 mL of diethyl ether. Place the tube in an ice water bath for 20 minutes after mixing the contents. The presence of polyunsaturated fatty acids is indicated by the appearance of precipitate[15].

2.3. Drug Excipient Compatibility Studies

2.3.1. Fourier Transform Infrared Spectroscopy

The FTIR analysis method scans test samples with infrared light to observe chemical properties. The FTIR spectra of extracted flaxseed oil samples from RTMNU, Nagpur were analysed using a Bruker FTIR spectrophotometer[16].

2.3.2. Standard Calibration Curve

Preparation of stock solution of drug: 100mg of pure fish oil weighed accurately and carefully transferred into 100 ml volumetric flask was dissolved in 100 mL of pure methanol and left to stand for 30 minutes.

Preparation of sample solutions for analysis: By diluting the stock solution with methanol, various drug concentrations ranging from 5 to 25 µg/ml were prepared and absorbance was taken at 203nm.

2.4. Procedure of Beads Formulation

The dripping method was used to create chitosan-pectin hydrogel beads, with calcium chloride (CaCl2) in deionised water serving as a continuous media. At 70 °C, chitosan solution was prepared by dissolving it in 5% (v/v) acetic acid. Using a magnetic stirrer, pectin was dissolved in deionized water at 40 °C. After dissolving 300 mg flaxseed oil in the pectin solution, the mixture was homogenised for 5 minutes with 0.5% (v/v) Tween 20 as an emulsifying agent. The pectin-containing oil was placed in a syringe and dripped into a $CaCl_2$ solution while magnetically stirring at 351 °C. After being washed with deionized water, the beads were immersed in chitosan for 30 minutes. The beads were washed again, oven-dried for 4 hours at 60 °C, wrapped in an aluminium foil bag, and stored at 4-6 °C for further testing[17–19].

2.5. Screening, Optimisation and Validation of Flaxseed Oil Containing Pectin Chitosan Beads

2.5.1. Quality by design (QbD)

A potential approach for the element of pharmaceutical development to provide a framework of the current status of QbD for pharmaceutical product is Quality Target Product Profile (QTPP), Critical quality Attributes (CQAs), Risk Assessment, Design space, Control strategy, Product life cycle Management, and Continual Improvement[20–22] (Table 1).

Table 1: Quality Target Product Profile (QTPP) with reference to in process critical quality attribute

IP CQAs	Quality Target Product Profile (QTPP)
Appearance	Off white to yellow coloured
Assay	80% to 105% of the label claim of composite blend sample
Average beads size	1.04 ± 0.20 mm
% Encapsulation Efficiency	NLT 30% NMT 100%
% Drug Release	NLT 20% NMT 100%

Depending on the RPN number based on FMEA and multivariate data analysis, the effect of CQA on finished product quality were analysed for establishment of design space with a goal to ensure the quality of product (Table 2).

Table 2: Failure Modes and Effects Analysis (FMEA)

Factors	RPN
Chitosan	38
Speed	36
Pectin	12
Calcium Chloride	8
Acetic acid	6
Tween 20	4

2.5.2. Design of experiment with Minitab 21.1.0 software

The Plackett-Burman factorial design was used to screen three variables in order to identify significant factors influencing the characteristics of the beads. Each variable was represented at two levels of range covered by each variable and the responses (1+ and -1). Response surface methodology was used to further optimise the variables that had a significant effect on critical quality attributes of the granules based on the regression analysis of the variables. The value of probe F less than 0.05 implied that model term was significant[23,24] (Table 3 & 4).

Table 3: Levels of Plackett- Burman screening experiment

Variables	Levels	
	Low level	High level
PECTIN	4.5%	7.5%
CHITOSAN	0.2%	0.7%
SPEED	100Rpm	400Rpm

Table 4: Two level Plackett– Burman Factorial Design

Std Order	Run Order	Pt Type	Blocks	PECTIN	CHITOSAN	SPEED
1	1	1	1	7.5	0.2	400
7	2	1	1	4.5	0.7	400
11	3	1	1	4.5	0.7	100
5	4	1	1	7.5	0.7	100
2	5	1	1	7.5	0.7	100
12	6	1	1	4.5	0.2	100
3	7	1	1	4.5	0.7	400
10	8	1	1	7.5	0.2	100
6	9	1	1	7.5	0.7	400
4	10	1	1	7.5	0.2	400
8	11	1	1	4.5	0.2	400
9	12	1	1	4.5	0.2	100

2.5.3. Optimization using Response surface methodology

The central composite design was used to optimise the two factors i.e. Chitosan and Stirring speed which was found significant in screening with plackett- Burman design[25](Table 5 & 6).

Table 5: Levels of design experiment

Variables	Levels	
	Low (-1)	High (+1)
CHITOSAN	0.2%	0.7%
SPEED	100 rpm	400rpm

Table 6: Three levels Central composite design

Std Order	Run Order	Pt Type	Blocks	CHITOSAN	SPEED
12	1	0	1	0.450000	250.000
13	2	0	1	0.450000	250.000
11	3	0	1	0.450000	250.000
3	4	1	1	0.200000	400.000
4	5	1	1	0.700000	400.000
10	6	0	1	0.450000	250.000
8	7	-1	1	0.450000	462.132
6	8	-1	1	0.803553	250.000
2	9	1	1	0.700000	100.000
5	10	-1	1	0.200000	250.000
7	11	-1	1	0.450000	100.000
1	12	1	1	0.200000	100.000
9	13	0	1	0.450000	250.000

2.5.4. Response Optimizer analyser

The Response optimization design was used to optimize the two factors screened using Central composite design i.e. Chitosan and Speed[26] (Table 7).

Table 7: Levels of design experiment

Variables	Levels		
	High-(T)	Current-(T1)	Low-(T2)
Chitosan	0.8036 g	0.20 g	0.20 g
Speed	462.1320 rpm	250 rpm	100 rpm

2.5.5. Validation

Six batches of optimized formulation were prepared and evaluated for % Drug release and % Encapsulation efficiency (Table 8).

Table 8: Validation batch of Optimized formulation

Sr. No.	Content	Quantity
1	Pectin	4.5 g
2	Chitosan	0.2 g
3	Calcium chloride	7.5 g
4	Acetic acid	10 ml
5	Tween 20	0.25 l

2.6. Evaluation of Pectin Chitosan Beads[27]

- **In-vitro Drug Release**

% Drug Release: $y = mx + c$

Where, c = y -intercept (the point in which the line crosses the y -axis).

y = Absorbance, x = concentration, m= slope

- **% Encapsulation Efficiency**

$$\% EE = V1/V2 * 100$$

Where, V1- Concentration of oil encapsulated in beads

V2- Total oil taken

2.7. Scanning Electron Microscopy (SEM):

SEM was used to examine the surface morphology of oil beads loaded with pectin and chitosan. A set of lenses in the electron column then focus the beam on the sample surface. The beads were smooth, distinct, round, and had sharp edges without any surface degradation or fissures[28].

2.8. Gas Chromatography Mass Spectroscopy (GCMS):

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. Oil samples were analysed using GCMS at ANACON Labs Pvt. Ltd. Butibori, Nagpur[29,30].

3. RESULTS AND DISCUSSION

3.1. Phytochemical Evaluation

The different phytochemical components were evaluated for the collected oil such as alkaloids, steroids, terpenoids, flavonoids, saponins, tannins, phenolic compounds, cardiac glycoside, proteins, and carbohydrates (Table 9).

Table 9: Phytochemical Test Results

Test	Flaxseed oil
Test for Alkaloids	-
Test for Steroids	-
Test for Terpenoids	+
Test for Flavonoids	-
Test for Saponins	-
Test for Phenolic compounds	-
Test for Tannins	-
Test for Cardiac Glycosides	+
Test for Proteins	+
Test for Carbohydrates	+

3.2. Thin Layer Chromatography

The solvent system used for sample compound was Benzene: Chloroform. The sample compound shows RF value (Table 10) near to that of standard compound as shown in Figure 1.

Table 10: RF value of sample and Standards compound

Sr.no	Sample	Mobile phase	No. of spots	Rf value
1	Flaxseed oil	Benzene: Chloroform	1	0.7
2	Standard compound	Benzene :Chloroform	1	0.8

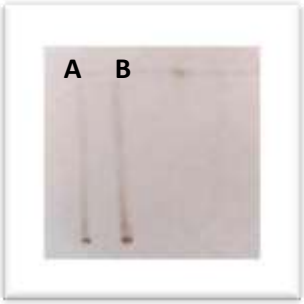

Figure 1: Comparative TLC of Sample compound and Standard compound

3.3. Test for Determination of Polyunsaturated Fatty Acid

To determine the level of linolenic acid present, a hexabromide test is performed. The test results show precipitates when fish oils, which are high in linolenic acid, are present. When examined in this manner, the vegetable oil fats result in white precipitate. Precipitate formed, which is a sign that unsaturated fatty acids are present (Figure 2).

Figure 2: Hexabromide Test Results

3.4. Drug Excipient Compatibility Studies

- **Standard Calibration Curve:**

Calibration curve is a regression model used to predict the unknown concentrations of analytes of interest based on the response of the instrument to the known standards. The figure 3, shows the standard calibration curve of fish oil. The linear relationship was evaluated by calculation of the regression line by the method of least squares.

- **Fourier Transform Infrared Spectroscopy:**

The chemical structure of the material as well as core-polymer interaction and degradation of beads were evaluated by FTIR (Figure 4-7) No substantial variation was observed between spectra of pectin and chitosan loaded beads. There was no shift of spectrum after the formulation of pectin loaded beads that revealed no strong chemical interaction of chitosan with pectin and oil was physically dispersed in the matrix of polymeric dispersion. The results thus obtained from these characteristic peaks indicated that oil was encapsulated by pectin and chitosan.

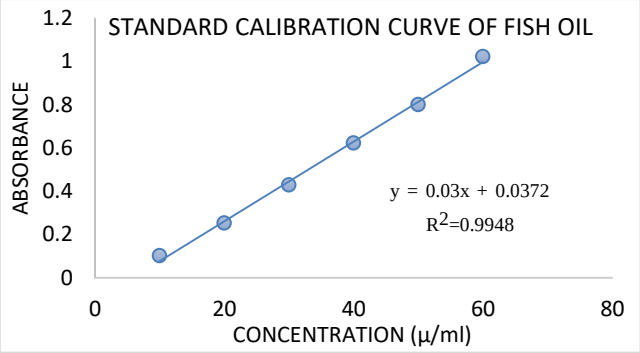

Figure 3: Calibration Curve of Fish oil

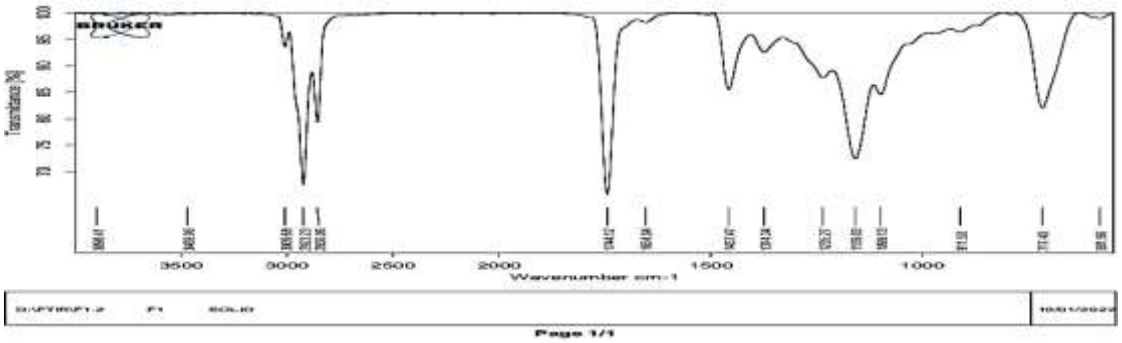

Figure 4: FTIR Spectra of Flaxseed Oil

The FT-IR spectra of flaxseed seed oil shows the characteristic **C-H stretch (~2923.23 cm-1), C=O stretch (~1744.12 cm–1), and C-O stretch (~1159.03 cm–1)** of triglyceride component which confirms the presence of polyunsaturated fatty acid in oil sample (Figure 4).

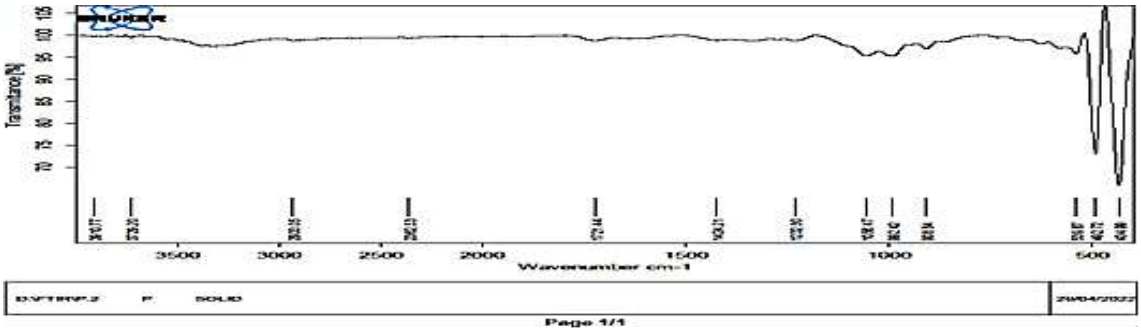

Figure 5: FTIR Spectra of Pectin

The spectrum of pectin indicated peak at **3729.20 cm^{-1}** due to stretching of **O-H** group, the peak at **2933.05 cm^{-1}** indicated **C-H** stretching vibration. The peak at **1424.21 cm^{-1} and 1232.30 cm^{-1}** could be assigned to CH_2 and OH bending vibrations (Figure 5).

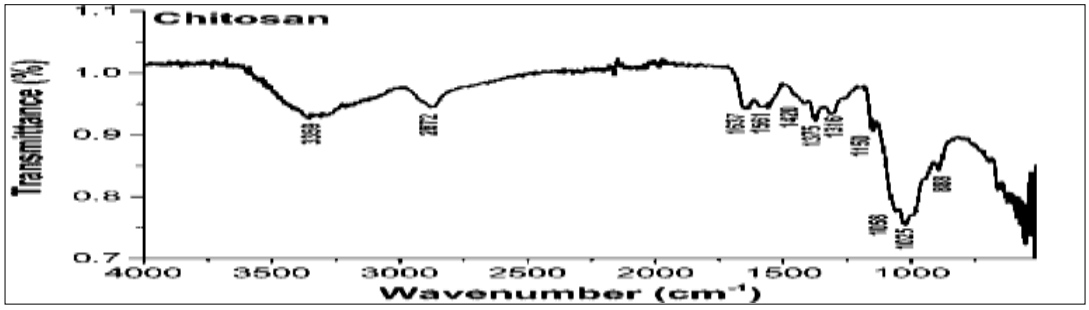

Figure 6: FTIR Spectra of Chitosan

FTIR spectra of chitosan shows the main corresponding peaks of chitosan at **3359 cm-1 (– OH stretch), 2872 cm-1 (C–H stretch), 1637 cm-1 and 1561 cm-1 (N–H bend), 1375 cm-1 (bridge O stretch) and 1025 cm-1 (C–O stretch)** (Figure 6).

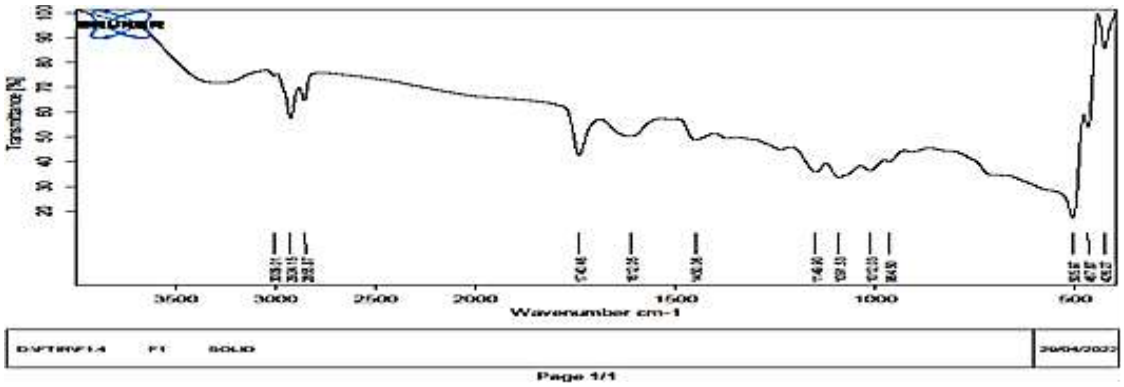

Figure 7: FTIR Spectra of Flaxseed oil beads

The FT-IR spectra of flaxseed seed oil beads shows the characteristic **C-H stretch (~2924.15 cm–1), C=O stretch (~1740.46 cm–1), and C-O stretch (~1149.90 cm–1)** of triglyceride component which confirms the presence of polyunsaturated fatty acid in oil sample (Figure 7).

3.5. Screening, Optimisation and Validation of Flaxseed Oil Containing Pectin Chitosan Beads

3.5.1. Screening

Screening of three factors (CMA's and CQA's) at two distinct levels was demonstrated for a flaxseed oil batch using Minitab 21.1.0 software and the Plackett-Burman Design of Experiment (DOE). Full factorial design, for example. Pectin, chitosan, and speed were the variables examined. Plackett-Burman design screenings of these variables were carried out using Minitab 21.1.0 software (Table 11). Total 12 batches of the Plackett-Burman design were given following the design screening. These 12 batches underwent formulation and, separately, encapsulation efficiency and in-vitro drug release evaluations.

Table 11: Evaluation of beads using Plackett-Burman Design

Std Order	Run Order	% Drug release	% Encapsulation Efficiency
1	1	47.98	46.60
7	2	69.22	67.37
11	3	81.47	80.51
5	4	38.29	37.89
2	5	37.10	38.82
12	6	80.01	79.93
3	7	78.90	77.81
10	8	63.17	63.26
6	9	46.85	44.33
4	10	46.66	47.09
8	11	79.01	78.24
9	12	81.11	81.07

Table 12: Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	3	3530.74	1176.91	1319.78	0.000
Covariates	1	3340.48	3340.48	3745.97	0.000
%EE	1	3340.48	3340.48	3745.97	0.000
Linear	2	6.38	3.19	3.58	0.078
CHITOSAN	1	1.40	1.40	1.57	0.245
SPEED	1	5.10	5.10	5.72	0.044
Error	8	7.13	0.89		
Total	11	3537.88			

The best outcomes after applying the Plackett-Burman design were F3, F6, F11, and F12. Chitosan and speed were found to be significant VS% drug release features when in-vitro drug release and encapsulation efficiency were tested. Since chitosan and speed both had P-values around 0.25, these 2 factors were analysed and found to be significant.

3.5.2. Optimization

- **Evaluation of Beads Using Response Surface Methodology**

Minitab 21.1.0 software, the Response surface method (RSM) was used to optimise these parameters. RSM distributed 13 batches following a design screening. These 13 batches were created and tested for the % DR and % EE characteristics, respectively. After these Response Surface Methodology (RSM) parameters were tested, as indicated respectively in table 13, no factor demonstrated any interaction with other factors.

Table 13: Evaluation of beads using Response surface methodology

Std Order	Run Order	% Drug release	% Encapsulation Efficiency
7	1	76.32	70.09
8	2	78.23	79.22
13	3	55.96	62.17
3	4	89.76	87.90
4	5	45.90	43.19
5	6	82.38	78.88
9	7	57.03	63.37
10	8	69.42	64.77
2	9	33.34	37.49
1	10	81.55	79.00
11	11	56.89	58.03
6	12	31.99	35.29
12	13	67.59	68.54

- **Response Surface Regression: % Drug Release versus %Encapsulation Efficiency, Chitosan, Speed** (Table 14 &15)

Table 14: Coded Coefficients (α = 0.25)

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	61.29	2.54	24.10	0.000	
CHITOSAN	-21.15	2.78	-7.61	0.000	1.00
SPEED	2.13	2.64	0.81	0.441	1.12
SPEED*SPEED	6.22	3.03	2.05	0.070	1.13

Table 15: Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	3	195006	65002	22.15	0.000
Linear	2	170798	85399	29.11	0.000
CHITOSAN	1	169831	169831	57.88	0.000
SPEED	1	1907	1907	0.65	0.441
Square	1	12383	12383	4.22	0.070
SPEED*SPEED	1	12383	12383	4.22	0.070
Error	9	26407	2934		
Lack-of-Fit	5	15398	3080	1.12	0.470
Pure Error	4	11008	2752		
Total	12	221413			

- **Regression Equation in Uncoded Units**

% DR = 113.1 – 84.6 Chitosan - 0.1240 Speed + 0.000276 Speed*Speed

- **Response Surface Regression: %Encapsulation Efficiency versus % Drug Release, Chitosan, Speed** (Table 16)

Table 16: Analysis of Variance (α = 0.25)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	4	159265	39816	34.94	0.000
Linear	2	140576	70288	61.69	0.000
CHITOSAN	1	138653	138653	121.69	0.000
SPEED	1	3680	3680	3.23	0.110
Square	2	12338	6169	5.41	0.033
CHITOSAN*CHITOSAN	1	5274	5274	4.63	0.064
SPEED*SPEED	1	7840	7840	6.88	0.031
Error	8	9115	1139		
Lack-of-Fit	4	5488	1372	1.51	0.349
Pure Error	4	3627	907		
Total	12	168380			

- **Regression Equation in Uncoded Units**

% EE = 93.32 – 13.2 Chitosan - 0.0909 Speed – 71.0 Chitosan*Chitosan + 0.000221 Speed*Speed

3.5.3. Factorial Plots

- **Factorial Plots For % Drug Release**

To illustrate the link between the response and the variables, factorial plots were created. For example, the responses vs. %DR variables were speed and chitosan, as shown in figure 8. However the outcome shows that the major effect was not statistically significant.

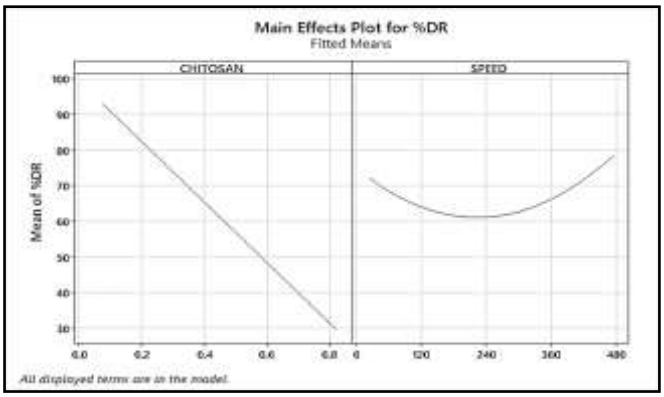

Figure 8: The response % DR vs. variables speed and chitosan

The variation in the components, level, and slope of the line may result from random change. Chitosan seems to be more linked to influencing the % DR in the main effect plot. But according to the results of the general linear model, the main effect was not statistically significant.

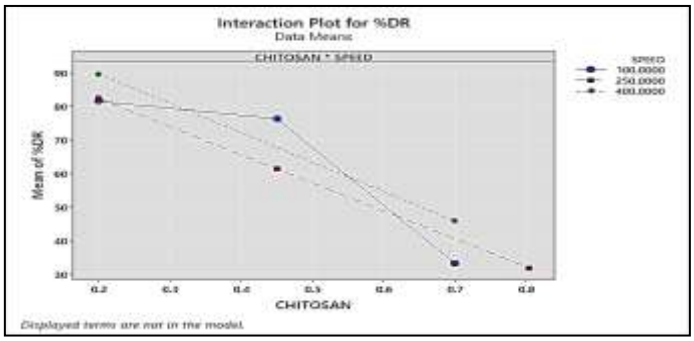

Figure 9: Interaction plot of % DR Vs chitosan

Examined the two-way interaction effect in the interaction plot. This graph demonstrates how the value of a second predictor affects the connection between a predictor and the response variable. The lines in this interaction plot were not straight. As demonstrated in figure 9, this interaction effect demonstrates that the relationship between chitosan and speed has no impact on the value of% DR. According to the interaction plot, chitosan does not interact with speed influence on %DR, however all other plots exhibited interaction because the lines were not parallel.

3.5.4. Counter Plots

Contour plots were used to plot the relationship between a fitted response and two continuous variables. A contour plot displays a two-dimensional view in which points that have the same response value were connected to produce contour lines. (Figure 10)

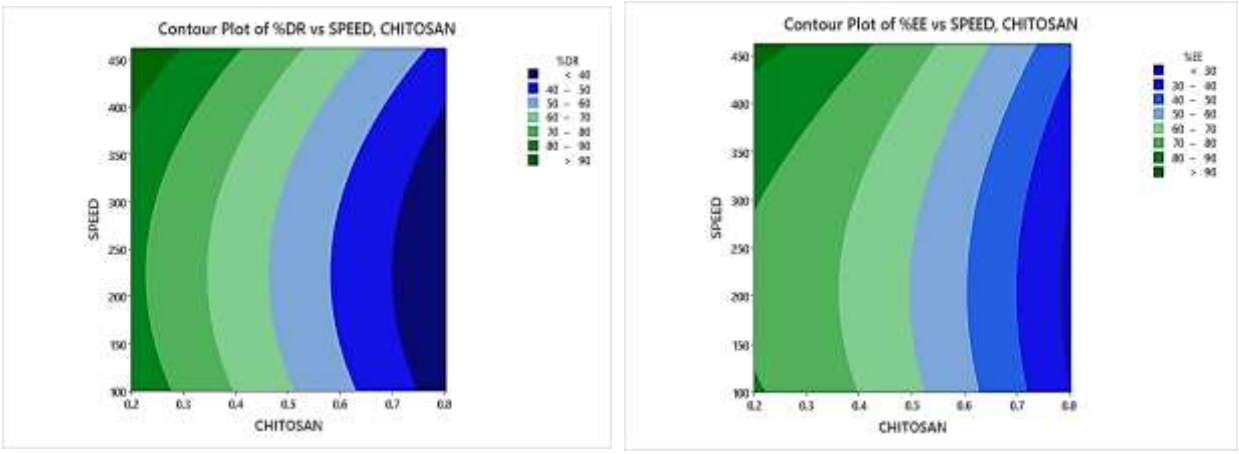

Figure 10: Counter Plots For %Drug Release Vs Speed, Chitosan and Counter Plots For %Encapsulation efficiency Vs Speed, Chitosan

3.5.5. Surface Plots

Surface plots were used to plot the relationship between a fitted response and two continuous variables. A surface plot displays the three-dimensional relationship in two dimensions, with the variables on the x-axis and y-axis and the response variable represented by a smooth surface. (Figure 11)

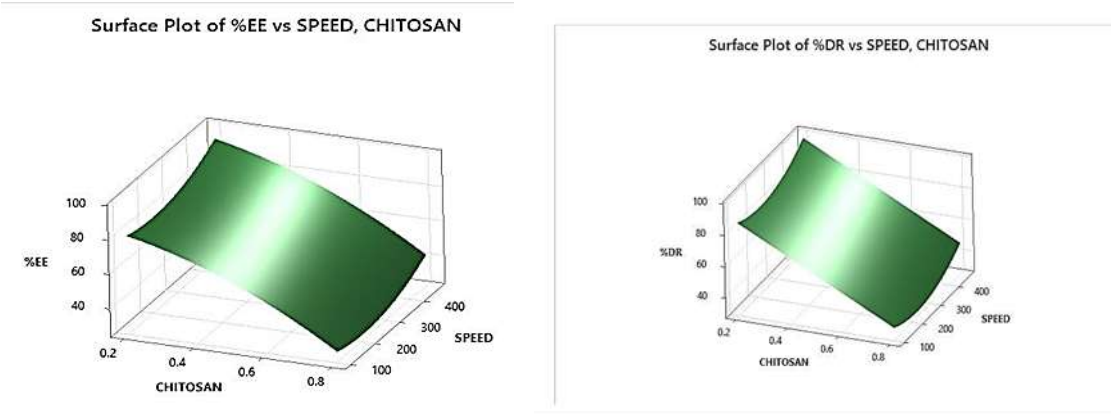

Figure 11: Surface Plots of %Drug Release Vs Speed, Chitosan and Surface Plot of % Encapsulation Efficiency Vs Speed, Chitosan

3.5.6. Response optimizer analyser

Table 17: Response Optimization: %Drug Release, %Encapsulation Efficiency

Response	Goal	Lower	Target	Upper	Weight	Importance
%DR	Target	90	95	100	1	1
%EE	Target	80	90	95	1	1

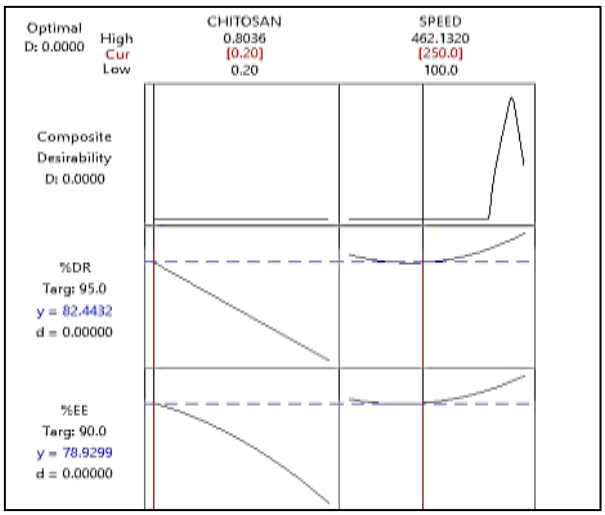

Figure 11: Response optimizer analyzer

If factorial plots and interaction plots graph shows any significant effect and have at least one stored model and want to find values that optimize one or more responses i.e. %DR and %EE use response optimizer for optimization of these responses as shown in figure11. The % DR target was set to 95% and % EE target was set to 90%. The Response optimizer had screened two factors at three different levels and gave the optimised values of Chitosan 0.2g and speed 250 rpm as shown in table 17. These batches were formulated and evaluate.

3.5.7. Formulation and Evaluation of Optimized Batch

After applying RSM to final batches it showed satisfactory results and the results matched Quality Target Product Profile (QTPP) with reference to In Process Critical Quality Attribute (IP CQAs) and Drug Product Critical Quality Attribute (FP CQA) so it was considered to optimize the formula in formulation optimization of pectin chitosan beads.

3.5.8. Validation of Optimized Batch (Formulation and Process)

Six batches of optimized formula were prepared and validated for % Drug Release and % Encapsulation Efficiency.

3.6. Scanning Electron Microscopy

Figure 12: Scanning Electron Microscopy of Flaxseed Oil Bead

SEM image of formed beads indicate complete encapsulation of oil as there are no oil globules on the surface of beads, indicating a positive result with smooth morphological surface, spherical shape at various magnifications under SEM with various diameters as shown in figure 12.

3.7. Gas Chromatography Mass Spectroscopy (GCMS):

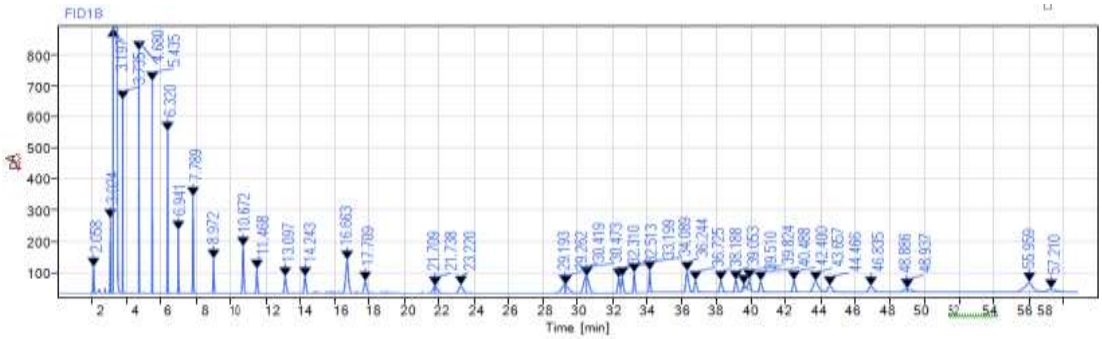

Figure 13: GCMS Standard graph of Fish oil

Table 18: Polyunsaturated Fatty acids present in fish oil

Signal RT [min]	Type	Width [min]	Area	Height	Area%	Name
32.310	BV	0.33	376.03	47.00	0.08	Linoleic acid
34.089	BV	0.41	391.31	68.89	0.09	α-Linolenic acid
36.244	BV	0.58	756.25	66.06	0.17	cis-11-Eicosenoic acid
39.510	BV	0.20	106.57	17.38	0.02	Arachidonic acid

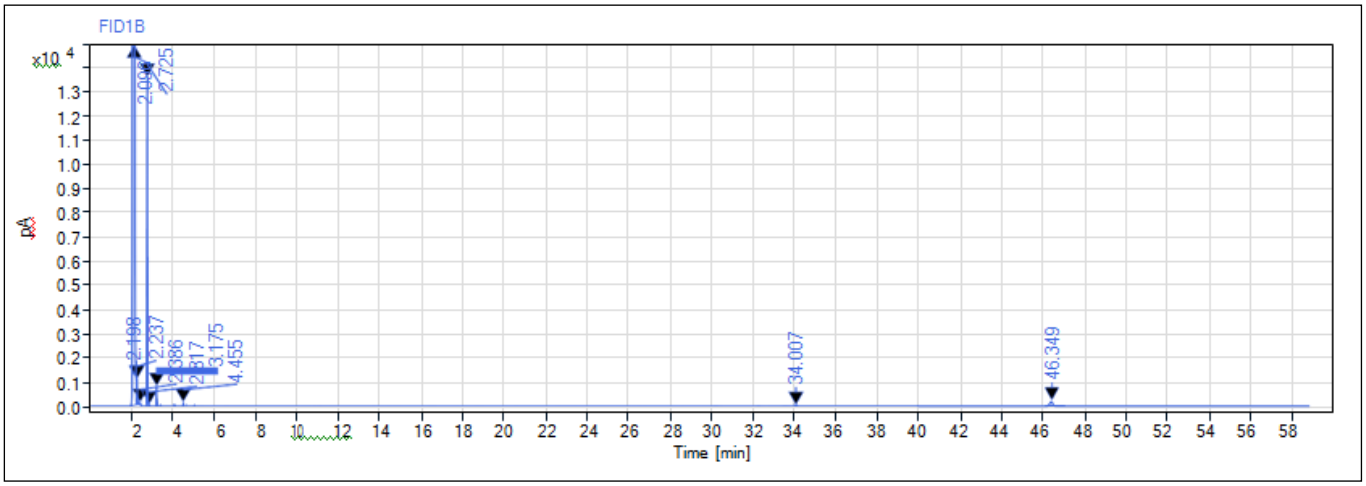

Figure 14: GCMS of flaxseed oil

Table 19: ω-3 and ω-6 fatty acids in flaxseed oil

Signal: RT [min]	Type	Width [min]	Area	Height	Area%	Name
34.007	BB	0.32	209.26	34.68	0.01	**α-Linolenic acid** (ω-3)
46.349	BV	0.93	2134.74	197.92	0.01	**α-Linoleic acid**(ω-6)

The extracted oils were evaluated for ω-3 and ω-6 fatty acids using GCMS keeping fish oil as standard. The percent compositions of omega fatty acid in extracted flaxseed oil were compared with standard, which was found to be approximate to the values of omega fatty acid composition of fish oil given in table 18. The ω-3 and ω-6 fatty acids from flaxseed can be the best alternative as nutraceutical to fish oil as shown in table 19 (Figure 13 & 14).

4. CONCLUSION

Nutraceutical flaxseed oil contains omega fatty acids but is prone to oxidation. The Present study was focused to formulate pectin-chitosan beads to entrap the flaxseed oil. In this study, flaxseed oil was extracted using cold maceration method. Extracted oil was evaluated for phytochemical evaluation. Pectin chitosan beads were prepared using Ionic gelation method. Minitab 21.1.0 was used to screen and optimize the process and formulation parameters. Plackett Burman's design was used for the initial screening. Twelve batches were made ready for screening, and each batch was evaluated for optimization based on the percentage of drug release and the percentage of drug encapsulation efficiency. The optimized batches F4, F6 and F10 were shown the satisfactory results complying with IP specifications. The SEM image of the produced beads shows that the oil has been completely encapsulated. The % compositions of omega fatty acids in extracted flaxseed oil were estimated using the GCMS Quantitative analysis. The ideal substitute for fish oil as a nutraceutical could be the omega-3 and omega-6 fatty acids found in flaxseed oil.

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