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RESEARCH ARTICLE

Development and Characterisation of Curcumin and Piperine Dual Drug-loaded Sodium Alginate Nanoparticles by Desolvation Technique

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Abstract

Background: The purpose of this research is to extract curcumin from *Curcuma longa* and Piperine from Black pepper and perform the simultaneous estimation of the mixture by the double beam Ultraviolet (UV) spectroscopic method. The aim is to develop a dual drug-loaded nanoparticulate system to enhance bioavailability and sustained release. **Aim:** To prepare and characterise the Curcumin and Piperine dual drug-loaded sodium alginate nanoparticle by desolvation technique and optimise the formulation using a 3^2 factorial design. **Method:** Curcumin and Piperine were extracted using conventional methods and quantified spectrophotometrically. Nanoparticles were prepared by desolvation using sodium alginate as a polymer. A 3^2 factorial design was applied with consideration of the concentration of polymer (Factor A) and Stirring speed (Factor B) as two independent variables, and three-level responses with particle size, *in vitro* drug release, and % drug entrapment efficiency were considered as dependent variables. The prepared Curcumin and Piperine nanoparticles were characterised by particle size, zeta potential, Polydispersity Index (PDI), compatibility studies, surface morphology and *in vitro* drug release studies. **Results:** The optimised nanoparticle morphology exhibited an irregular, smooth surface and non-porous particle size ranging from 168.5nm to 194nm. Zeta potential values were negative, indicating good stability due to electrostatic repulsion. FTIR peaks proved there were no drug-excipient interactions. The entrapment efficiency was found to be between 70% to 81%. The drug release using the *in vitro* method showed sustained drug release. **Conclusion:** Curcumin and Piperine-loaded sodium alginate nanoparticles were successfully formulated and optimised using a factorial design approach. The system exhibited favourable physicochemical properties and a sustained drug release pattern, suggesting potential for enhanced therapeutic efficacy in topical applications.

Major Finding: The formulated nanoparticles exhibited high drug entrapment efficiency, sustained drug release, and good stability as confirmed by zeta potential analysis. The dual-loaded system has potential for enhanced therapeutic delivery.

Keywords: Curcumin, Desolvation Method, 3^2 Factorial Design, Nanoparticle, Piperine

1. Introduction

Curcumin, a natural medication previously separated from the rhizomes of *Curcuma longa* Linn[1]. Curcumin is widely examined and has assorted pharmacological properties[2]. Clinical and pre-clinical trials have shown curcumin is incredibly protected even at extremely high sectors[3]. US Food and Medication Organisation likewise proclaimed curcumin as "for the most part viewed as protected". Curcumin, present in turmeric, is an aquaphobic polyphenol, which is considered the most remarkable anti-inflammatory constituent. It assists in

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fixing and cleaning. This property might help in the re-pigmentation of skin because of its intense cancer prevention agent and mitigating properties. Turmeric can demonstrate advantages in the management of persistent skin issues of vitiligo[4,5]. Curcumin in turmeric is a strong, regular immunomodulator. Piperine is separated and segregated from Dark pepper by two unique strategies[6,7]. They are soxhlation and reflux strategy. Phenolic amides from Piperine likewise display an unrivalled cell protection limit compared to manufactured compounds. It can animate melanocyte expansion and works with the re-pigmentation of de-pigmented skin[8]. The important points in framing a network in the form of nanoparticulate technology are to accomplish the size of particle and topology, and to deliver pharmacologically active speciality to accomplish the site-targeted impact of the compound at the remedially ideal time with a small frequency of dose[9]. The development of medication-loaded nanoparticles is an exceptionally promising methodology. Molecule size decreases to the nanometre range can be accomplished utilising the desolvation technique[10].

2. Materials and Methods

2.1 Materials

Curcumin and Piperine were extracted and isolated. Sodium alginate as a polymer, ethanol as a desolvating agent and Glutaraldehyde as a cross-linking agent were purchased from SD Fine Chemicals Limited, Mumbai. All other chemicals were of analytical grade.

2.2 Multicomponent Analysis of Curcumin and Piperine

2.2.1 Preparation of Standard Stock Solution of Curcumin and Piperine

The stock solutions of 100mcg/ml of Curcumin and Piperine were prepared by adding 10mg of both APIs in 100 ml of ethanol separately. Further aliquots were made with Ethanol[11].

2.2.2 Determination of λmax

Solutions of Curcumin (10 µg/mL) and Piperine (10 µg/mL) prepared in ethanol were subjected to an overlay scan in a UV spectrophotometer from 200-800nm. The λmax for Curcumin and Piperine were confirmed at 421 and 342nm, respectively.

2.2.3 Calibration Curve of Curcumin and Piperine

According to Beer's range, the aliquots of Curcumin were prepared, and the absorbance was noted against a blank at 421nm. Similarly, Piperine was subjected to the same procedure, and absorbance was measured at 342nm.

2.2.4 Iso-Iso-Absorptive Point Determination

The standard stock solutions of both drugs concerning their dilution in ethanol were prepared, which furnished 10 µg/ml each of Curcumin and Piperine. It was scanned using the UV spectrophotometer between 200 to 400nm[12].

2.2.5 Multi-component Analysis

The spectra of Curcumin (10 µg/ml), Piperine (10 µg/ml) and the mixture of Curcumin-Piperine solution (10 µg/ml of each), absorption maxima of both, viz., 421nm as λmax of Curcumin and 324nm as λ max of Piperine, were considered as the wavelength for the simultaneous analysis. The absorptivity was also determined at 341nm and 421nm. Values were subjected to the equation noted below:

$$C1 = \frac{B2bn1 - B1bn2}{Bm2bn1 - bm1bn1} \quad \text{.....Eq.-1}$$

$$C2 = \frac{B1bm2 - B2bm1}{Bm2bn1 - bm1bn1} \quad \text{.....Eq.-2}$$

Where C1 and C2 are concentrations of Curcumin and Piperine in µg/mL, respectively, in the known sample solution. B1 and B2 are absorbances of sample solutions at 421nm and 324nm, respectively, bm1 and am2 are the absorptivity of Curcumin at 421nm and 324 nm, and bn1 and bn2 are the absorptivity of Piperine at 342nm and 421nm.

2.3 Fabrication of Nanoparticles by Various Methods

Nanoparticles of Curcumin and Piperine were prepared by using four different methods. As mentioned, the effect of manufacturing parameters was studied, and a suitable method for the preparation of nanoparticles was identified.

- Thin film hydration technique[13]
- Solvent evaporation technique[14]
- Desolvation method[15]
- Inotropic gelation method[16]

2.3.1 Desolvation Method of Developing Nanoparticles

Curcumin and Piperine-embedded polymeric nanoparticles were fabricated by adapting the Desolvation method. A known quantity of curcumin and Piperine was loaded in polymers like Chitosan, Bovine Serum Albumin (BSA) and sodium alginate labelled as P1, P2, and P3, respectively. A 1% polymer solution was formulated, and the pH was adjusted to 7. Curcumin and Piperine were mixed with 20ml of desolvating agent (ethanol) and subjected to sonication for 20 minutes. The desolvating agent was added dropwise into the 40ml polymeric solution continuously while mechanically stirring at 800rpm. The appearance of turbidity was noted as an endpoint. 0.3ml of 25% glutaraldehyde was added as a cross-linker and then subjected to stirring for up to 12 hours. The solvent and water were evacuated entirely using a rotary evaporator. Then, resultant free-flowing nanoparticles were collected and characterised.

2.4 Screening of Polymers

Nanoparticles of Curcumin and Piperine were prepared by using three different polymers, like Chitosan, Bovine Serum albumin and Sodium alginate with the desolvation method[17-19]. The nanoparticles were evaluated based on particle size and drug entrapment efficiency.

2.5 Characterisation of Cur – Pip Loaded Nanoparticles by Desolvation Method

2.5.1 Compatibility Analysis

The compatibility between polymer and drug was analysed using the Fourier Transform Infrared Spectroscopy (FT-IR) spectroscopic technique (Bruker alpha) and the KBr pelletisation method[20,21].

2.5.2 Determination of Particle Size, Polydispersity Index and Zeta Potential

The average size and distribution of Cur- Pip embedded sodium alginate NPs were measured by Dynamic Light Scattering (DLS) using a Malvern Zetasizer (ver. 7.13 model number: MAL1200116)[22,23].

2.5.3 % Entrapment Efficiency of Drug

20 mg equivalent weight of the prepared nanoparticles was solubilised in 20ml of methanol and kept for stirring at 600rpm for 3 hours. The total content of the drug in the nanoparticles was examined using a UV spectrophotometer at 366nm[24].

2.5.4 In Vitro Release of Drug

The nanoparticles were subjected to drug release studies using a constant equilibrium method for 12 hrs. The formulation was dissolved in the 7.4 pH buffer. Once it was poured into a beaker containing buffer solution, it was kept stirred continuously at 100rpm, and the required samples were removed at specific intervals. The samples collected were analysed at 366nm by UV-visible spectrophotometry[25].

2.5.5 Drug Release Kinetics

The optimised nanoparticle can be reported by studying the drug release kinetic models (zero order, first order, Higuchi's, Korsmeyer-Peppas and Hixon-Crowell). The best-fitted kinetic model was selected as per the value of the regression coefficient[26].

2.6 Optimisation of Formulation, Data Analysis and Desirability Function Using 3^2 Factorial Design

A 3^2 factorial design for two factors at three levels was upgraded to optimise the response of variables. Two factors, concentration of polymer (X1), stirring speed

Table 1. Formulation with an independent variable of a 3^2 factorial design

Formulation Code	Run	Concentration of polymer	Stirring Speed
FCP1	12	0.2	400
FCP2	9	1	400
FCP3	13	0.2	800
FCP4	3	1	800
FCP5	2	0.2	600
FCP6	4	1	600
FCP7	8	0.6	400
FCP8	6	0.6	800
FCP9	7	0.6	600
FCP10	1	0.6	600
FCP11	10	0.6	600
FCP12	11	0.6	600
FCP13	5	0.6	600

(X2), particle size, % drug entrapment efficiency and drug release, were taken as response variables. In this design, two factors and three responses show thirteen possible combinations in that 9-formulation processing variable and four combinations given the same factors are shown in Table 1.

2.6.1 Optimisation of Formulation, Data Analysis and Desirability Function Using 3^2 Factorial Designs

Various response surface methodologies and computations were performed by software named Design-Expert 13 (Version 13.0.5.0 Stat-Ease). Linear, quadratic and polynomial models were generated for all responses. 3D and Contour plots were generated by the Design expert software[27].

2.7 Scanning Electron Microscopy

The morphology and size of optimised Cur-Pip-loaded sodium alginate nanoparticles were characterised by Scanning Electron Microscopy (SEM), SAIF, Cochin University, Kochi, Kerala)[28].

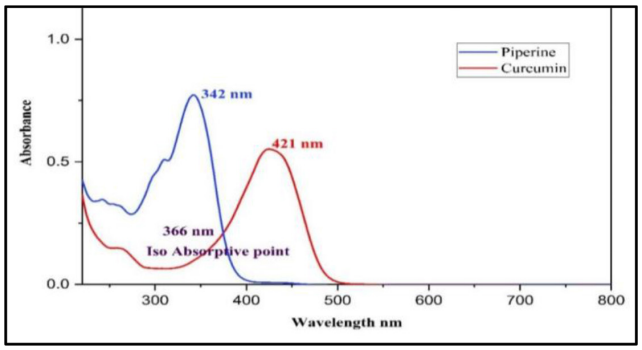


Figure 1. UV overlay spectra of Curcumin and Piperine.

3. Result and Discussion

3.1 Iso-Absorptive Point Determination

The maximum absorption of Curcumin was at λmax 421nm, while the maximum absorption of Piperine was at λmax 342nm and from overlain spectra, proved that the Iso-absorptive point is 366nm, as shown in Figure 1. Standard calibration curves for curcumin and Piperine were linear with correlation coefficient values of 0.9988 and 0.9991. Standard calibration curves for Curcumin and Piperine were found to have linear correlation coefficient values of 0.9992 and 0.9984, respectively, at the iso-absorptive point, as shown in Figure 2.

3.2 Fabrication of NP by Various Methods

Among four methods, it was concluded that the desolvation method produces the minimum average particle size with excellent polydispersity index and optimal zeta potential as well as good entrapment efficiency, which falls within the acceptable limits of the study.

3.3 Screening of Polymer

The formulation P3 shows the best result compared to the other polymers, based on the results with sodium alginate-loaded nanoparticles, particle size 187.4nm with zeta potential -52.4mV and drug entrapment efficiency 79%. Hence, a considerable and ideal polymer for preparing curcumin and piperine dual drug-loaded nanoparticle is Sodium alginate, as shown in Table 2.

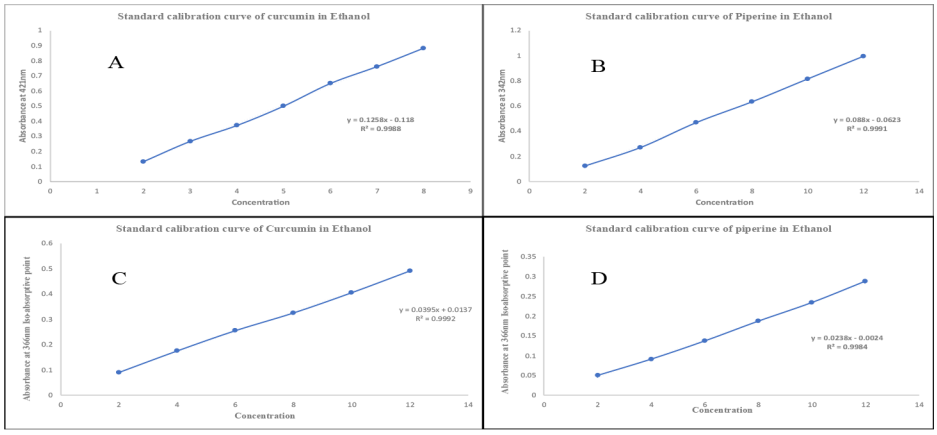


Figure 2. Standard calibration curve of **(A)** Curcumin in ethanol at wavelength 421nm, standard calibration curve of **(B)** Piperine in ethanol at wavelength 342nm, **(C)** Curcumin in ethanol at iso-absorptive point 366nm, **(D)** Piperine in ethanol at iso-absorptive point 366nm.

3.4 Physicochemical Characteristics of Cur-Pip Nanoparticles

3.4.1 Compatibility Studies

After spectra analysis, which were shown in Figure 3 and Tables 3A and 3B, showed no significant changes in characteristic peaks of extracted Curcumin and Piperine and Curcumin and Piperine loaded nanoparticles by the desolvation method.

3.4.2 Determination of Particle size, Polydispersity Index, Zeta potential, % DEE and Drug Release

The particle size of the formulation (FCP1 to FCP9) was found to be 194 to 168.5nm. The polydispersity index ranges from 0 to 1. Value 0 indicates homogeneous dispersion, and above 0.3 indicates heterogeneity. The polydispersity index was found to be 0.516 to 0.930. zeta potential -40.6 to -57.9, as shown in Table 4 and Figures 4A, 4B and 4C.

The % Drug Entrapment Efficiency (DEE) of all (FCP1-FCP13) formulations ranges from 70 to 81%, as shown in Table 4. The drug entrapment was found to be a maximum of 81% in (FCP4) with a concentration of 1% polymer (Sodium alginate) and 800 rpm rotation speed, which was found to increase the speed of rotation and polymer concentration.

The % Cumulative Drug Release (CDR) of all formulations (FCP1 – FCP13), as shown in Table 4 and Figure 5. The drug-polymer and speed of rotation composite influence the *in vitro* drug release rate

Table 2. Screening of various polymers

Formulation Code	Polymers	Size of the particle (nm)	PdI	Zeta Potential	Entrapment Efficiency (%)
P1	Chitosan	746.4	0.759	-53.2	68
P2	Bovine serum albumin	662.4	0.585	-51.5	71
P3	Sodium alginate	187.4	0.930	-52.4	79

Table 3A. Major peaks of extracted Curcumin and Curcumin and Piperine loaded nanoparticle in the IR spectra

Functional Groups	Phenolic OH	C=O Ketonic	C=C aliphatic	CO enol	C-O phenol
Extract Curcumin	3507 cm^{-1}	1627 cm^{-1}	1506 cm^{-1}	1428 cm^{-1}	1278 cm^{-1}
CUR-PIP Nanoparticle(F1)	3566 cm^{-1}	1651.61cm^{-1}	1507.54 cm^{-1}	1418 cm^{-1}	1245 cm^{-1}

Table 3B. Major peaks of extracted Piperine, Curcumin, and Piperine-loaded nanoparticle in IR spectra

Functional Groups	Alkenes	CH_2	Amines	C-C-O	Ketonic	C-N Stretching
Extract Piperine	3008 cm^{-1}	2851 cm^{-1}	1632 cm^{-1}	1249 cm^{-1}	1580 cm^{-1}	1029 cm^{-1}
CUR-PIP Nanoparticle (F2)	2924.54 cm^{-1}	2853.97cm^{-1}	1651.61cm^{-1}	1211.98cm^{-1}	1588.47cm^{-1}	1045.26 cm^{-1}

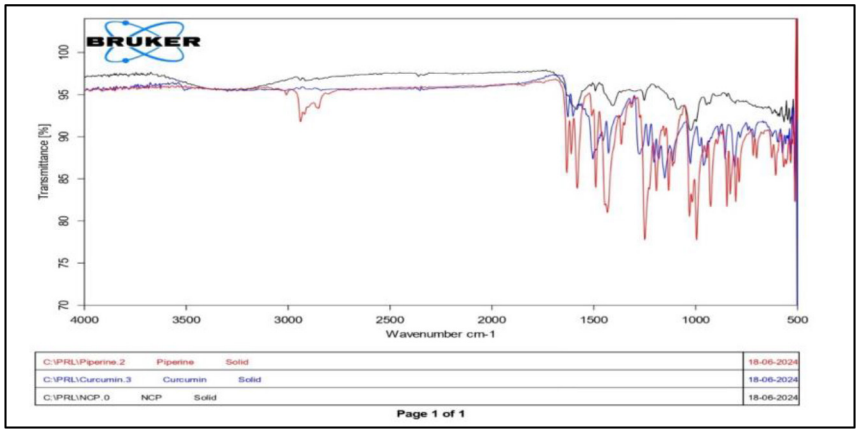


Figure 3. Fourier transform infrared spectroscopy of Curcumin and Piperine nanoparticles.

Table 4. Summary of size, dispersity, charge analysis, drug entrapment efficiency and drug release

Formulation Code	Size of particle in nm	Polydisperse Index	Zeta Potential (mV)	DEE (%)	Drug release % at the end of 720 min
FCP1	191.2	0.806	-40.6	70	77.52±5.68
FCP2	189	0.516	-48.9	78	81.29±0.55
FCP3	187.4	0.930	-52.4	75	79.43±1.23
FCP4	168.5	0.692	-57.9	81	85.52±0.56
FCP5	175	0.723	-49.8	72	78.36±0.88
FCP6	175	0.722	-50.7	80	77.42±1.39
FCP7	194	0.584	-52.0	73	83.01±0.17
FCP8	183.7	0.621	-53.6	79	80.17±1.73
FCP9	171.5	0.725	-48.3	77	80.54±0.31
FCP10	175.8	0.612	-49.4	77	80.17±1.54
FCP11	175.5	0.630	-54.0	76	80.19±1.87
FCP12	176	0.625	-52.0	77	80.17±1.055
FCP13	175.5	0.617	-53.4	77	80.17±1.69

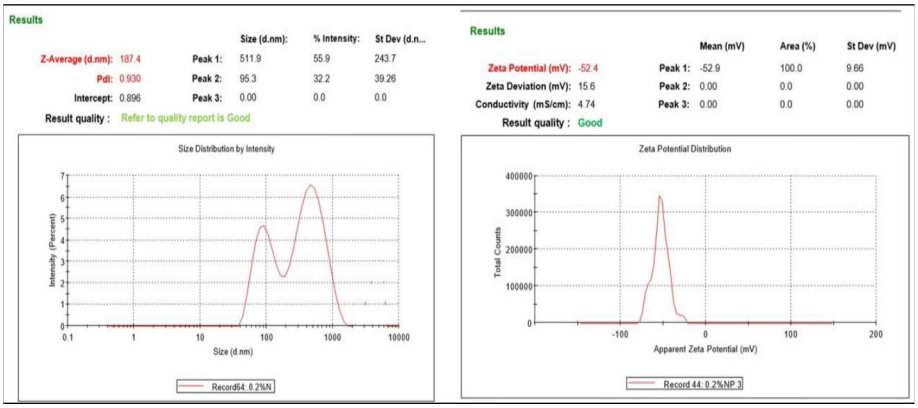


Figure 4A. Particle size, polydispersity index and zeta potential FCP3.

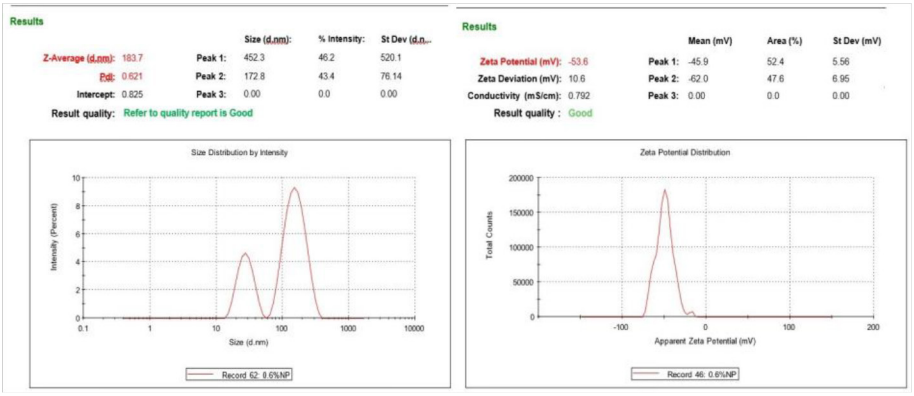


Figure 4B. Particle size, polydispersity index and zeta potential of FCP8.

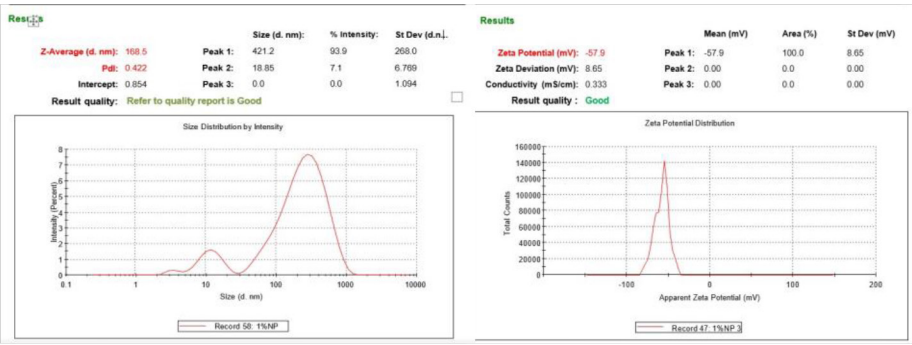


Figure 4C. Particle size, polydispersity index and zeta potential of FCP4.

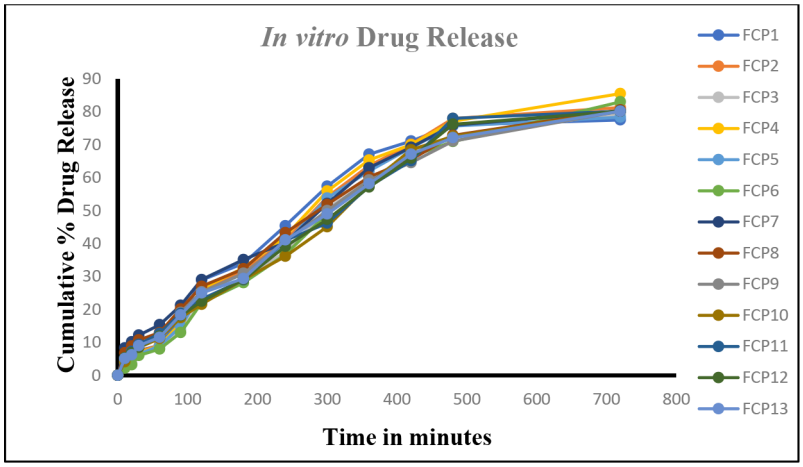


Figure 5. Kinetic release of prepared NPs by the desolvation method (FCP4).

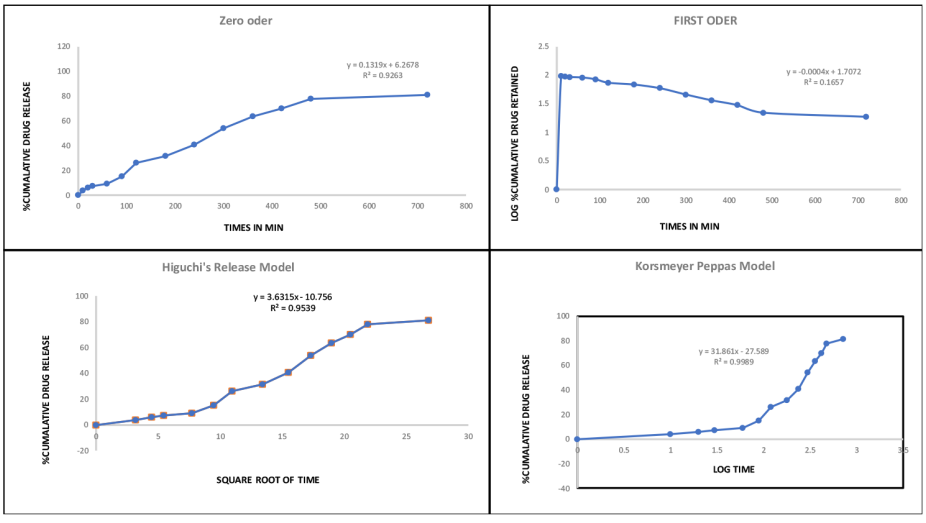


Figure 6. Kinetic release of prepared NPs by the desolvation method (FCP4).

of nanoparticles. The smaller the particle size of nanoparticles of 1% concentration and 800rpm speed of rotation exhibits the higher drug release (85.52%) in (FCP4).

3.4.3 Kinetics Pattern of Drug Release

The regression coefficient of formulation (FCP4) is shown in Figure 6. Higuchi model and Korsmeyer's model were the best-fit models for the selected batch of

nanoparticles, and plots show the linearity (R^2 0.9539 and 0.9989).

3.5 Optimisation of Nanoparticle Formulation Using 3^2 Factorial Design

3.5.1 Effect of Factor on Particle Size (Y_1)

Two significant variables were selected using the 3^2factorial design, including the variations of polymer concentration (A) and stirring speed (B), used to get smaller particle sizes and increase the loading efficiency and drug release using the factorial design. ANOVA results were analysed by Design of Experiments (DOE). One quadratic and two linear models were found when particle size, DEE and drug release were regarded as the responses, respectively. The results fitting the polynomial equation to the data, when particle size is the response, are shown in Table 5. The model is highly statistically significant ($P< 0.05$) with insignificant lack of fit ($F= 0.0736$; $P>0.05$); hence, the p-value is more than 0.05 and was considered to be not significant. It is concluded that the quadratic model is fitted to the data appropriately, as shown in the equation.

Polynomial Equation $Y_1= 175.54-3.52A-5.77B-4.17AB-2.26A^2+11.59B^2$ ……. Eq.-3

Where Y_1 is particle size and A and B are the ratios of polymer and stirring speed, respectively.

In order to analyse the interaction between variables, a 3D surface curve and a counter plot were plotted by examining the particle size between the ratio of polymer concentration(A) and stirring speed(B).

In Figures 7A and 7B, 3D surface design and counter plots show the particle size of nanoparticles by the desolvation method using sodium alginate. Particle size shows that increasing the amount of polymer concentration and stirring speed decreases particle size. Particle size influences the concentration of polymer(A) and stirring speed(B). The counter plot shows the blue region, which shows the smaller

Table 5. ANOVA results for the response (Y_1)

Source	Sum of squares	*df*	Mean Square	F= value	p-value	
Model	729.72	5	145.94	14.69	0.0014	Significance
A – polymer concentration	74.20	1	74.20	7.47	0.0292	Significance
B – stirring speed	199.53	1	19.53	20.097.0	0.0029	Significance
AB	69.72	1	69.72	17.02	0.0330	Significance
A2	14.07	1	14.07	1.42	0.2728	Not significant
B2	371.20	1	371.2	37.37	0.0005	Significance
Residual	69.53	7	9.93			
Lack of fit	55.24	3	18.41	5.15	0.0736	Not significant
Pure Error	14.29	4	3.57			
Cor Total	799.25	12				

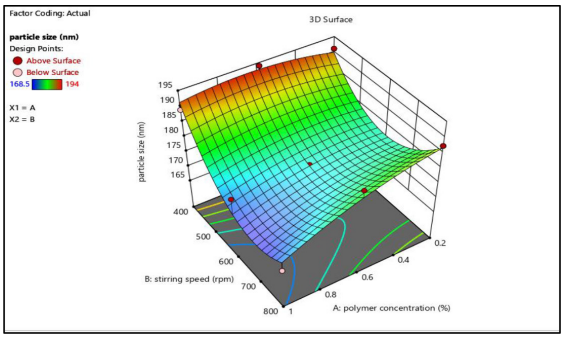


Figure 7A. 3D surface graph showing the effect of polymer concentration and stirring speed on particle size.

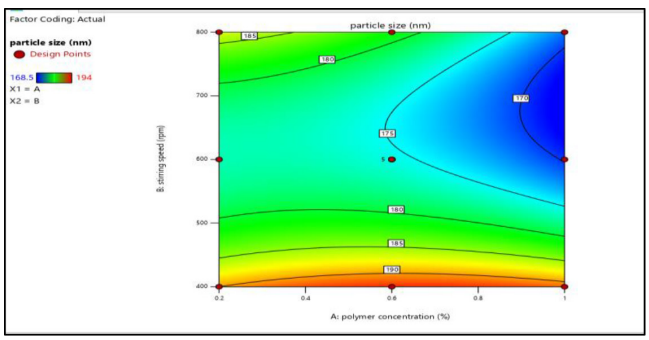


Figure 7B. Counterplot showing the effect of polymer concentration and stirring speed on particle size.

particle size and the green region, which shows the bigger particles, based on the polymeric concentration and stirring speed.

3.5.2 Effect of Factor on Drug Entrapment Efficiency (Y_2)

Drug loading efficiency is considered the response; the linear model is fitted to the data according to the ANOVA F-value of DOE. The results of the fit model summaries are shown in Table 6.

The ANOVA F-value of the model showed that the model P-value is less than 0.05 and considered significant, while the value is more than 0.05 and the lack of fit of the model is not significant (F=0.1059; P>0.05). Hence, it can be concluded that the linear equation can fit the data appropriately, as shown in the equation

Polynomial Equation $Y_2 = 80.59-2.42A+1.08B$ …. Eq.-4

Where Y_2 is the DEE, and A and B are the polymer and stirring speed ratio, respectively.

The entrapment efficiency of nanoparticle interaction is shown by the three-dimensional response and counter plots. The % drug entrapment efficiency increased with polymeric concentration is increases, and stirring speed also affects the entrapment efficiency as stirring speed increases, with an increase in the entrapment efficiency shown in Figure 8A. In the counterplot, the blue region shows low entrapment efficiency, and the red region shows more entrapment efficiency with increasing polymer concentration and stirring speed, as shown in Figure 8B.

3.5.3 Effect of Factor on Drug Release (Y_3)

The ANOVA F- F-value of the model indicated that the model P-value is less than 0.05 and considered to be significant, while the value is more than 0.05 and the lack of fit of the model is not significant (F=0.1105; P>0.05), as shown in Table 7. Hence, it can be concluded that the linear equation can fit the data appropriately, as shown in the equation.

Polynomial Equation $Y_3 = 73.31+3.67A+2.33B$. Eq.-5

Where Y_3 is the drug release and A and B are the polymer and stirring speed ratios, respectively.

Table 6. ANOVA results for drug entrapment efficiency as the response (Y_2)

Source	Sum of squares	*df*	Mean Square	F= value	p-value	
Model	113.33	2	56.67	104.25	<0.0001	Significance
A – polymer concentration	80.67	1	80.67	148.40	<0.0001	Significance
B – stirring speed	32.67	1	32.67	60.09	<0.0001	Significance
Residual	5.44	10	0.5436			
Lack of fit	4.64	6	0.7726	3.86	0.1059	Not significant
Pure Error	0.8000	4	0.2000			
Cor Total	118.77	12				

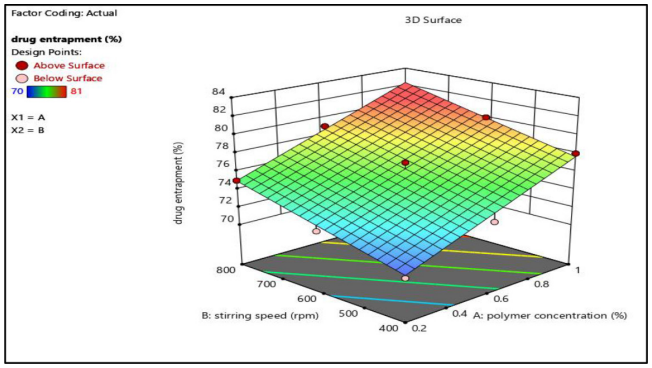


Figure 8A. 3D Surface graph shows the effect of polymer concentration and stirring speed on % drug entrapment efficiency.

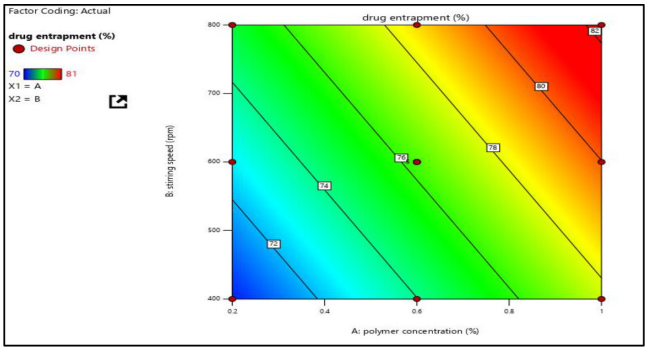


Figure 8B. Counter plot shows the effect of polymer concentration and stirring speed on drug entrapment efficiency.

The % drug release response is elucidated by 3D and counter plots; the impact observed responses of % drug release upon the polymer concentration and stirring speed. The concentration of polymer increased (- 0.2 to +1.0), and there was an increase in their %drug release, as shown in Figure 9A. In the counterplot, the blue region shows a decrease in % drug release, and the red region shows an increase in % drug release, as shown in Figure 9B.

3.5.4 Validation of Optimisation Design

The optimisation of the formulation is generally aimed at implementing systemic approaches to achieve the best possible combination of the process characteristics. The motto of studying the impact of the selected variable on system response by changing one variable at a time is shown in Table 8. An optimisation tool was preferred for understanding the effect of different formulation variables on the quality of the pharmaceutical formulation after obtaining the best-performing one. After applying the desirable constraints on the % drug release. A numerical optimisation analysis was then employed by Stat Ease - 13 using the desirability function to develop the counter plot shown in Figure 10. The optimal formulation was 0.99% polymer concentration and 620.29 rpm stirring speed, which was suggested to prepare the nanoparticle. Hence, it was prepared and evaluated. As shown in Table 9, the residual between expected and experimental values was small, demonstrating the reasonableness of the optimisation process. Thus, the optimal nanoparticle formulation was considered promising to be used in further investigation.

3.6 Surface Morphology

The SEM images of the optimised formulation-FCP4 are shown in Figure 11. The results show that the formulated nanoparticles are non-porous and irregular in shape.

Table 7. ANOVA results for drug release as the response (Y_3)

Source	Sum of squares	*df*	Mean Square	F= value	p-value	
Model	42.15	2	21.08	38.54	<0.0001	Significance
A – polymer concentration	35.09	1	35.09	64.16	<0.0001	Significance
B – stirring speed	7.06	1	7.06	12.91	0.0049	Significance
Residual	5.47	10	0.546			
Lack of fit	4.64	6	0.7741	3.76	0.1105	Not significant
Pure Error	0.8245	4	0.2061			
Cor Total	47.62	12				

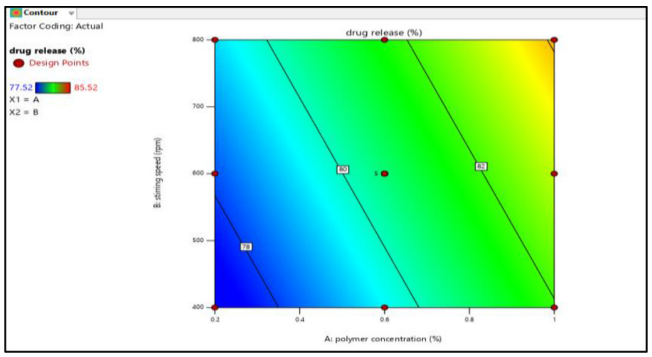


Figure 9A. Counterplot showing the effect of polymer concentration and stirring speed on drug release.

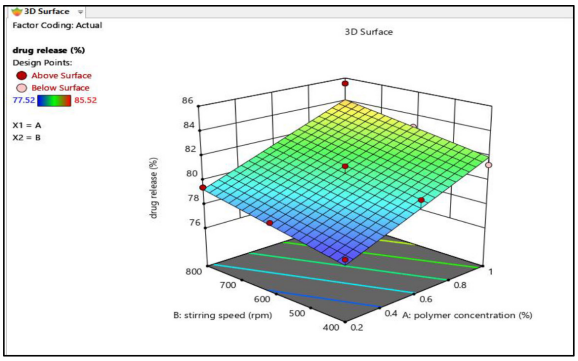


Figure 9B. Counterplot showing the effect of polymer concentration and stirring speed on drug release.

Table 8. Validation of optimisation design by varying the effect of time

Formulation Code	Concentration of polymer	Stirring Speed	Drug release at 4hrs	Drug release at 10hrs	Drug release at 12hrs
FCP1	0.2	400	24.84	64.6	77.52
FCP2	1	400	27.09	66.9	81.29
FCP3	0.2	800	26.47	66.19	79.43
FCP4	1	800	28.5	71.26	85.52
FCP5	0.2	600	26.12	65.3	78.36
FCP6	1	600	27.67	69.17	83.01
FCP7	0.6	400	26.72	66.8	80.17
FCP8	0.6	800	26.84	67.11	80.54
FCP9	0.6	600	26.73	66.82	80.19
FCP10	0.6	600	26.72	66.8	80.17
FCP11	0.6	600	26.73	66.82	80.19
FCP12	0.6	600	26.72	66.8	80.17
FCP13	0.6	600	27.72	69.17	80.17

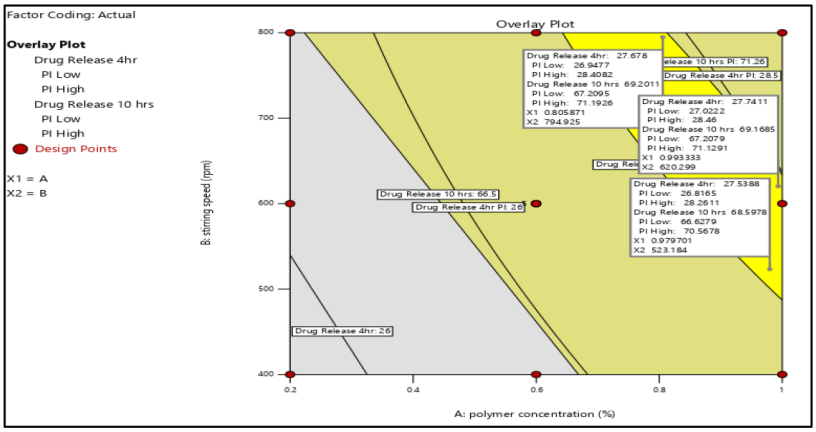


Figure 10. Overlay plot illustrates the effect of particle size, drug entrapment and drug release at the polymer and the speed of rotation of the prepared optimised nanoparticle.

Table 9. ANOVA finding the point of prediction value for prepared nanoparticles

Batch Code	Composition		Response	Predicted Value	Experimental Value	% Error
	Concentration of polymer	Stirring Speed				
DNCP-A	0.80	794.95	% Drug Release 4hrs	27.6	27.4	0.98
			% Drug Release 10hrs	69.20	68.9	0.43
			% Drug entrapment	78.89	78.6	0.37
			Particle size (nm)	178.44	178	0.25
DNCP- B	0.99	620.29	% Drug Release 4hrs	27.74	27.5	0.87
			% Drug Release 10hrs	69.16	69.13	0.04
			% Drug entrapment	74.20	73.9	0.40
			Particle size (nm)	176.81	176.5	0.18
DNCP- C	0.97	523.18	% Drug Release 4hrs	27.53	27.3	0.84
			% Drug Release 10hrs	68.59	68.4	0.28
			% Drug entrapment	76.85	76.8	0.07
			Particle size (nm)	183.35	182.5	0.46

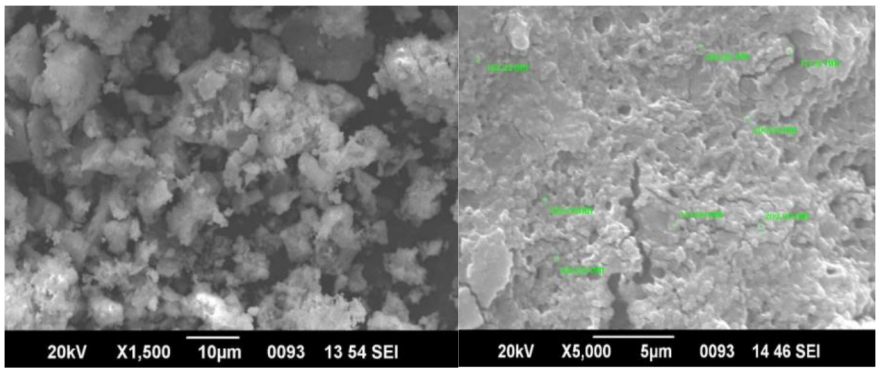


Figure 11. SEM analysis of optimised formulation.

4. Conclusion

In the current work, Piperine and Curcumin were extracted and isolated successfully, and nanoparticles were prepared using the Desolvation method using sodium alginate as the polymer. FT-IR studies were performed to find out the possible interaction between the selected Herbal drug and polymers. FT-IR studies revealed that there was no interaction between the selected herbal drug and polymer. 3^2 F was employed for optimisation to prepare the nanoparticles. The selection of different polymers gave the best results for sodium alginate, which fetched the lowest particle size. The ratio of the concentration of polymer and the stirring speed was an effective variable in size, % DEE, and release of the drug. The polynomial equation was used to analyse the quantitative impact of the factor at different levels. It is one of the best combinations of characteristics, which was then examined using an optimisation method to prepare nanoparticles with identical particle size, good entrapment efficiency and drug release. The recognised drug release mechanism, Higuchi and Korsmeyer Peppas model, was the best-fit model for the selected batch of nanoparticles, and plots show the linearity (R^2 0.9539 and 0.9989). The SEM image is revealed to be non-porous and irregular in shape.

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6. Author Contribution

Madhuri H R contributed to the original idea and concept of the study and assisted in the writing and structuring of the manuscript. Dr. Bijal Prajapati provided critical corrections and suggestions during manuscript preparation and contributed to the conceptual development of the research idea. All authors have read and agreed to the published version of the manuscript.

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