RESEARCH ARTICLE

Formulation and Optimization of Colon-Specific Nanoparticles Containing A Herbal Anticancer Agent

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ABSTRACT

Colon cancer is one of the major ailments associated with increased death rate globally. This study aimed to design and optimize a nanoparticulate system for delivering sulforaphane (SFN), a dietary phytochemical with anticancer properties, to the colon. Eudragit S100, a pH-sensitive polymer was utilized to fabricate polymeric nanoparticles for delivering the drug specifically to the colon. The nanoparticles were formulated by nanoprecipitation technique and applied design of experiments (DoE) for optimization and validation. The optimized formulation was characterized for its physicochemical properties, such as particle size, shape, surface morphology, encapsulation efficiency, zeta potential, polydispersity index, *in-vitro* drug release, and stability at refrigerated conditions. The prepared NPs revealed spherical and smooth surfaces and value of polydispersity index, particle size, zeta potential and encapsulation efficiency were found to be 0.255, 119.46 ± 0.09 nm and -35.68 ± 0.75 mV and 64.17 ± 0.56%, respectively. The *in-vitro* drug release study showed that the optimized nanoparticles exhibited minimal drug release at pH 1.2 and pH 6.8, but significant and sustained drug release at pH 7.4 corresponded to the colon's pH. The stability study indicated that the prepared formulation was stable at refrigerated condition for six months. These findings suggest that the developed delivery system could be a promising strategy for effectively encapsulating and delivering the phytochemical anticancer agent to the colon.

Keywords: Sulforaphane, Biodegradable polymer, Colon, Cancer, Site-specificity.

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INTRODUCTION

Cancer is one of the serious health issues prevailing worldwide. It is one of the alarming causes of death with increasing new cases and low survival rate.[1] The major factors responsible for the ailments include lack of balance diet, poor lifestyle, genetic factors and other inflammation1. Though several treatment approaches, chemotherapy and surgery are available but are effective only over little extent. Surgery can cure the disease between stages 0 to stage II, later stages require additional therapies along with chemotherapy.[2] The challenge encountered in the treatment of cancer is that the cancerous tissues develop resistance to drug. With the advancement in the treatment with chemotherapy or adjuvant therapy the response rate of patients has increased. However, almost all patients suffer from resistance thereby restricting the efficacy of the anticancer agents and thereby failure of the treatment. In addition, these therapies are associated with a number of adverse side effects. Therefore, to solve the issues related to available therapy, there is a rising need to develop targeted drug delivery systems.[3,4] In addition, these therapies are associated with number of adverse side effects. Therefore, to overcome the issues related to conventional therapy, there is a rising need to develop targeted drug delivery systems. The advantages of targeted delivery systems include reduced frequency, site specificity, increased solubility, bioavailability and fewer side effects.[5-8] Besides, herbal anticancer agents are gaining importance over traditional anticancer drugs due to their fewer side effects. Several investigations have revealed the potent anticancer properties of several herbal plants. But the herbal anticancer drugs posses low solubility, less side specificity and hence low bioavailability. Owing to these all underlying issues, the use of these herbal drugs provides limitations in achieving desired therapeutic effect. The formulation of targeted drug delivery system containing herbal anticancer drugs could provide a newer approach to treat colon cancer. The targetable systems may provide higher amount of drug

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at the distal colon region, thereby increasing the therapeutic efficacy.[9-13] Sulforaphane is a dietary phytochemical obtained from cruciferous vegetables like broccoli, cabbage, etc. The anticancer property of sulforaphane has been evident from several reported investigations by various researchers.[14-18] In the current study, sulforaphane loaded polymeric nanoparticles have been prepared for treatment of colon cancer. Eudragit S100 was employed as a polymer for targeted delivery to colon region.[19,20] In this current study, the optimization of the formulation was performed using Box Behnken design as a response-surface technique of experiments and various response graphs were obtained. Box–Behnken design is an response surface methodology in which each independent variable is assign with three equally spaced values i.e -1, 0, +1. The experimental design can fit to the quadratic model.[21-24]

MATERIALS AND METHODS

Materials

Eudragit S100 was obtained from Evonik (India). Sulforaphane was procured from Carbosynth (United Kingdom). Polyvinyl alcohol was procured from Sigma Aldrich (USA). The other chemicals of extra pure grade were used in the experiment.

Formulation of Sulforaphane Loaded Nanoparticles (S-NPs)

The nanoparticles were formulated using nanoprecipitation method.[25] The mixture of SF and ES100 was dissolved in acetone. The solution of drug and polymer was further added drop by drop into PVA solution over magnetic stirrer and allowed for complete evaporation of acetone. Further, the nanoparticles were received by subjecting the solution to refrigerated centrifugation (8,000 rpm) for 30 minutes (ELTEK). The collected nanoparticles were finally washed thrice using deionized water followed by freeze drying (Labcono, Model: FreeZone 4.5L).

Optimization of Sulforaphane Loaded Nanoparticles

The Box-Behnken experimental design (BBD) was used to optimized the prepared nanoparticles with Design-Expert® Software 11 (Trial Version) of 17, 3-factor, 3-level. The independent variables and dependent variables are given in Table 1. The interaction between various variables was analyzed. The following is the quadratic equation generated by experimental design:

$$Y= A0 + A1*X1 + A2*X2 + A3*X3 + A4*X4 + A5*X1*X2 + A6*X1*X3 + A7*X1*X4 + A8 *X2 *X3 + A9*X2*X4 + A10*X3*X4 + A11 *X12 + A12 *X22 + A13*X32 + A14*X42$$

Where, Y, A0, A1 to A14, X1 to X4, Xa Xb and Xi2 are values of response variables, intercept that is taken as arithmetic mean of entire runs, regression coefficients, codes allotted to independent variables, interaction terms and quadratic interaction terms, respectively. The synergistic effect as well as opposite effect of the process variable is indicated by signs (negative or positive) to the value of coefficients in polynomial equation on dependent variables. The optimized batch was further finalized obtained on basis of maximum encapsulation percentage efficiency and lowest particle size.

Characterization of Sulforaphane Loaded Nanoparticles

SEM studies

The evaluation of morphology of S-NPs was assesed by scanning electron microscopy (Zeiss, Germany). The nanoparticles so prepared were subjected to gold coating over metal stubs while setting at suitable resolution of 2 nm, applied voltage of 2.2 Kv, 20 mV and secondary electronic image display.

Particle size and surface charge

To prepared S-NPs were assessed by dynamic light scattering technique (Nanoplus) at 25°C. A homogenous suspension of nanoparticles was prepared in deionized water by sonication and was subjected to determine particle size, zeta potential and polydispersity index in triplicate.

Entrapment efficiency

The percentage encapsulation of developed nanoparticulate formulation was determined by HPLC method. The solution of nanoparticles in mobile phase (acetonitrile/water) was prepared, followed by centrifugation (5 minutes). The drug concentration was determined over HPLC system (Shimadzu). The following is the formula for calculations:

$$\text{Percentage Encapsulation efficiency} = \frac{\text{Drug (mg) in nanoparticles}}{\text{Total drug (mg)}} \times 100$$

Drug release study

The *in-vitro* drug release investigation of S-NPs was carried out at 37 ± 1°C using phosphate buffer pH 1.2, phosphate buffer pH 6.8, and phosphate buffer pH 7.4 as a dissolution medium. Samples were taken at different time intervals, and the same volume was replenished. HPLC analysis was used to determine the drug concentration in the samples.

Stability evaluation

Instability assessment, the prepared formulation was placed for 90 days at refrigerated temperature. The samples were further determined for particle size, encapsulation efficiency and zeta potential.

RESULTS AND DISCUSSION

Optimization of Formulation

The results of the optimization design by Design Expert® software are given in Table 2. The choosen independent variables have exhibited remarkable interaction and influence on the response variables viz., particle size and encapsulation efficiency. The statistics of 17 formulations was set to number of mathematical equations using Design Expert® Software 11 and various 3D graph were generated. The interaction between the variables was assessed and the quadratic model was found to be the most suitable for assesment of S-NPs.

Table 1: BBD with their levels

Variables	*Levels*					
			Units	*-1 (Low)*	*0 (Medium)*	*+1 (High)*
Independent variables	X1	Organic solvent	mL	2.5	5	7.5
	X2	Theoretical drug loading	%	10	20	30
	X3	Concentration of surfactant	%	0.5	1	1.5
Dependent variables	Y1	Size of particle	nm	Lowest		
	Y2	Drug entrapment Efficiency	%	Highest		

Table 2: Summary of experimental design

Formulation code	*Independent variables*			*Dependent variables*	
	X1	*X2*	*X3*	*Y1 (nm ± SD)**	*Y2 (% ± SD)**
SFP1	0	-1	1	128.94 ± 0.46	61.24 ± 1.19
SFP2	0	1	-1	170.42 ± 1.37	62.16 ± 0.15
SFP3	-1	0	1	142.96 ± 2.16	58.82 ± 0.88
SFP4	1	1	0	182.56 ± 0.46	55.69 ± 1.91
SFP5	1	0	1	175.86 ± 0.29	60.85 ± 0.65
SFP6	0	0	0	116.25 ± 0.55	68.64 ± 1.56
SFP7	0	1	1	155.64 ± 1.75	44.78 ± 2.43
SFP8	1	-1	0	158.72 ± 1.12	52.36 ± 1.37
SFP9	0	0	0	121.12 ± 2.68	64.28 ± 0.25
SFP10	-1	-1	0	158.94 ± 0.17	50.86 ± 0.69
SFP11	0	0	0	119.38 ± 0.09	65.88 ± 0.48
SFP12	-1	1	0	160.12 ± 1.69	57.86 ± 1.22
SFP13	0	0	0	124.64 ± 0.47	61.65 ± 0.74
SFP14	0	0	0	118.16 ± 0.08	66.54 ± 0.60
SFP15	1	0	-1	178.34 ± 0.66	60.86 ± 1.52
SFP16	-1	0	-1	180.54 ± 0.34	61.86 ± 0.74
SFP17	0	-1	-1	182.37 ± 0.71	42.51 ± 1.8

* Data represent n = 3, mean ± standard deviation

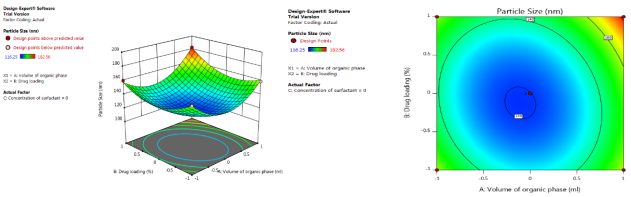

Figure 1a

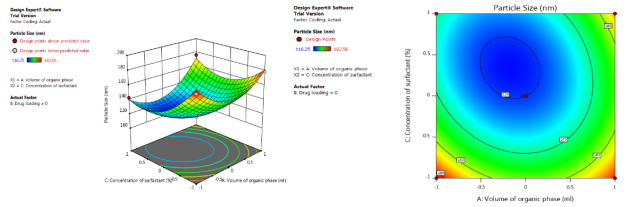

Figure 1b

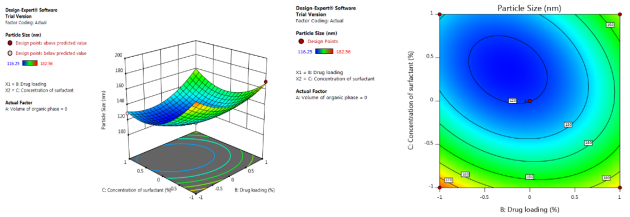

Figure 1c

Figure 1: 3-D response surface plots for S-NPs showing effect of various independent variables on particle size

Particle morphology

The following is the quadratic equation obtained for the size of particle for S-NPs-

Y1 = +119.910+6.615X1 +4.971X2 -13.534X3 +5.665 X1 X2 +8.775 X1 X3 +9.663X2X3 +27.63 X12+17.546 X22+ 21.886 X32

The polynomial equation show that organic phase volume synergistically affects particle size. Higher volume of acetone leads to particle size growth, and drug loading also has a positive impact on particle size. In addition, drug loading has also shown positive impact on size of particles. However, the amount of surfactant has led to decrease in the particle size which can be attributed to the property of surfactant to form droplets with reduced size. The ANOVA for the quadratic model for particle size are shown in Table 3 and the three-dimensional graphs shown in Figure 1 a-c.

Entrapment Efficiency

The following is the quadratic equation obtained for the size of particle for SNPs-

Y2 = +65.398+0.045X1+1.69X2 -0.212 X3-0.9175 X1X2 +0.7575 X1X3 -9.0275X2 X3 -1.6403X12-9.5653X22- 3.1603X32

The above quadratic equation showed that the encapsulation efficiency increases by enhancing the organic phase volume from level -1 to 0. But further increment in the volume would not affect the encapsulation substantially. Further, the theoretical drug loading has positively influenced the percentage efficiency. The increment in encapsulation at upper level was very less showing the saturation of polymer as a matrix. Later, an increase in surfactant concentration has lowered the drug entrapment in prepared nanoparticles. The data of ANOVA for particle size are shown in Table 4 and three-dimensional graphs are shown in Figure 2 a-c.

Table 3: ANOVA analysis of S-NPs for response Y1

Source	*Sum of squares*	*df*	*Mean square*	*F-value*	*p-value*	
Model	10081.97	9	1120.22	48.46	< 0.0001	significant
A-Volume of organic phase	350.07	1	350.07	15.14	0.0060	
B-Drug loading	197.71	1	197.71	8.55	0.0222	
C-Concentration of surfactant	1465.30	1	1465.30	63.39	< 0.0001	
AB	128.37	1	128.37	5.55	0.0506	
AC	308.00	1	308.00	13.33	0.0082	
BC	373.46	1	373.46	16.16	0.0051	
A^2	3214.10	1	3214.10	139.05	< 0.0001	
B^2	1296.30	1	1296.30	56.08	0.0001	
C^2	2016.88	1	2016.88	87.26	< 0.0001	
Residual	161.80	7	23.11			
Lack of fit	121.23	3	40.41	3.98	0.1075	not significant
Pure error	40.58	4	10.14			
Corr total	10243.77	16				

Table 4: ANOVA analysis of S-NPs for response Y2

Source	Sum of Squares	df	Mean Square	*F-value*	*p-value*	
Model	820.12	9	91.12	17.48	0.0005	significant
A-Volume of organic phase	0.0162	1	0.0162	0.0031	0.9571	
B-Drug loading	22.85	1	22.85	4.38	0.0746	
C-Concentration of surfactant	0.3613	1	0.3613	0.0693	0.8000	
AB	3.37	1	3.37	0.6458	0.4480	
AC	2.30	1	2.30	0.4402	0.5283	
BC	325.98	1	325.98	62.52	< 0.0001	
A^2	11.33	1	11.33	2.17	0.1840	
B^2	385.24	1	385.24	73.88	< 0.0001	
C^2	42.05	1	42.05	8.07	0.0250	
Residual	36.50	7	5.21			
Lack of fit	9.15	3	3.05	0.4463	0.7333	not significant
Pure error	27.34	4	6.84			
Cor total	856.62	16				

Optimization of Formulation

The data from optimization by BBD have revealed that all the independent variables has greatly influenced the particle size and entrapment efficiency. On the basis of the criteria i.e., to achieve lowest size of particle and highest encapsulation efficiency and by using point prediction method, an optimized formulation was obtained. The selected optimized S-NPs batch contain acetone, PVP, SF and desirability value of 5.11 mL, 1.3% w/v, 20% and 0.962, respectively. The experimental values of responses, i.e. particle size (119.46 nm) and entrapment efficiency (64.17%) were obtained to be in line with the predicted values. The values for dependent variables obtained by prediction method were found to be particle size (116.64 nm) and entrapment efficiency (65.02%).

Characterization of Sulforaphane Loaded Nanoparticles

The S-NPs were found to be spherical in shape with even and smooth surface texture. The values of average size of particles, polydispersity index and zeta potential of formulated nanoparticles was found to be 119.46 ± 0.09 nm, 0.255 and -35.68 ± 0.75 mV, respectively (Figures 3 and 4). The size of formulated in nanosize range indicates desirable permeability and accumulation of nanoparticles by enhanced penetration and accumulation in the tumor. The results therefore revealed that S-NPs possess potential for successful colon targeting.

Table 5: Stability study data

Storage Condition	*Parameters*	*Days*			
		0	30	60	90
Refrigerated temperature	Size of Particle (nm)	119.46 ± 0.09	119.98 ± 0.16	120.42 ± 0.05	122.15 ± 0.18
	Zeta Potential (mV)	-35.68 ± 0.75	-35.68 ± 0.75	-35.68 ± 0.75	-35.68 ± 0.75
	Encapsulation Efficiency (%)	64.17 ± 0.56	63.56 ± 0.23	63.45 ± 1.56	62.82 ± 0.48

Data represent n = 3, mean ± standard deviation

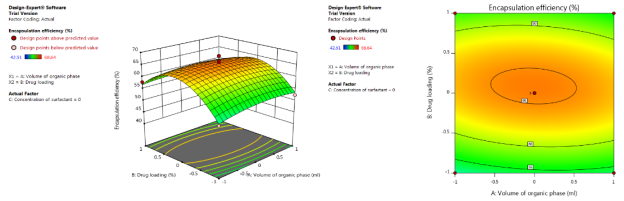

Figure 2a

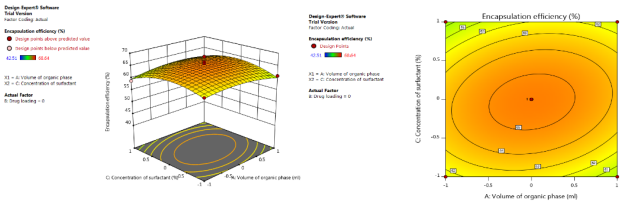

Figure 2b

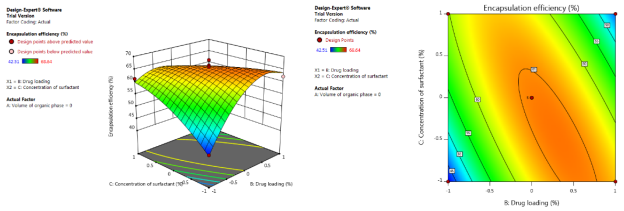

Figure 2c

Figure 2: 3-D response surface plots for S-NPs showing effect of various independent variables on particle size

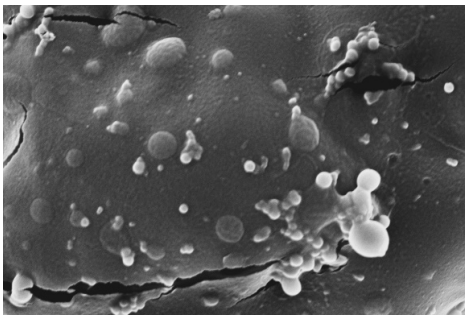

Figure 3: Scanning electron microscopic image of S-NPs

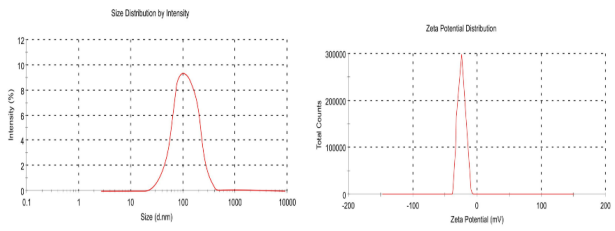

Figure 4: Particle size and Zetva potential of S-NPs (n=3)

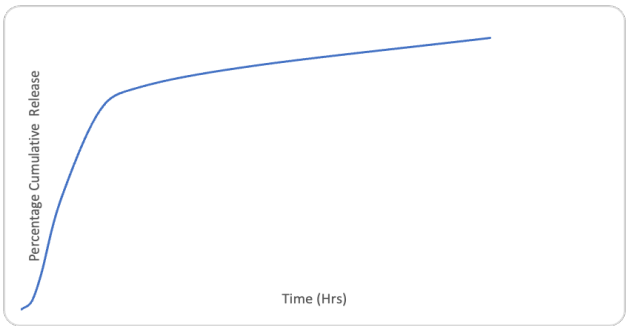

Figure 5: In-vitro release of drug from S-NPs

Further, the PdI value indicates monodispersibility whereas zeta potential with negative value assures stable nanoparticles formulation. The percentage encapsulation was found to be 64.17 ± 0.56%.

Drug Release Study

The drug release study of S-NPs was carried out in different pH ranges, i.e., from pH 1.2 to 6.8 (Figure 5). The drug release profile exhibited negligible drug release (<10%) at pH of 1.2 and 6.8, respectively. Further, at pH 7.4 it was found that drug release from NPs matrix was slow and steady. The higher release of drug at pH 7.4 assures the colon targeting of the prepared formulation.

Stability Study

The data from the stability study of S-NPs when subjected to refrigerated conditions for 90 days are summarized in Table 5. The result of the study indicated negligible or insignificant change in the properties of optimized SF-NPs. Thus, it can be concluded that the refrigerated condition to be an appropriate storage condition for prepared SF-NPs for maintaining stability.

CONCLUSION

The optimization of prepared S-NPs by using BBD experimental design showed substantial interaction between all the selected independent and dependent parameters. The particle size in nm range assured the site-specific delivery of drug to the colon. The data of encapsulation efficiency and zeta potential revealed the potential of prepared formulation in effective colon targeting. It was evident from the results of *in-vitro* drug release that there was almost negligible loss of drug in gastrointestinal tract, thereby assuring higher drug concentration in the colonic area. On the basis of above results, it is evident that the sulforaphane-loaded nanoparticles formulation has the potential as an effective approach for the delivery of herbal anticancer agents in the treatment of colon cancer.

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