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Original Article

FORMULATION AND OPTIMIZATION OF CHITOSAN NANOPARTICLES OF DIMETHYL FUMARATE USING BOX-BEHNKEN DESIGN

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ABSTRACT

Objective: Dimethyl fumarate (DMF) is a methyl ester of fumaric acid. It has been approved by USFDA recently for the treatment of an autoimmune disorder, multiple sclerosis (MS). The objective of present study was to synthesize and optimize chitosan loaded nanoparticles of DMF by box-behnken design (BBD), to provide a better drug delivery system for the management and treatment of MS.

Methods: Polyelectrolyte complex coacervation technique was used to prepare Chitosan (CS) loaded DMF nanoparticles and box behnken design using 3 factors and 3 levels were selected for optimization of the formulation. Effect of three independent factors that is, polymer CS concentration, polymer dextran sulfate (DS) concentration and the amount of drug were studied on two dependent responses that is particle size and % drug entrapment efficiency. The analysis of variance (ANOVA) was performed to evaluate the significant differences between the independent variables.

Results: The optimized batch showed the highest % drug entrapment (65.36) and an average particle size (355 nm). Zeta potential value was optimum to maintain the stability of the formulation. *In vitro* drug release behavior followed Korsmeyer-Peppas model which showed the initial release of 21.7±1.3% with prolonged drug release of 69.5±0.8% from optimized CS nanoparticle up to 24 h. The % cumulative drug release (% CDR) of optimized nanoparticles was 84%.

Conclusion: The optimized nanoparticles of DMF with improved properties could be a promising formulation for the treatment and management of MS.

Keywords: Multiple sclerosis, dimethyl fumarate, chitosan nanoparticle, optimization, box behnken design, complex polyelectrolyte conservation.

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INTRODUCTION

Nanoparticles represent an effective nanocarrier platform for the delivery of hydrophilic and hydrophobic drugs since the drugs are protected from possible degradation by enzymes [1].

MS is an autoimmune disease the body's own immune system spearheads the attacks. The disorder is mediated by a complex interaction of individual's genetics and as yet unidentified environmental insults. In multiple regions, the myelin sheaths deteriorate to sclerosis, which are hardened scar or plaques [2, 3].

Nanotechnology by manipulation of characteristics of materials such as polymers and fabrication of nanostructures is able to provide superior drug delivery systems for better management and treatment of diseases [2]. Drug targeting by nanoparticles has been getting much attention by the researchers for the treatment of various central nervous system disorders [4]. DMF is a white, nonhygroscopic BCS class 1 drug [5]. DMF has been approved by USFDA in 2014 as the first-line oral treatment for Multiple Sclerosis [6]. DMF is almost completely absorbed in the small intestine and extensively metabolized by esterases before it reaches the systemic circulation. The half-life of DMF is approximately 1 hour. CS is a promising candidate for preparation of nano and microparticulate drug delivery systems owing to its low toxicity, better stability, simple and reproducible preparation methods and provides versatile routes of administration as drug delivery carrier [7]. CS is one of the most abundant biopolymers, poly [β-(1,4)-2-amino-2-deoxy-d-glucopyranose], possesses unique structural features. In the present method, an organic phase containing the polymer and drug is added dropwise to a dispersing phase which is a nonsolvent for the dispersed polymer but is miscible with the diffusing solvent. The formation of nanoparticles happens spontaneously [8]. This method does not require vigorous shearing or stirring rates, ultrasonication and is mostly suitable for the compounds having hydrophobic nature [9-11]. In the present study, CS loaded DMF nanoparticles were formulated and optimized by box-behnken design. This work has a novel and promising approach for the use of DMF in the treatment and management of MS.

MATERIALS AND METHODS

Materials

CS (degree of acetylation=80.45%) and dextran sulphate (DS) were procured from chemsworth chemicals, Surat. DMF was obtained from Alfa Aesar; a Johnson matthey company. Methanol, glacial acetic acid, and acetone were of the suitable analytical grade. Double distilled water was used in the preparation of solutions and dispersion of chitosan nanoparticles.

Preparation of CS DS nanoparticle

CS Nanoparticles were prepared by polyelectrolyte complex coaservation technique [12-15]. A solution of CS was prepared by dissolving required quantity of CS in 2% v/v acetic acid solution. DS solution was prepared by dissolving required quantity of DS in double distilled water. To DS solution required a quantity of DMF was added and dissolved completely. Now DS containing dissolved drug solution was added dropwise to CS solution under magnetic stirrer for 1 hour. Tween 80 was added to stabilize the resultant particles followed by continuous stirring. The ratio between the volumes of DS Solution and CS solution was 1:4. The nanoparticle batches were prepared as per box-behnken design.

Optimization of CS nanoparticles by box Behnken design

Design Expert® 9.0.5.1 software was used to developing a box-behnken statistical design, response surface methodology (RSM) with 3 factors, 3 levels, and 15 runs for the optimization of CS nanoparticles [16-18]. Optimization was performed to investigate the level of independent variables (X_1, X_2, and X_3) that would yield a minimum value of the particle size (R_1) and the maximum value of EE (R_2). The design was used to explore the quadratic response surfaces, and the polynomial equation was generated by the experimental design is as follows:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}X1^{2^1}{}_1 + b_{22}X2^{2^1}{}_1 + b_{33}X3^{2^1}{}_1$$

Where Y is the independent variable, b_0 is the intercept and b_1, b_2, b_3 are regression coefficients which were calculated from the experimental results of independent variables and dependent variables. The independent variables were a concentration of CS (X_1), the concentration of DS (X_2) and concentration of DMF (X_3) and dependent variables was particle size (R_1) and entrapment efficiency (% EE) (R_2) with high, medium and low level. The independent and dependent variables are listed in table 1 and table 2 respectively. The box behnken design was used with 3 formulation variables at 3 levels and all the batches of CS nanoparticles were evaluated statistically ($p<0.05$). A checkpoint analysis was performed to confirm the role of the derived polynomial equation and contour plots in prediction the responses [19, 20].

Table 1: Independent variables

Independent variables	level		
	-1	0	+1
A % CS (w/v)	0.025	0.0625	0.1
B % DS (w/v)	0.02	0.04	0.06
C % Drug amount (w/v)	0.1	0.3	0.5

Table 2: Dependent variables

Dependent variables	Constraints
R1 (Particle Size)	Minimum
R2 (% EE)	Maximum

Characterization of DMF loaded nanoparticles

Size determination

The average particle diameter, polydispersity index (PDI) and zeta potential of the polymeric nanoparticles were determined by dynamic light scattering (DLS) analysis using Zeta Sizer Nano ZS90 (Malvern Instruments Limited, U. K.) The samples of CS nanoparticle were placed in disposable cuvettes for size and zeta potential measurement. The nanoparticles were dispersed in an appropriate volume of HPLC grade water at 25 °C, at a detection angle of 90 ° for measuring the size and PDI and 120 ° for zeta potential measurement.

Drug entrapment efficiency (% EE)

DMF loaded nanoparticles were separated from the solution by centrifugation 2000 rpm for 1 hour. Supernatants recovered from centrifugation were decanted. DMF content in the supernatant was analyzed by a UV-Vis spectrophotometer (Shimadzu UV 2700) at 208 nm. The percentage drug entrapment efficiency (%EE) was calculated using the following formula [21, 22].

$$\% \text{ EE} = [(\text{Total amount of DMF added} - \text{Free DMF in supernatant}) \div \text{Total amount of DMF}] \times 100$$

***In vitro* drug release**

In vitro drug release study of the optimized DMF loaded CS nanoparticles was carried out using the equilibrium dialysis technique at 37±1 °C. Nanoparticles (equivalent to 1 mg DMF) were suspended in 5 ml of phosphate buffer (PBS) having pH 7.4 and placed in a dialysis membrane bag. The membrane bag containing DMF loaded CS nanoparticle suspension was placed in 500 ml PBS. The agitation speed was set at 50 rpm. At regular time intervals, 5 ml of the aliquots were collected and replaced with an equal volume of fresh PBS to maintain the sink condition. The collected aliquots were centrifuged, and the supernatant was analyzed to calculate the % release of DMF using UV-Visible Spectrophotometer at 208 nm. *In vitro* drug release study was also performed using 0.1 N HCl as dissolution fluid. All the experiments were repeated in triplicate.

FT-IR spectroscopy

In order to evaluate the chemical interaction between CS, DS and DMF spectra of the pure CS, pure DS, pure DMF and optimized nanoparticle were obtained (by KBr Pellet Method) on FT-IR (Perkin Elimer Spectrum II).

Electron microscopic examination

The optimized batch of nanoparticles was formulated and examined under scanning electron microscope (SEM) on FEI quanta 250 to study the morphology of prepared nanoparticles.

RESULTS AND DISCUSSION

Total 15 runs as per BBD and 3 checkpoint batches were formulated. Different concentrations of CS, DS and DMF were used to optimize the best concentration on the basis of % entrapment efficiency and particle size. The observed values of both dependent variables that are particle size and % EE are shown in the table 3.

Table 3: Result showing effect of independent variables on responses (mean value±SD) (n=3)

Run	Factor 1 A % CS(w/v)	Factor 2 B % DS (w/v)	Factor 3 C %Drug amount (w/v)	Response 1 Size (nm)	Response 2 % EE
Batch 1	0	0	0	245±1.8	68±0.25
Batch 2	-1	-1	0	365±2.6	53±0.43
Batch 3	1	0	1	235±1.4	69±0.52
Batch 4	1	0	-1	238±1.6	67±0.32
Batch 5	0	1	1	255±2.1	58±0.62
Batch 6	1	-1	0	240±2.6	54±0.53
Batch 7	0	-1	-1	260±1.5	56±0.72
Batch 8	0	1	-1	258±1.7	58±0.24
Batch 9	1	1	0	242±1.8	70±0.32
Batch 10	-1	0	1	364±2.0	52±0.26
Batch 11	-1	0	-1	358±2.1	50±0.23
Batch 12	0	-1	1	264±1.6	54±0.83
Batch 13	0	0	0	245±1.7	68±0.62
Batch 14	-1	1	0	362±1.4	55±0.33
Batch 15	0	0	0	245±1.9	68±0.62

Effect of independent variables on particle size

Polymer concentration affects particle size and release of drug from the nanoparticle matrix. Average particle size of developed nanoparticles was found in the range of 235 nm (Batch 3) to 365 nm (Batch 2) for different variable combinations. The effect on *z*-average can be explained by the following quadratic equation:

$$\text{Size} = 245.00 - 11.75 \times A - 1.50 \times B + 0.50 \times c + 1.25 \times AB - 2.25 \times AC - 1.75 \times BC - 1.62 \times A2 + 8.88 \times B2 + 5.37 \times C2$$

The Model F-value 0f 57.85 and prob>F less than 0.05 implies the model is significant. The predicted R-squared is in a reasonable range with the adjusted R-squared with a difference of less than 0.2.

Effect of independent variable on % entrapment efficiency (% EE)

The percentage drug entrapment of developed nanoparticles was found in the range of 52 (Batch 10) to70 (Batch 9). Final quadratic equation for prediction of % EE is as follows:

$$\%\ \text{EE} = 68.00 + 6.25A + 3.00B + 0.25C + 3.5AB - 5.55AC + 0.05BC - 3.50A2 + 6.5B2 - 5.00C2$$

The Model F-value of 6.11 implies the model is significant again prob>F, less than 0.05 implies the model terms are significant.

Checkpoint analysis

A total of three checkpoint DMF loaded CS nanoparticle formulations were prepared and evaluated for the responses. Predicted values were also calculated from the polynomial equation and compared with measured values.

As per the optimization results of BBD the independent variables for optimized batch are % CS is 0.651 w/v, % DS is 0.481 w/v and % DMF is 0.502 w/v. The predicted values of responses are average particle size is 334.63 nm and % drug entrapment efficiency of 69.47 (fig. 1). DMF loaded CS nanoparticles were formulated with optimized concentration and evaluated for % EE and average particle size. % EE of the optimized batch was found to be 65.36% and average particle size was found to be 355 nm which is in good correlation with predicted values of responses.

Table 4: Checkpoint batches with predicted and measured value (mean value±SD)(n=3)

Batch code	Factor 1	Factor 2	Factor 3	Particle size		% EE	
	% CS	% DS	% Drug	Predicted	Measured	Predicted	Measured
CP1	1	0	-1	260	248±1.23	56	52±0.15
CP2	0	0	1	235	230±1.4	69	63±0.24
P value				0.986		0.965	

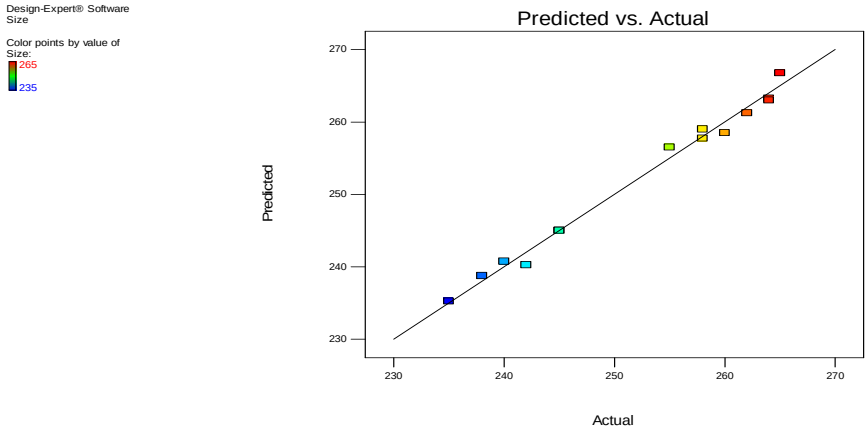

Fig. 1: Graph showing correlation between predicted and actual results

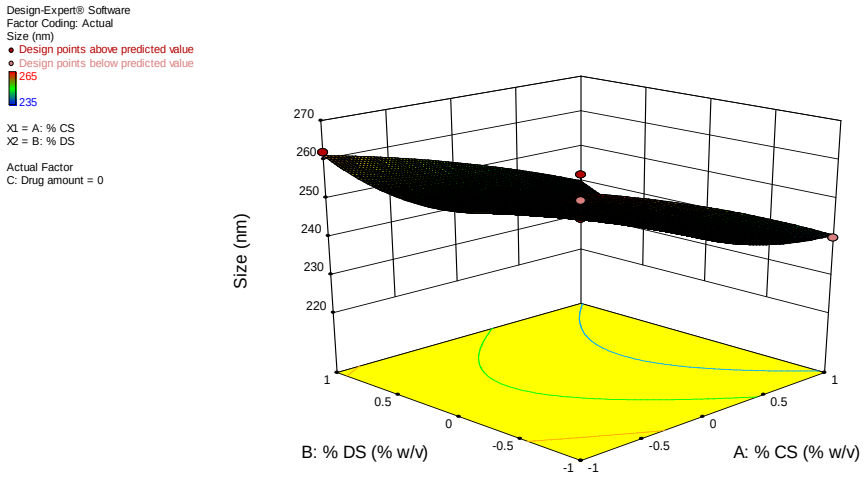

Fig. 2: 3D response surface plot showing effect of % CS and DS on size

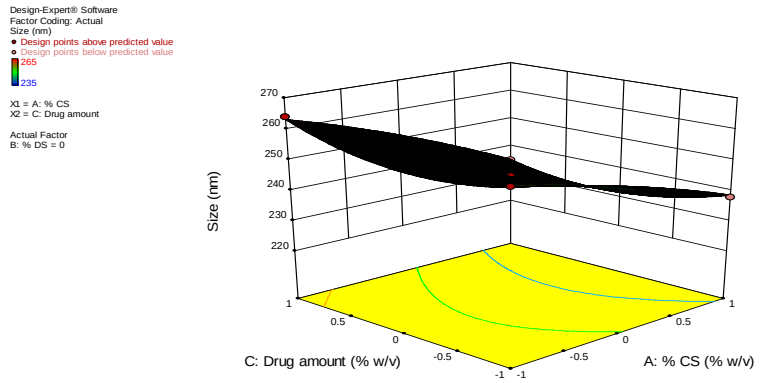

Fig. 3:3D response surface plot showing effect of % CS and drug amount on size

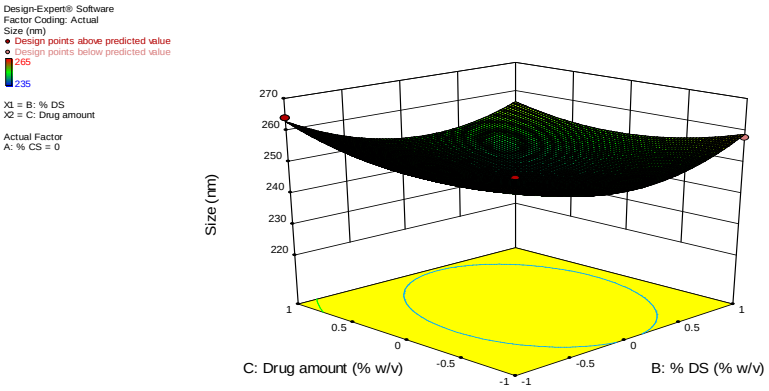

Fig. 4: 3 D response surface plot showing effect of % drug amount and % DS on size

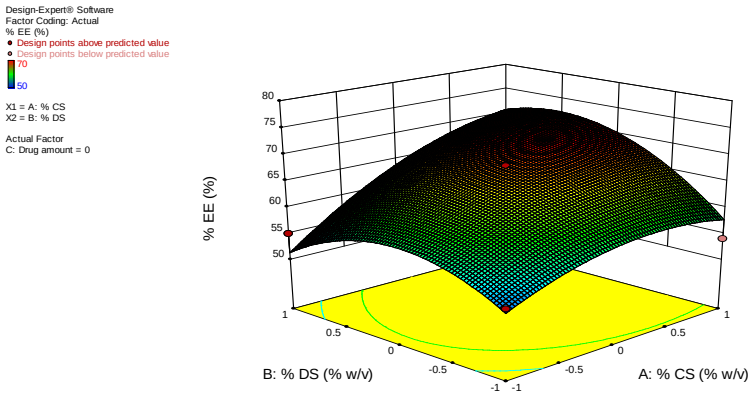

Fig. 5: 3-D response surface plot showing effect of % CS and % DS on % EE

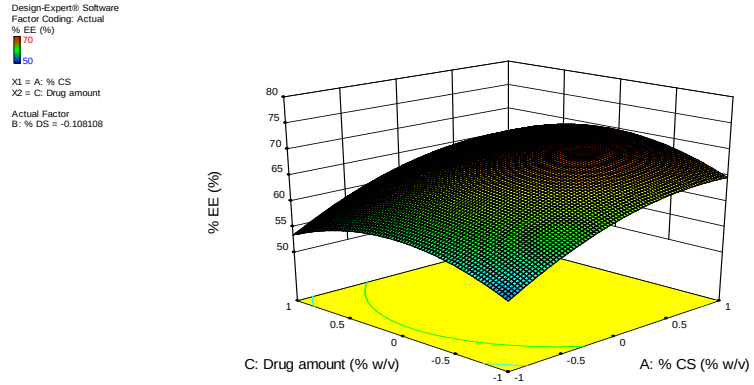

Fig. 6: 3-D response surface plot showing effect of % CS and Drug amount on % EE

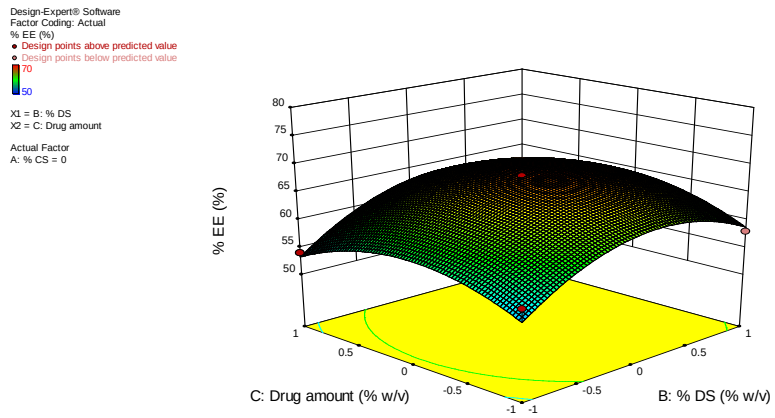

Fig. 7: 3D response surface plot showing effect of % DS and drug amount on % EE

Drug-excipient compatibility

In FTIR study it was observed that there was no interaction between DMF and the polymers. DMF is compatible with the nanoparticle components.

In vitro drug release study

In vitro drug release was analyzed by dialysis method and the data was expressed as mean±SD where n = 3 (fig. 8). The release data was fitted with different kinetic models of dissolution such as zero order, first order, Higuchi and Korsmeyer-Peppas model (table 5).

The cumulative % drug release for an optimized batch of DMF loaded chitosan nanoparticle was 84% over a period of 24 h. Korsmeyer-peppers model was observed to be a best fit model with R^2 value 0.981 on 0.1 N HCl and 0.923 in 7.4 PBS.

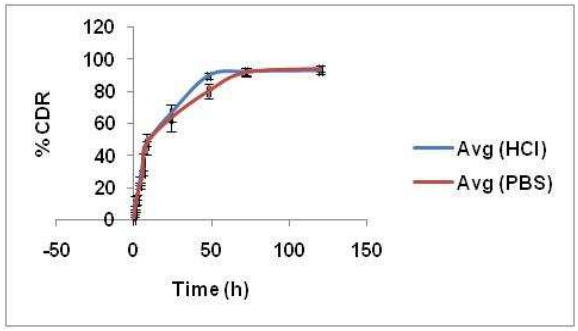

Fig. 8: *In vitro* drug release profile of optimized CS nanoparticles (n=3)

Table 5: Release kinetics of chitosan nanoparticles of the optimized batch (mean value±SD), *n*=3

Dissolution medium	Zero order		First order		Hixson		Higuchi		Korsmeyer-Peppas	
	k	R^2	k	R^2	k	R^2	k	R^2	k	R^2
0.1N HCL	2.558	0.835	0.017	0.911	0.053	0.882	14.18	0.950	0.616	0.981
7.4 PBS	2.429	0.916	0.016	0.851	0.649	0.896	13.32	0.9488	0.677	0.923

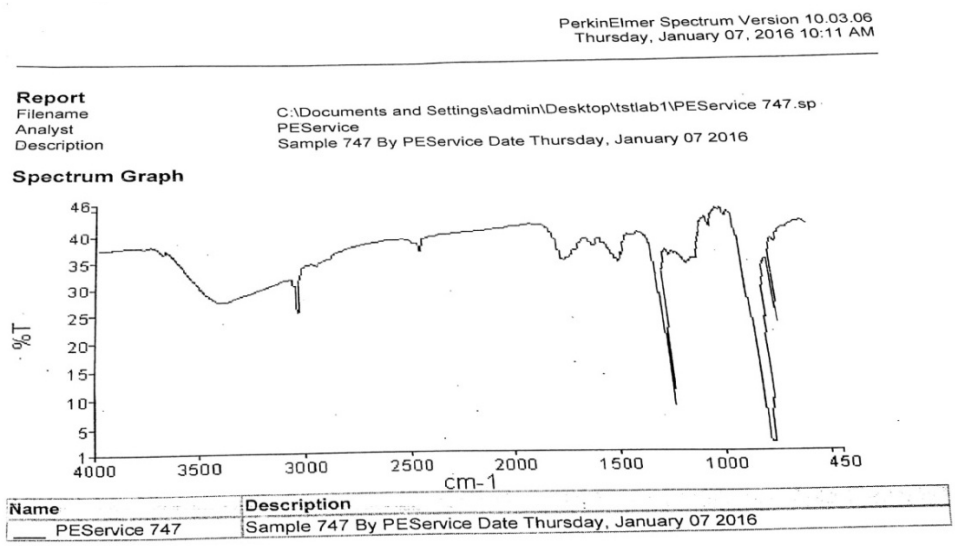

Fig. 9: FTIR of pure chitosan

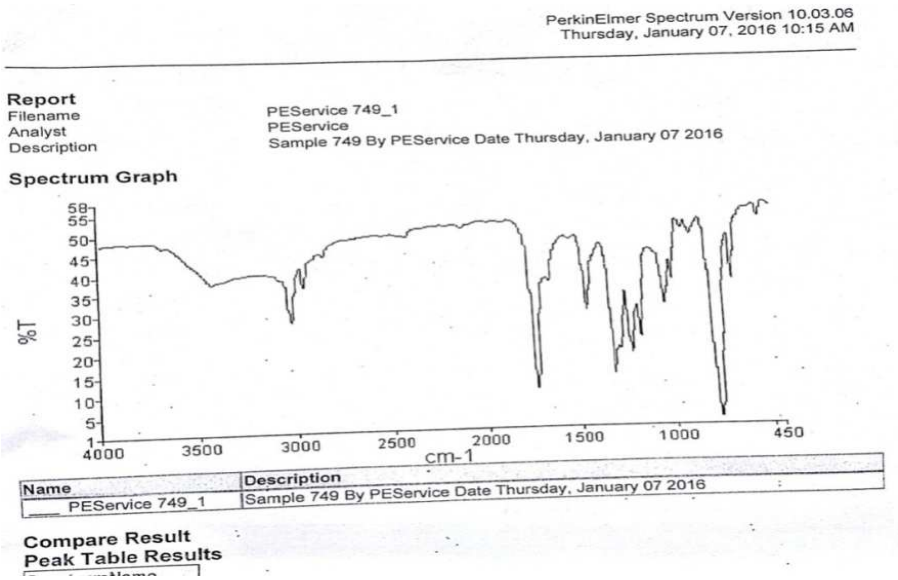

Fig.10: FTIR of pure DMF

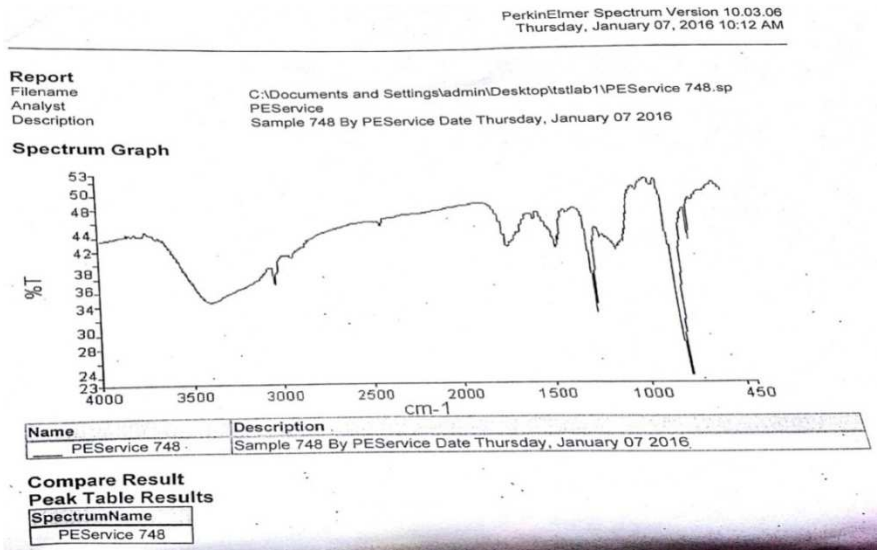

Fig. 11: FTIR of pure dextran sulphate

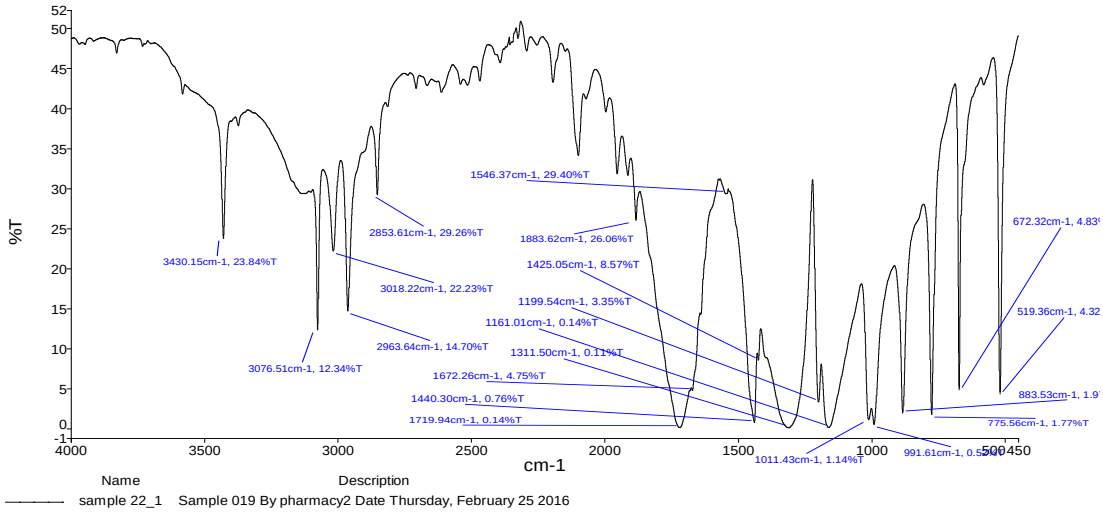

Fig. 12: FTIR of optimized DMF loaded CS nanoparticles

The aim of present study was to synthesize and optimize a biocompatible CS-DS nanoparticle of DMF for the treatment and management of MS. DMF loaded CS-DS nanoparticles can be produced by the polyelectrolyte complex coacervation technique and tween 80 was used to prevent the aggregation of nanoparticles prepared by above method. The particle size was found to be increased with increasing amount of polymer DS and CS. An equimolar ratio of drug and both the polymers yield an optimum sized nanoparticle. The average zeta potential value is-40 mv which is optimum to maintain the stability of nanoparticles at an equimolar ratio. All the measured zeta potential values for different batches are negative. A negative zeta potential indicates the CS-DS coating of DMF nanoparticles. A higher value of zeta potential implies that high energy will be needed to bring two particles in contact. Thus the prepared CS-DS coated DMF nanoparticles have a high energy barrier for the aggregation of nanoparticles. The entrapment efficiency was also improved with the use of tween 80.

In vitro release kinetics was fitted with dissolution models zero. First, hixon, Higuchi and Korsmeyer-peppers and on the basis of the correlation coefficient (R^2) value the best fit model was selected. Korsmeyer-peppers model showed a higher R^2 value and the n value indicated that release kinetics follows diffusion coupled with polymer matrix relaxation. Results of SEM image showed that the particles are spherical in shape with a smooth surface. The particle size varied from 240 to 320 nm.

Fig. 13: SEM image of optimized batch

		Size (d.nm):	%intensity	Width (d. nm):
Z-Average (d.nm): 324.5	Peak 1:	323.7	97.9	89.94
Pdi: 0.367	Peak 2:	0.000	0.0	0.000
Intercept: 0.950	Peak 3:	0.000	0.0	0.000
Result quality: Good				

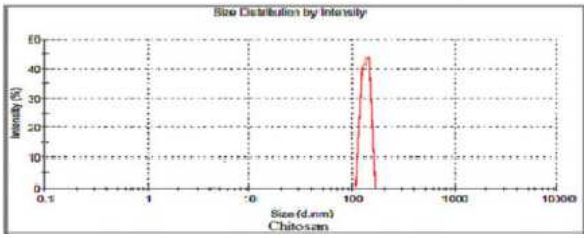

Fig. 14: Particle size distribution of optimized batch

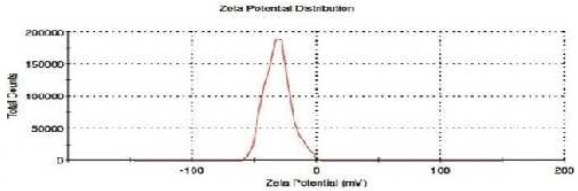

Fig. 15: Zeta potential of optimized batch

CS is a biocompatible and natural biodegradable polymer which shows its compatibility with entrapped DMF. Based on the results it is clear that an equimolar ratio of CS and DS produces a nanoparticle formulation with least particle size and an optimum zeta potential. All optimized nanoparticles showed a mean diameter of 320 nm and thus these are a promising formulation to reduce the side effects of currently available tablet and pellets of DMF.

CONCLUSION

In the present study, a novel nanoparticle formulation of DMF was investigated. The effect of three independent variables which are a concentration of CS, concentration of DS and amount of DMF was investigated on two dependent variables which are particle size and % entrapment efficiency. An optimized batch of nanoparticles was formulated and characterized as per RSM. The observed values were found to be promising when compared with the design expert software results. The *in vitro* release kinetics shows a controlled and sustained release profile. From the present research work it could be concluded that DMF nanoparticles can be successfully prepared with CS and DS polymers. The SEM image also confirms the formation of smooth and spherical nanoparticles. The prepared nanoparticles carry a good poly dispersibility index and zeta potential values.

CONFLICTS OF INTERESTS

Declare none

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