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Polymeric Micelles of Oregano - Formulation and *In-Vitro* Evaluation

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Abstract:

Polymeric micelles are most popular and promising drug delivery systems in recent years. Polymeric micelles are self assembled nano sized colloidal particles and size is ranges from 10-100 nm. The aim of the present study is to formulate oregano polymeric micelles to improve its aqueous solubility. Oregano loaded polymeric micelles were prepared with different polymers like PVA, PEG4000, and Pluronic F127 using solvent diffusion technique. The drug and polymer ratio were taken by Box-Behnken design. Optimization of the micellar formulation was done by using response surface method (RSM). The active constituents of oregano were analyzed by HPLC at 274 nm. The retention time of oregano in HPLC was found at 11min. The prepared polymeric micelles have low CMC value, the particle size and zeta potential was found to be in between 53.9±6.4 to 255±7.2 and -11.7±2.2 to 42.9±0.9 respectively. *In vitro* drug release studies revealed that 70.1 ± 1.81 % of drug released from micelles after 24h. The entrapment efficiency was found different for all formulations due to different concentration of drug and PEG used. The optimized oregano polymeric micelle formulation was further evaluated by phase contrast microscopy, FT-IR analysis and *in-vitro* drug release kinetics. Optimized micelle formulation displayed a spherical- shaped morphology, no drug-excipient interactions and follows first order so the formulations released in sustained manner, and it also follows Higuchi and Hixson crowell release mode. It is concluded that the prepared oregano polymeric micellar system has an excellent potential delivery with increased solubility to be used orally.

Keywords: Oregano; Amphiphilic copolymers; Pluronic F127; Polymeric micelles.

Introduction

Polymeric micelles (PMs) are the great potential drug delivery system for the hydrophobic compounds which have poor bioavailability. Polymeric micelles are self-assembling nanoscopic core-shell structures formed by amphiphilic copolymers the size range generally between 1 to 100nm. (1) The PMs contains an inner hydrophobic core in which hydrophobic drugs can be entrapped and protected, while hydrophilic shell supports and stabilize the hydrophobic core in the aqueous medium and enhance water solubility of the polymers. (2) CMC is the minimum concentration of amphilic polymers required to self-assemble into micelles in solution. CMC is critical indicator of micellar solubility and stability.(3,5,6) Diblock copolymers, like polystyrene and poly (ethylene glycol) PEG: triblock copolymers like poly (ethylene oxide), and graft copolymers like stearic acid and

G-chitosan used in the preparation of polymeric micelles.(4) PEG/PEO chain embedded in copolymer backbone has ability to prolong circulating time of drugs. The hydrophilic corona of micelles can prevent opsonization of protein or blood components and thus prolonging micelles circulation in blood. PVA, PEG 4000 is a water soluble polymer which is non-toxic and non-immunogenic. Pluronic F127 is also called as poloxamer 407 which is a block amphiphilic synthetic polymer. which has PEO block hydrophilic &PPO block hydrophobic in nature arrange in triple block assembly.(7,8) Most commonly used methods for preparation of polymeric micelles are direct dissolution, solvent casting and dialysis.(2)

Oregano (origanum vulgare) composed primarily of monoterpenes, major compounds (80%) are carvacrol, and thymol while lesser compounds include rosmarinic acid, p-cymene, caryophellene etc. Oregano is naturally occurring plant which posses multiple pharmacological actions such as anti-inflammatory, antiviral, ant diabetic and other possible health benefits like cough, asthma, painful menstrual cramps etc. it has disadvantages such as poor aqueous solubility and poor bioavailability.(9,10)

The proposed research work is to enhance the solubility and dissolution rate of oregano by encapsulating it into polymeric micelles using amphiphilic polymers. The enhanced solubility would help to reduce dose of drug in formulation. The prepared oregano polymeric micelles have superior control release property and tissue penetration capability. In addition to that they have versatile loading capacity and are stable even at variable physiological conditions.

Materials and Methods

Oregano (sozo) was purchased from Madangir, DR Ambedkar nagar, New delhi. PVA, PEG 4000, Pluronic F127 were obtained as a gift samples from S.d fine chemical Pvt, Ltd, Mumbai. All other reagents and chemicals used were of analytical grade and purchased from Indian scientific company pvt ltd, Tirupati.

Extraction of chemical constituents from oregano dried leaves

Dried oregano leaves powder was suspended in 90%v/v ethanol in round bottom flask. Extraction carried out by a soxhlet apparatus in heat reflux technique in water bath at 95℃ for 6hr. The extracts were filtered and dried. The residue obtained was stored in freezer till further use. (11)

Determination of chemical constituents by HPLC

The active chemical constituents of oregano were determined by HPLC (Isocratic Shimadzu LC 20) method. Extracted Sample was reconstituted, sonicated for 60 minutes, centrifuged for 15 min and then the supernatant was filtrated before spiking in to the HPLC column with nylon membrane filter (0.2µ) and analyzed at λ_{max} of 274 nm. Data acquisition and processing were, performed and samples were run with mobile phase composed of ethanol-water (60:40 v/v) with flow rate of 1.0 ml/min and column oven temperature was maintained at 25℃. Sample volume used for injection was 20µL (100µg /ml) (12).

Formulation of oregano polymeric micelles

Oregano polymeric micelles were prepared by solvent diffusion technique. Accurate amount of drug and polymer were dissolved in 20 ml of acetone and water respectively, under sonication (UP 200, Ultraschall processor). Drug solution was added drop wise to an aqueous polymer dispersion using programmable syringe infusion pump under magnetic stirring (Remi equipment, Mumbai). The setup was kept under room temperature overnight to ensure acetone evaporation. Final volume of polymeric micelles dispersion was filtered (0.45 µm) and stored in amber glass vials till further use.(13)

Characterization of oregano polymeric micelles

Critical Micelle Concentration (CMC) Determination: The CMC value of polymer mixture was determined by UV-Visible spectroscopic method (Shimadzu, 1801 Japan) using iodine as a hydrophobic probe. In this method different concentrations from 10-100µg/ml solutions were prepared, 25ml of KI/I_2 solution was added to each of polymer concentration, and mixture was left in dark room temperature for 12hrs and absorbance was measured at 366nm.(14)The polymer concentration at which the absorbance increases rapidly, that concentration was taken as CMC value. The graph was plotted by polymer concentration (µg/ml) V/s absorbance.

Particle size and zeta potential

The average particle size and their poly dispersity index (PDI) of prepared oregano polymeric micelles were determined by zetasizer (Nanoparticle SZ 100, Horiba scientifics) method (DLS) angle of 173^0. All experiments were carried out in triplicate and data are represented as mean ± standard deviation (SD).(15)

Lyophilization of oregano polymeric micelles

To improve the stability of oregano loaded micelles, those samples were lyophilized. Mannitol was used as a cryoprotectant to preserve the particle size and shape. The oregano micelles were freeze-dried in two steps. In the first step, the micellar solution was converted to dry ice cake at -70°C for 24h in the Deep freezer (Scientific Lab Instrument, Chennai, India) and in the second step these vials containing a micellar solution in form of dry ice cake were transferred to the lyophilizer (Scientific Lab Instrument, Chennai, India) and lyophilized at −70°C for 24h.(7)

$$\% \text{ cumulative re-lease} = \frac{\text{(amount of oregano in the medium)}}{\text{(amount of oregano loaded in the micelles)}} \times 100$$

Encapsulation efficiency (% EE)

After lyophilization of 1mg of polymeric micelles were accurately weighed then, 1 ml of ethanol was added, sonicated for 3min and kept them in dark for 1hr at room temperature, and then samples were measure by UV absorbance at 274nm. % EE was calculated using the following equation. (16)

% EE= (Total wt of the drug encapsulated in micelle)/ (Total wt of drug added initially) × 100

Particle shape and morphology

Morphological studies of Oregano-loaded polymeric micelles (10 mg/mL) conducted by phase contrast microscopy. Samples were dispersed on glass slide and observed at high magnification with an Olympus optical microscope (model CH30RF200, Olympus Tokyo, Japan).

In vitro drug release studies: Membrane dialysis method was used to establish the *in-vitro* release profile of freshly prepared oregano polymeric micelles. 2ml of micelle solution was placed in dialysis bag (Mol. Wt. cut-off 12000 Da). The bag was closed on both sides and immersed in a glass container containing 50ml of released medium (phosphate buffer pH 7.4). The container was placed at 37℃. About 2ml of release medium was withdrawn at different time intervals (1, 2, 3, 4, 8, 12, 16 and 24 hr). The amount of oregano was determined from the UV absorption at 274nm, after every withdrawal, release medium was replaced with fresh buffer. Percentage cumulative release of oregano at different time intervals was calculated from the below equation. Then, the oregano release profiles were obtained by plotting the mean values ±S.D. versus time. (16,7)

Release kinetic study

To evaluate the release mechanism of oregano from polymeric micelles, the drug release profile was fitted to zero-order, first-order, Higuchi's, Hixson–Crowell's, and

Korsmeyer–Peppas's models (Microsoft®Excel 2007 software). In this case, the model with the highest correlation coefficient (R2) was considered as the best fit model.(16,7)

FT-IR studies

FT-IR spectra of pure extract and drug loaded micelle were recorded in FTIR spectrometer (Shimadzu 8400S, Japan). Dispersion of drug was prepared with KBr (1:100 ratio). The prepared dispersion was placed in a sample holder for analysis. The samples were scanned over a range of 4400-400cm^{-1}. (7)

DSC studies

Thermal analysis was performed using a DSC (METLER, STAR^0 SW 8.10). DSC thermograms were recorded for oregano extract, and extract and polymer mixture. 10mg of each sample was weighed and sealed in standard aluminum pin holed pan, with a heating rate of 10ºC/min from 50 to 300ºC throughout the measurement.(18,20)

Stability studies

To evaluate the storage stability of prepared polymeric micelles, lyophilized oregano loaded micelles was stored at room temperature for 3 months. At the end of 3 months, the sample was collected. This sample was subjected to particle size, % entrapment efficiency and pH determination. (19)

pH determination

The determination of hydrogen potential (pH) was performed directly on the polymeric micelles in the pH meter. Potentiometer previously calibrated wit PH 4.0 and 7.0 buffer solution. The pH was evaluated during the stability test.(17)

Experimental design

In the present work Box-Behnken response surface methodology was selected in order to investigate the effect of independent variables on response. The factors were selected according to the literature review. Drug concentration (mg) (X1), polymer ratio (mg) (X2), stirring time (X3) were chosen as independent variables varied at three different levels of low, medium, and high (-1, 0, +1) respectively as summarized in table 1 . The dependent variables estimated were particle size (Y1), % entrapment efficiency (Y2), % cumulative drug release (Y3). The mathematical relationship of responses and independent variables were modeled by polynomial equation. By means of this equation it would be possible to investigate the linear, quadratic and interactive effects of the independent variables on responses. (7,20,21,22)

Table -1: Independent variables and there levels

S.NO	Independent variables	Levels		
		(low)-1	(medium) 0	(high)+1
1	Drug concentration(mg) (x1)	50	100	150
2	Polymer ratio(mg) PEG(x2)	250	500	750
3	Stirring time(min) (X3)	15	30	45

Statistical Data analysis

The prepared oregano polymeric micelle formulations of F1- F13 (table-2) were optimized by Box-Behnken design (RSM) using DoE Software (Design Expert @ v.13). By inserting the values of dependent variables in design expert software which will examine the formulations and gives the optimized formulation through regression analysis, ANOVA, surface response plots, contour plots and over-lay plots.

Results and Discussion

Determination of chemical constituents by HPLC

The chemical constituents of oregano were analyzed using high performance liquid chromatography (HPLC) at 274nm. The retention time of oregano extract was found

Table-2 :Composition of factorial layouts using Box-Behnken response surface method:

Runs	Coded values			Actual values			Response (Y1, Y2,Y3)		
	X1	X2	X3	X1	X2	X3	Particle size±SD	%entrapment efficiency±SD	%cumulatve drug release±SD
F1	+1	+1	0	150	750	30	219.1± 3.8	88.7±0.9	66.7±2.6
F2	-1	-1	0	50	250	30	53.9±6.4	56±5.7	49±3.3
F3	-1	+1	0	50	750	30	190.5 ± 8.8	86.4±2.8	69.2±4.2
F4	0	+1	-1	100	750	15	255± 7.2	89.3± 1.5	70.1±1.5
F5	0	-1	-1	100	250	15	85.5±8.8	65.1±3.2	47.5±5.9
F6	-1	0	+1	50	500	45	92±4.0	84.4±2.2	52.5±4.6
F7	0	-1	+1	100	250	45	70.8±8.8	59.3±6.9	45±7.5
F8	0	+1	+1	100	750	45	216±5.5	92.3±4.4	64.9±3.8
F9	-1	0	-1	50	500	15	144±4.9	76.3±7.3	54.7±6.3
F10	+1	0	+1	150	500	45	125.7±9.5	78.9±6.2	50.3±2.3
F11	+1	-1	0	150	250	30	67.6±15.2	55.6±9.1	46.7±4.9
F12	+1	0	-1	150	500	15	152±8.6	81.3±3.5	51±5.2
F13	0	0	0	100	500	30	110.4±6.4	76.4±2.7	52.9±3.5

to be at 11min.which is a characteristic peak for active constituent present in oregano i.e. carvacrol.

Optimization of polymeric micelles

The prepared thirteen formulations were evaluated for parameters like particle size, %EE, %CDR. The responses of each batch for parameters were fitted to various model by DoE Software (Design Expert @ v.13). It was observed that the quadratic models were best fit of particle size, %EE, %CDR responses.

All values of the correlation coefficient (R^2), standard deviation, percentage coefficient of variation, and results of ANOVA are shown in Table 3. Results of ANOVA and R^2 value for the dependent variables confirmed that the model was significant for the specified response variables

Effect of independent variables (drug concentration (X_1), polymer ratio (X_2), stirring time(X_3)) on responses

Table-3: Summary of Analysis of variance for dependent variables

Parameters	df	SS	MS	F	p-value	R^2	SD	Coeff. of Variance(%)
Mean Particle Size	9	51494.26	5721.58	104.58	0.0014 Significant	0.9968	7.4	5.39
Residual	3	164.14	54.71					
Total	12	51658.40						
%Entrapment Efficiency	9	2018.18	224.24	45.72	0.0047 Significant	0.9928	2.2	2.91
Residual	3	14.71	4.90					
Total	12	2032.89						
%CDR	9	968.51	107.61	87.43	0.0018 Significant	0.9962	1.1	2.00
Residual	3	3.69	1.23					
Total	12	972.20						

Particle size (Y_1)

From polynomial regression equation1 the ß coefficient indicated with positive sign which revealed that the mean particle size (Y_1) was significantly increased with increases in the concentration of drug(X_1) and polymer ratio(X_2). Therefore probability of particle-particle subsequent aggregation at high concentration which increased particle size. Stirring time showed negative effect which means increase in stirring time decreases in the particle size.

Particle size Y_1=+110.40+10.50(X_1)+75.35(X_2)-16.50(X_3)+3.72(X_1X_2)+6.43(X_1X_3)-6.07

(X_2X_3)- 3.01 ($X_1^{2)}$+25.39($X_2^{2)}$+21.04($X_3^{2)}$…… (1)

The coefficient value of interaction terms X_1 and X_2 has the significant effect on Y_1, indicated a simultaneous increasing the drug concentration and polymer ratio was significant effect on particle size. But on further increasing the drug concentration there was no significant improvement in particle size. The coefficient of variable X_2 revealed that the particle size was highly influenced by polymer ratio.

% Entrapment efficiency Y_2

From polynomial regression equation 2, the increases in polymer ratio (X_2) showed in increases %entrapment efficiency (Y_2), increasing drug concentration(X_1) and stirring time (X_3) showed slight positive value indicate the X_1 and X_3 has less significant effect on % entrapment efficiency. The coefficient value of interaction terms X_1X_2 has slight significant effect on Y_2. But on further increasing the drug and polymer ratio there was no significant improvement in % EE.

% EE Y_2=+76.40+0.1750X_1+15.09X_2+0.3625X_3 +0.6750X_1X_2-2.63X_1X_3+2.20X_2X_3

-0.5000X_1^2-4.2X_2^2+4.32X_3^2…… (2)

% Cumulative drug release (Y_3)

From polynomial regression equation 3, The positive coefficient of X_1 and X_2 indicated the increase in cumulative drug release (Y_3) by increasing the drug concentration and polymer ratio(X_2), the negative coefficient (X_3) indicated the increases stirring time (X_3) decreases cumulative drug release (Y_3), and also further increasing the drug and polymer ratio it has significant improvement in % cumulative drug release.

Y_3 = + 52.90 + 1.34 X_1 + 10.34 X_2 - 1.32X_3+0.0500X_1X_2-0.3750X_1X_3-0.6750X_2X_3

+0.1250X_1^2+4.88X_2^2-0.9000X_3^2…… (3)

ANOVA Study for Y_1, Y_2, and Y_3 responses

From statistical ANOVA results, the p-value< 0.05 indicated the model was significant and factors that gives the higher p values i.e. above 0.05 was taken as the non- significant. In the response Y1 the drug concentration, polymer ratio and stirring time was found to be significantly affecting particle size and remaining factors were not significantly affecting the particle size. The regression coefficient for response Y_2 only polymer ratio X_2 was found to be significantly affecting % EE. For response Y_3 the regression coefficient of all independent variables was found to be significantly affecting %CDR. Further increasing the proportion of polymer ratio it has significant

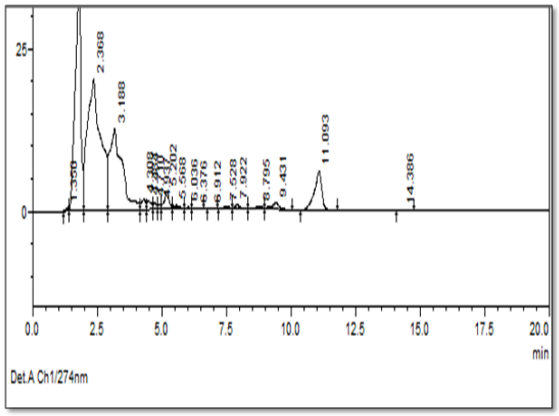

Figure-1: HPLC chromatogram oregano extract

improvement in % cumulative drug release.

2-D and 3-D contour plots of response Y_1, Y_2 and Y_3

2D contour plots and 3D response surface plots were generated for easy visualization and to show the relationship between dependent and independent variables.

2-D, 3-D and cube plots of response Y_1

The contour plots of particle size reveals linearity was shown in **figure-2** indicates all independent variables on response (Y_1) i.e particle size was found be linear in nature. When the drug concentration, polymer ratio and stirring speed kept at low level the particle size decreases. The particle size increases when the drug and polymer ratio increased by keeping medium stirring speed.

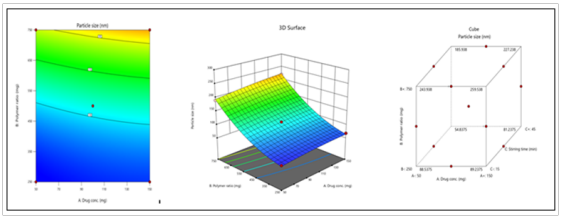

Figure-2: 2-D, 3-D surface and cube plots of Response Y1-particle size

2-D, 3-D and cube plots of response Y_2

2-D, 3-D surface and cube plots were shown in **figure-3**. They reveal that the relation between X_1 and X_2 is linear in nature. It indicates that increased drug concentration and polymer ratio, increases the %EE and stirring time shows non linearity in nature on % entrapment efficiency (Y_2). By keeping drug and polymer ratio low level 50 and 250 the %EE was decreased, then increased %EE by keeping drug and polymer ratio 150 and 750. Thus conclusively the both X_1 and X_2 influencing on the % entrapment efficiency at high level.

3.4.3. 2-D, 3-D and cube plots of response Y_3

The contour plots of % cumulative drug release reveals linearity and shown in **figure-4** indicates all independent variables on response (Y_3) i.e % cumulative drug release was found be linear in nature. The % cumulative drug release (Y_3) increases when the drug concentration, polymer ratio and stirring time by kept at high level.

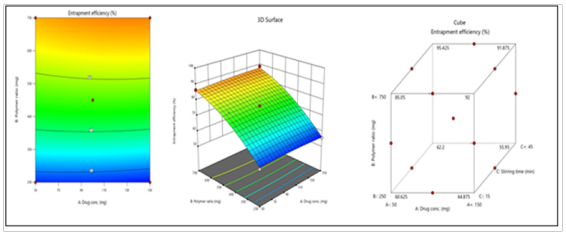

Figure-3: 2-D, 3-D surface and cube plots of Response Y1- %Entrapment efficiency

Desirability and overlay plot

From the shown response surface plots and polynomial equations, the desirability and overlay plots were obtained and were shown in **figure-5**. Then the software produced several solutions to determine the optimized formulation among that the one of solution was taken and considered as the best. Composition of formulation was obtained through this overlay plot and optimized formulation was formulated and compatibility and stability studies were performed.

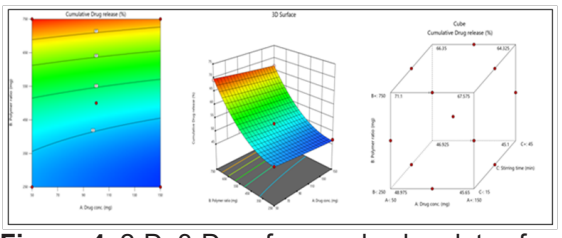

Figure-4: 2-D, 3-D surface and cube plots of Response Y1- % CDR

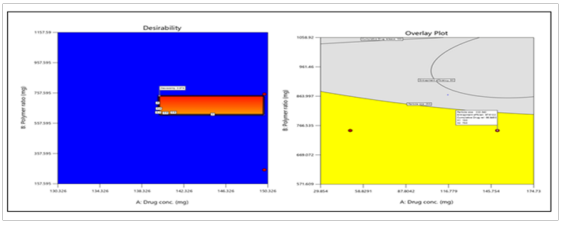

Figure-5: Desirability and overlay plot

Characterization of optimized oregano micelle formulation

CMC Determination of optimized formulation: The polymer concentration at which the absorbance increases rapidly, that concentration was taken as CMC values. The CMC of optimized formulation was found to be 40µg/ml. At this point micelles begin to form as vesicles. As the polymer concentration increases the absorbance also increases. At the point of 40 µg of concentration of polymer the threshold of micelle prepared were very much faster. If increases in polymer concentration number of micelles formed will increases.

Particle Size, Polydispersity Index Analysis and zetapotential: Oregano polymeric micelles were characterized for particle size analysis using Zetasizer (Nanoparticle SZ 100, Horiba scientifics). The mean particle size of formulated polymeric micelles was shown in table 2. The formulation (F1) shows mean particle size and polydispersity index was found to be 219±3.8 and 4.6±0.4 respectively, PDI value is increased indicating that prepared polymeric micelles are not uniformly dispersed and aggregation of particles in the formulation. Zeta potential was found to be -32.6± 1.2. The zeta potential is important factor to ensure stability; high charged micelles are capable of remaining stable as colloidal suspension.

% Entrapment efficiency (%ee)

The % EE of prepared oregano polymeric micelles was shown in Table-2. % entrapment efficiency was measured by UV-visible Spectrophotometry. In the preparation of oregano polymeric micelle, it was observed that drug and polymer concentration increases, the % entrapment efficiency increased respectively. But on further increasing the drug and polymer ratio there was no significant improvement in % EE.

Particle shape and morphology**:** The morphology of polymeric micelles were studied by phase contrast microscopy was shown in **figure-6**, and the results shows that, well distributed particles and mostly spherical in shape.

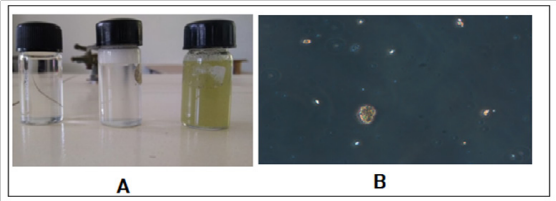

Figure-6: (A) Comparison of water, blank micelles and oregano polymeric micelles, (B) Phase contrast image of optimized formulation

In vitro drug release study

In vitro Drug Release profile of oregano polymeric micelles was shown in **figure-7** after 24h 66.7±2.6 % drug released from micelles, whereas, 24.7±3.2% drug released from plain oregano in 24h, indicating improved release of oregano from micelles. In this study observed that drug and polymer concentration increases, % drug release was increased respectively, and also further increasing the drug and polymer ratio it has significant improvement in % cumulative drug release.

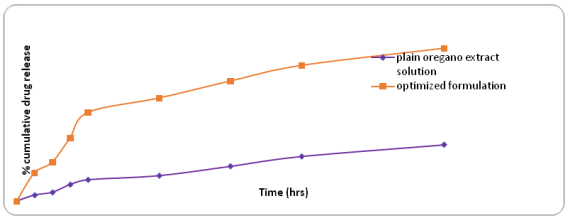

Figure-7:Cumulative % drug release of pure oregano extract and optimized oregano polymeric micelles formulation.

Drug release kinetic studies

In this study, *In-vitro* oregano polymeric micelles release profile of optimized formulation was fitted to different kinetic models. The mathematical analysis comprised the fitting to zero-order, first-order, Higuchi's, Hixson–Crowell's and Korsmeyer– Peppas models and R^2 values obtained for each model were 0.815, 0.922, 0.964, 0.951 and 0.635

respectively. Depending on the good fit (R^2), the model meeting criterion is first order so the polymeric micelles showed a sustained release. The release mechanism could be best fitted to Higuchi and Hixson-crowell, the release mechanism of oregano from micelles might be and dissolution.

Drug- polymer compatibility studies

FT-IR Studies: The FT-IR spectra of oregano extract (A), oregano+ polymeric mixture (B) and oregano polymeric micelles(C) were shown in **figure-8**. Three spectra were compared, and absence of extra peaks indicates that no interactions between drug and polymers. Thus the selected polymers and drug were compatible with each other.

Differential Scanning Calorimetery: The sharp peak oregano extract observed at 163.6º and oregano polymeric mixture of pluronic F127 and PEG was observed at 55.10ºC, peak of extract was observed at 159.8ºC and PVA shows peak at 185.5º. DSC thermograms of extract, extract polymer mixture were shown in Fig. 9A and 9B respectively. From the result of DSC, it was clarified that drug and polymer were compatible with each other.

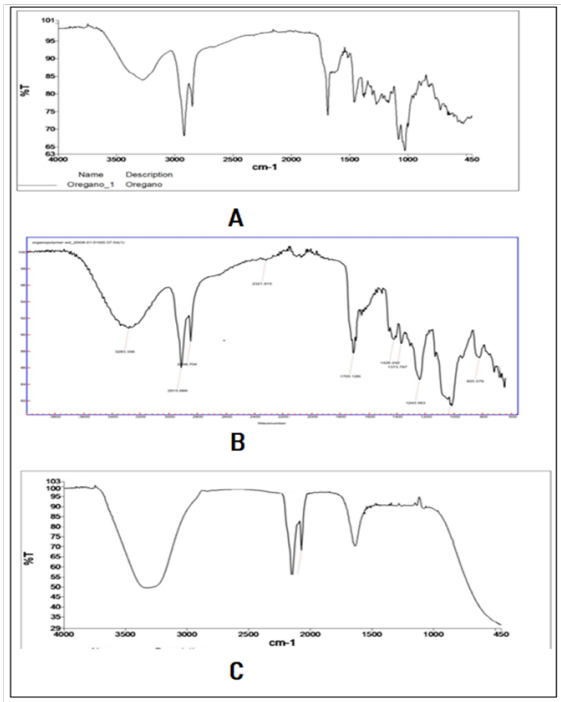

Figure-8 FTIR spectra of (A) oregano extract (B) Extract+ polymer mixture (C) oregano polymeric micelle.

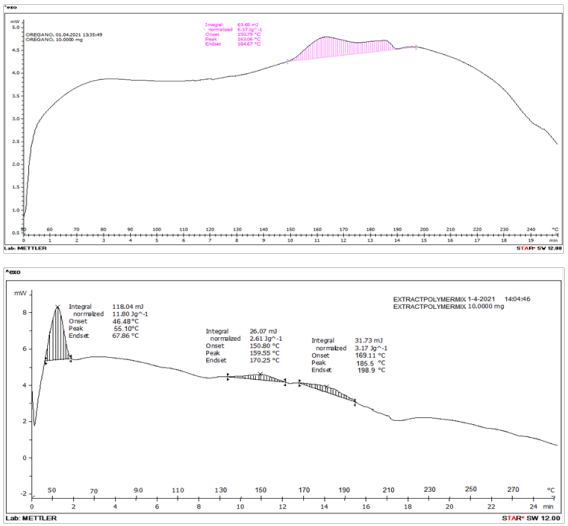

Figure -9 (A) DSC of oregano extract and (B) extract polymer mixture

Stability Study

The optimized polymeric micelle formulation was kept for stability study for 3 months. The stored lyophilized sample was taken, re dispersed and subsequently checked for the particle size, % entrapment efficiency and pH. As shown in Table 4, formulated polymeric micelles remained stable over this period of study as there was no alteration in mean particle size, % EE and pH results.

Table-4: Stability Studies for optimized formulation

Parameters	Immediately after prepa-ration	After 3 months
Particle size(nm)	211.4	219.7
Entrapment effi-ciency (%)	88.7	85.2
pH	7.1	6.9

Conclusion

Oregano polymeric micelles were formulated using solvent diffusion technique and Box-Behnken response surface methodology was used to investigate the effect of independent variables on responses. The optimized formulation of polymeric micelles has low CMC, smaller particle size and negative zetapotential, high %EE and they are spherical in shape. Low CMC suggested stability of micelles upon dilution. Oregano polymeric micelles shows high drug release when compared to crude drug, In-vitro drug release kinetics follows first order, so formulation shows sustained release characters and the drug release mechanism drug diffusion and dissolution of polymer material. The FT-IR and DSC studies demonstrated no drug-excipient interactions. Formulated polymeric micelles were stable during period of study and there was no drug deterioration. The results demonstrated that prepared oregano polymeric micelles were more soluble and subsequently they have enhanced bioavailability.

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