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Article

Formulation and Optimization of Diclofenac Sodium Loaded Ethylcellulose Nanoparticles

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Design of experiment (DoE) is a useful time and cost-effective tool for analyzing the effect of independent variables on the formulation characteristics. The aim of this study is to evaluate the effect of the process variables on the characteristics involved in the preparation of Diclofenac Sodium (DC) loaded ethylcellulose (EC) nanoparticles (NP) using Central Composite Design (CCD). NP were prepared by W/O/W emulsion solvent evaporation method. Three factors were investigated (DC/EC mass ratio, PVA concentration, homogenization speed) in order to optimize the entrapment efficiency (EE) and the particle size of NP. The optimal formulation was characterized by Fourier Transform Infrared (FTIR), Scanning Electron Microscopy (SEM), Differential Scanning Calorimetry (DSC), and *in vitro* release. Optimized formulation showed an EE of 49.09 % and an average particle size of 226.83 nm with a polydispersity index of 0.271. No drug-polymer interaction was observed in FTIR and DSC analysis. SEM images showed that the particles are spherical and uniform. The in vitro release study showed a sustained release nature, 53.98 % of the encapsulated drug has been released over 24hours period. This study demonstrated that statistical experimental design methodology can optimize the formulation and the process variables to achieve favorable responses.

Keywords: Design of experiment. Nanoparticles. Diclofenac sodium. Sustained release.

INTRODUCTION

Nanoparticles are one of the promising drug delivery systems for controlling particle size, surface properties and release of therapeutic ingredients in order to reach the target at the therapeutic desirable proportion and rate regimen (Mohanraj, Chen, 2006). Biocompatible, biodegradable and non-biodegradable polymers as Chitosan derivatives, PLA, PLGA, EC, are used for the preparation of polymeric nanoparticles by dissolution, entrapment, encapsulation or attachment of a drug to a nanoparticle matrix (Nagavarma*et al,* 2012). NP matrix carriers can improve the encapsulation efficiency and stability of the drugs inside the NPs and provide effective drug levels over longer periods of time compared to traditional therapy (Cooper, Harirforoosh, 2014). Different methods are used for the preparation of nanoparticles.One of the most used is the solvent evaporation method. Uniform concentration of drug at the site of absorption, maintaining of stable plasma concentration and reducing toxic effects can be achieved by developing controlled-release drug delivery systems (Barzegar-Jalali*et al*, 2012).

Diclofenac sodium (DC) is a non-steroidal anti-inflammatory drug used for treatment of inflammatory diseases. DC has a short half-life of 1-2h and should be administered frequently at a high dose, which leads to severe undesirable effects and rises the possibility for missing a dose (Arias *et al*, 2009). The development of sustained dosage release forms was needed to ovoid

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theses inconveniences (Krishna Sailaja, Nandini, 2016). The design of experiment (DoE) is a valuable tool used for optimization. It allows the finding of the optimal conditions for the best responses of experiments and understand the relationship between the dependent and independent variables in the formulation or process development (Vera Candioti*et al*, 2014). The response surface methodology (RSM) is the combination of statistical and mathematical techniques based on the recapitulation of experimental data from experimental design (Trivedi *et al*, 2015). One of the promising RMS used in DoE is central composite design (Yang *et al*, 2014).

The objective of this study was to formulate and characterize DC-loaded EC-NP with the aim to evaluate the effect of the process variables on the characteristics involved using Central Composite Design.

MATERIAL AND METHODS

Materials

Diclofenac sodium was purchased from CAYMAN chemical company. Ethylcellulose (viscosity 22cP, 48% ethoxyl), Polyvinylalcohol (87-90% hydrolyzed, average mol wt. 30.000-70.000) and dialysis bags (cut-off 12 kDa) were procured from Sigma Aldrich USA. All other solvents and ingredients used were of analytical grade.

Methods

Preparation of nanoparticles

DC loaded EC nanoparticles were prepared by the W/O/W emulsion solvent evaporation method. First, 1mL of a DC aqueous solution (the internal aqueous phase) was emulsified by vigorous magnetic stirring into a 5mL of EC organic solution (ethyl acetate). Then this primary emulsion (W/O) was diluted in 10 mL of PVA aqueous solution (the external aqueous phase) while stirring using a homogenizer (KINEMATICA, Polytron PT 2500 E) in order to create the W/O/W emulsion. The NP suspension was obtained after solvent evaporation under magnetic stirring at room temperature. NP were separated by centrifugation (Sigma 3-30 KS, Germany) at 20.000 rpm for 20 min. The supernatant was kept for drug assay as described later.

Characterization of nanoparticles

Entrapment efficiency(EE)

For measuring drug entrapment efficiency in the NPs, the supernatant part of the centrifuged NPs sample was carefully removed and examined to determine the amount of non-encapsulated drug after dilution with purified water and analysis by UV-visible spectroscopy (Shimadzu, UV 1800, Japan) at 276 nm. Entrapment efficiency (EE) was calculated as follows:

$$EE = \frac{\text{Intial weight of feeding drug} - \text{Weight of not encapsulated drug in supernatant}}{\text{Intial weight of feeding drug}} \times 100 \quad (1)$$

Average particle size

The particle size of nanoparticles was determined using dynamic light scattering technique at 25ºC using a Zetasizer (Horiba scientific, nano partica SZ-100). All measurements were performed in triplicate.

Fourier Transform Infrared spectroscopy (FTIR)

FT-IR spectra for DC, EC and the optimized nanoparticles were generated by means of FT-IR spectrophotometer (JASCO, FT/IR-6300, United States). This was used to investigate whether there was any degradation or chemical interaction between the polymer and the active component after formulation. Spectra were recorded from the powder in the range of 400–4000 cm^{-1}, at room temperature.

Differential scanning calorimetry (DSC)

DSC thermograms of DC, EC, and the optimized NPs were determined by a differential scanning calorimeter (DSC 131, SETARAM instrumentation, France). Each sample, 2 to 3 mg, was accurately weighed into a close aluminum solid pan. The scanning rate was

run at 10°C /min from 20 to 300°C under argon purge. DSC analysis of pure DC and EC was performed to identify the drug melting point peak and polymer glass transition temperature (Tg), respectively. The optimized NPs of DC–EC were also analyzed to observe the change of the melting endotherm of DC.

SEM

Morphological observation of optimized NPs was carried out using Scanning Electron Microscopy (JSM-7100F). NPs powder was mounted onto metal stubs using double-sided adhesive tape. The stubs were then coated with conductive carbon black. The morphology of the particles was then examined.

In vitro dissolution

In vitro dissolution studies were performed using USP Type II dissolution test apparatus (Paddle) (Distek 2500, Inc., USA) at 50 rpm and a temperature of 37 °C ± 0.5. In a dialysis bag, 18.7 mg of the optimized NPs containing 4.6 mg of DC was diluted by Phosphate buffered saline solution (PBS, pH=7.4), then was immersed into a Pyrex flask that contains 500 mL of PBS (pH=7.4). At predetermined intervals, 3mL of aliquots were withdrawn and replaced by the same volume of PBS (pH= 7.4). Then the aliquots were filtered using a 0.45 μm membrane filter, diluted suitably, and analyzed by a UV spectrophotometer at 276 nm. The dissolution study was carried out in triplicate and their average was used for determining the release kinetics.

Kinetics of drug release

The *in vitro* drug release data was analyzed according to zero order, first order, Higuchi and Korsemeyer-peppas model. The selection of the most suitable model was based on the regression coefficient.

Experimental design

The effects of formulation factors on the NPs characteristics and the optimization procedure were examined by employing a CCD. The design and statistical analysis were performed by Minitab 18® Software for design of experiments (DOE).

TABLE I - Variables in CCD

Independent variables (Factors)	**Level used, real and coded values**		
	Low (-1)	**Intermediate (0)**	**High (+1)**
X_1: Mass Ratio of DC / EC (%)	66.66	73.33	80
X_2: PVA concentration (%)	0.5	0.7	0.9
X_3: Homogenization speed (rpm)	8000	9000	10000
Dependent variables (responses)	**Constraints**		
Y1: Entrapment Efficiency (EE)	Maximize (20-100)		
Y2: Particle size (nm)	Minimize (100-400)		

Experimental factors and their levels were determined in preliminary studies using full factorial design (data not shown). The factors evaluated in this investigation, were the mass ratio of DC/ EC (X_1: $\frac{mEC}{mDC+mEC} \times 100$), the PVA concentration (X_2: w/v %) and the homogenization speed (X_3: rpm) with different levels for each factor as described in Table I (coded and real values). The evaluated responses were the entrapment efficiency (Y1) and the average particle size (Y2).

TABLE II - Observed responses in CCD for DC nanoparticles

Batch	Depend ent variables			Independent variables		D
	X_1 (%)	X_2 (w/v %)	X_3 (rpm)	Y1 (%)	Y2 (nm)	
1	66.66	0.5	8000	41.50	224.06	0.393
2	80	0.5	8000	44.30	236.66	0.409
3	66.66	0.9	8000	43.36	271.73	0.351
4	80	0.9	8000	45.40	275.30	0.347
5	66.66	0.5	10000	40.73	215.00	0.407
6	80	0.5	10000	42.50	209.70	0.421
7	66.66	0.9	10000	40.70	241.30	0.366
8	80	0,9	10000	43.25	264.50	0.364
9	62.11	0.7	9000	42.80	252.70	0.373
10	84.54	0.7	9000	45.75	265.10	0.384
11	73.33	0.36	9000	42.25	199.60	0.427
12	73.33	1.03	9000	46.75	303.10	0.343
13	73.33	0.7	7318	46.70	275.30	0.382
14	73.33	0.7	10681	43.50	227.40	0.406
15	73.33	0.7	9000	53.43	241.60	0.446
16	73.33	0.7	9000	49.06	247.30	0.446
17	73.33	0.7	9000	48.70	241.30	0.446
18	73.33	0.7	9000	51.25	237.50	0.446
19	73.33	0.7	9000	52.81	268.30	0.446
20	73.33	0.7	9000	50.90	237.60	0.446

The CCD design and the data obtained are summarized in Table II. The quadratic non-linear model generated by the design is in thefollowing form:

$$Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_{12}X_1X_2 + A_{23}X_2X_3 + A_{13}X_1X_3 + A_{11}X_1^2 - A_{22}X_2^2 + A_{33}X_3^2 \quad (2)$$

Where Y is the measured response associated with each factor level combination; A_0 is an intercept, A_1, A_2, A_3 are the linear regression coefficients, A_{12}, A_{13}, A_{23} are the interactive regression coefficients, andA_{11}, A_{22}, A_{33} are the quadratic regression coefficients; X_1, X_2 and X_3 are the studied factors; X_1^2, X_2^2, X_3^2 are the quadratic effects, X_1X_2, X_2X_3, X_1X_3 represent the interaction between the variables (Vera Candioti*et al*, 2014) An analysis of variance (ANOVA) was performed to establish the optimum conditions.

The desirable optimum region was selected by the desirability function method, wherein the constraints for choosing an optimum formulation were further narrowed as shown in Table I. First, the responses Y1 and Y2 are transformed into individual desirability function d1 and d2 that vary over the range $0 \leq (d1, d2) \leq 1$ (Fitrianto, Midi, 2012). For the first response, the desirability function d1 should be maximized as follow:

$$d1=\left(\frac{Y1-L}{T-L}\right) \quad (3)$$

For the second response, the desirability function d2 should be minimized as follow

$$d2=\left(\frac{Y2-U}{T-U}\right) \quad (4)$$

Where, T is the target value desired, U, L are the upper and lower acceptable values of response. The overall desirability value (D) is calculated by the following equation

$$D=\sqrt{d1\times d2} \quad (5)$$

The validation of the derived polynomial equations and the optimized formulation selected was carried out by the preparation of four optimum checkpoint formulations based on their predicted values for the response variables. The error prediction was calculated by comparing experimental values of the responses with the predicted values (Motwani *et al*, 2008).

RESULTS AND DISCUSSION

Experimental design

The results were assessed for R^2, adjusted R^2, p-values (model and lack of fit) as quality indicators for the model. Comparing the second order model and full quadratic model, the second order model had the highest R^2 values, highly significant model *p*-value ($p<0.001$), and insignificant lack of fit p-value ($p> 0.10$) as listed in Table III.

For thefitted model, the experimental response might be represented by the following regression equation for both responses Y1 and Y2:

$$Y=A_0+A_1X_1+A_2X_2+A_3X_3+A_{11}X_1^2+A_{22}X_2^2+A_{33}X_3^2 \quad (6)$$

TABLE III - Regression analysis of the studied responses

	model	Adjusted R^2	R^2	*p*-value		Observation
				model	Lack of fit	
Y1	Second order	0.859	0.904	<0.001***	0.892	Suggested
	Full Quadratic	0.828	0.906	<0.001***	0.723	-
Y2	Second order	0.792	0.857	<0.001***	0.528	Suggested
	Full Quadratic	0.737	0.861	<0.001***	0.329	-

The followings are the regression equations of the fitted model with statistically significant terms:

$$Y_1=51.07+1.03X_1+0.82X_2-0.93X_3-2.7X_1^2-2.62X_2^2-2.41X_3^2$$

$$Y_2=245.99+4.02X_1+25X_2-11.55X_3+2.15X_1^2-0.51X_2^2-0.51X_3^2$$

ANOVA was carried out to evaluate the significance of the fitted models on the responses and their quantitative effects. The effects of the model terms (intercept and coefficient) and associated *p*-values for the two responses are listed in Table IV. The value and sign of the quantitative effect correspond to the extent and the trend of the terms influence on the response, respectively. The term with a positive value in the regression equation show synergistic effect, whereas a negative value shows an antagonistic effect between the factor and the response.

TABLE IV - The coefficients of responses in the CCDdesign

Term		Responses	
		Y1	Y2
X_1	Coefficient	1.034	4.022
	p-value	0.025*	0.225
X_2	Coefficient	0.823	25.004
	p-value	0.064	< 0.001***
X_3	Coefficient	-0.934	-11.555
	p-value	< 0.001***	0.003**
X_1^2	Coefficient	-2.707	2.152
	p-value	< 0.001***	0.496
X_2^2	Coefficient	-2.627	-0.518
	p-value	< 0.001***	0.869
X_3^2	Coefficient	-2.415	-0.518
	p-value	< 0.001***	0.869
Intercept	Coefficient	51.074	245.990
	p-value	< 0.001***	< 0.001***

For the optimized formulation, the observed value of drug loading (DL) is DL = 24.59%, the predicted maximum values of the responses are EE= 49.09% and particle size = 226.83 nm along with an individual desirability of 0.36 and 0.57, respectively. The overall desirability has a value of 0.458 with factors setting at 73.66% for the mass ratio, 0.56% for the PVA concentration and 9220.84 rpm for homogenization speed (Figure 1).

For the four checkpoint formulations, the results of the evaluation for EE and particle size were found to be within acceptable limits as listed in Table V. The validity of the generated regression equations was evaluated by determination of the error prediction.

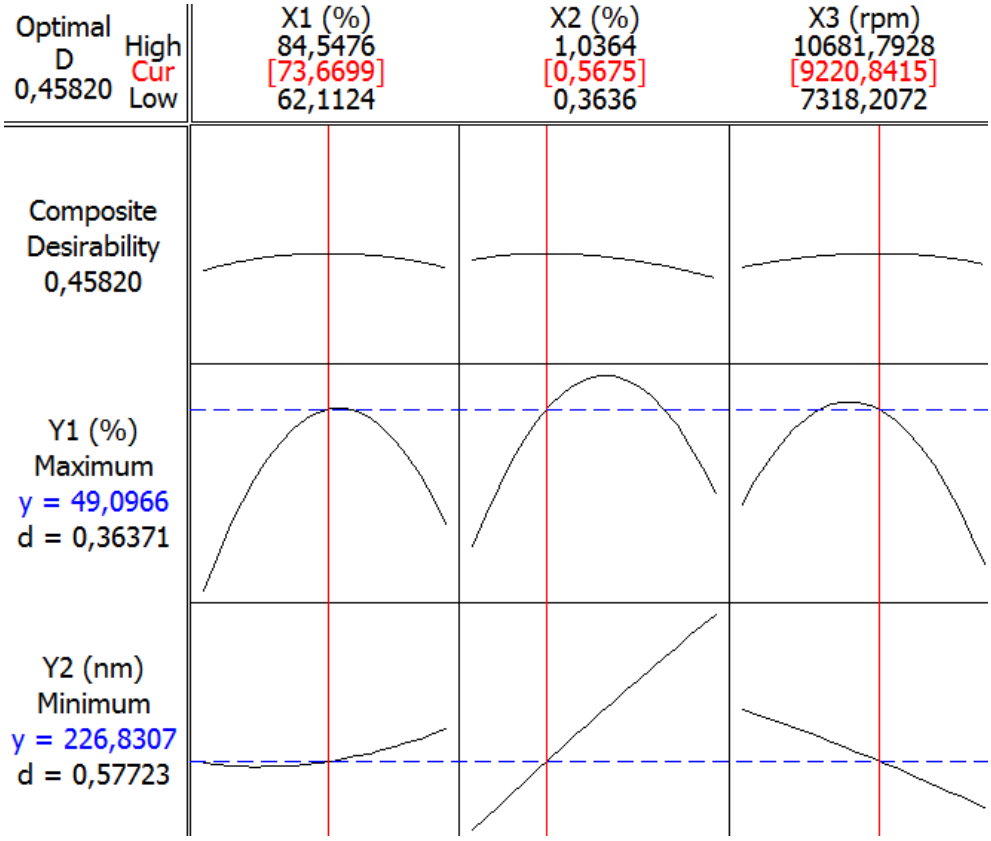

FIGURE 1- Optimum levels of factors and responses

TABLE V - Observed and predicted values and error prediction for the optimized formulation

Response variable	Observed value	Predicted value	Residual	Error prediction (%)
EE(%)	46.32	49.09	2.77	5.64
Particle Size (nm)	224.16	226.83	2.67	1.17

Fourier Transform Infrared

The FTIR spectra revealed that there was no interaction between DC and the polymer. The characteristic absorption peaks of Diclofenac Sodium were obtained at wave numbers of 3384.46 cm^{-1} (NH stretching of the secondary amine),1570. 74 cm^{-1} (–C=O stretching of the carboxyl ion), 1554.34 cm^{-1} (C=C ring stretching) and at 741.49 cm^{-1} (C-Cl stretching) (Kebebe, Belete, Gebre-Mariam, 2010). The absorption peaks of EC were obtained at 1051.01 cm^{-1} (C–O–C stretching), 2969.84 cm^{-1}(C–H stretching) (Madni*et al*, 2014). In the IR spectrum of NPs, peaks corresponding to DC, NH stretching of the secondary amine (3387.35 cm^{-1}), C-Cl stretching (743.42 cm^{-1}) still present, in contrary to C=O stretching of the carboxyl ion and C = C ring stretching which disappear or are buried in the peaks of EC indicating drug entrapment and the absence of chemical interaction between polymer and drug in nanoparticles as shown in Figure 2.

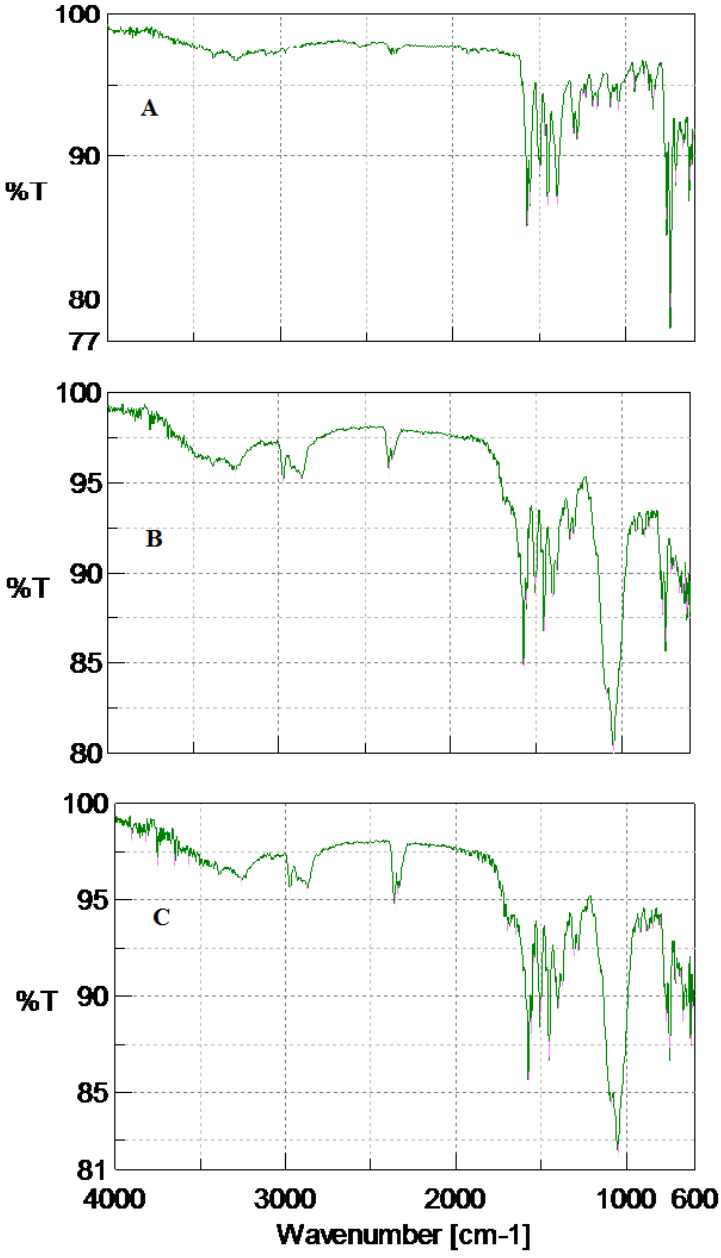

FIGURE 2- FTIR spectra of (A) Diclofenac sodium, (B) ethylcellulose, (C) diclofenac sodium loaded ethylcellulose nanoparticles

DSC

Thermal analysis is a supportive tool for determining the dispersion of the drug in polymeric materials. DSC thermograms of the DC, EC and optimized NPs are represented in Figure 3. The pure drug showed a high endothermic peak indicating its melting point at around 280°C which was absent in NPs.

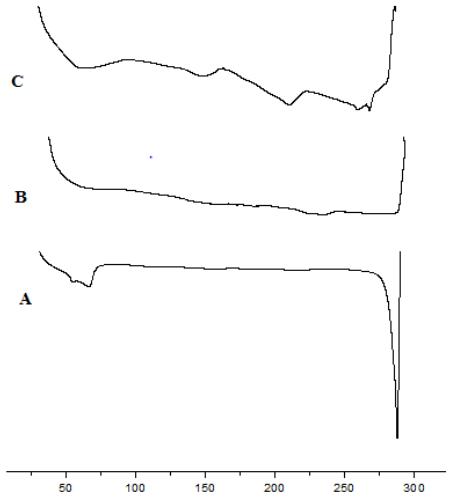

FIGURE 3 - DSC thermograms of DC (A), EC (B), optimized NPs (C)

SEM

The SEM micrographs showed that uniform NPs were successfully prepared using the solvent evaporation method. The optimized nanoparticles have a spherical shape and a smooth surface as shown in Figure 4.

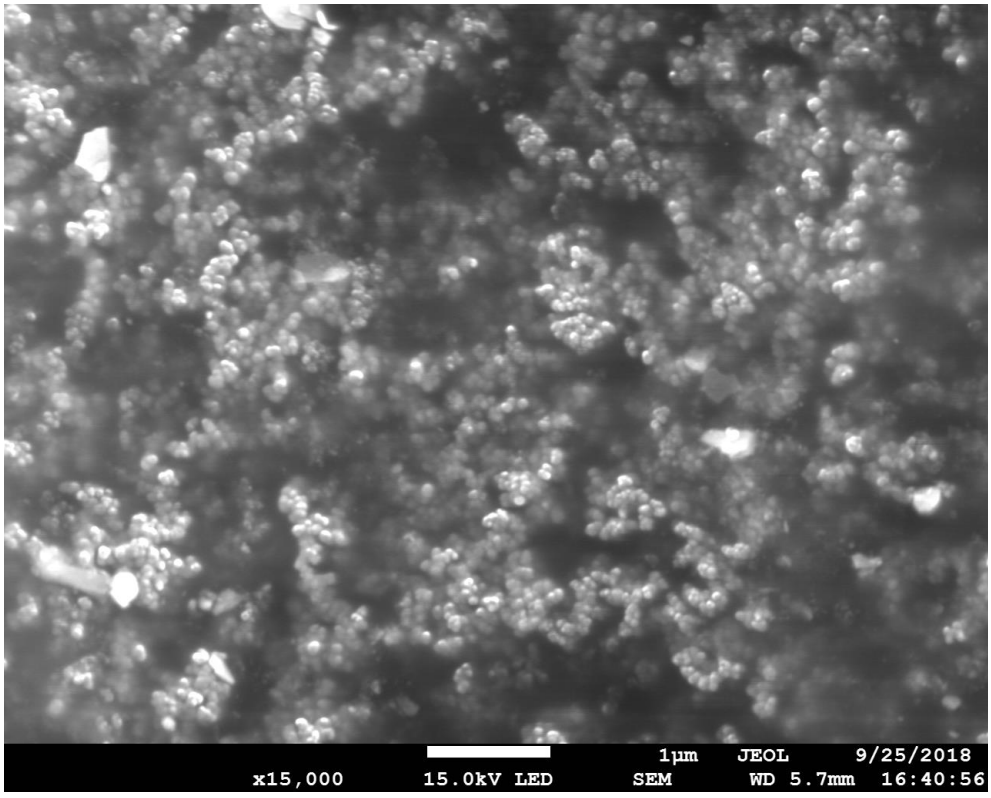

FIGURE 4- SEM micrographs of the optimized Diclofenac Sodium nanoparticles

In vitro release kinetic evaluation

In vitro drug release studies were performed to determine the sustained release nature of the formulation. In EC Formulation the drug release was slow and the spread extended. In fact, over a time period of 24 hours only 53.98% of the drug has been released from the EC Formulation (Figure 5).

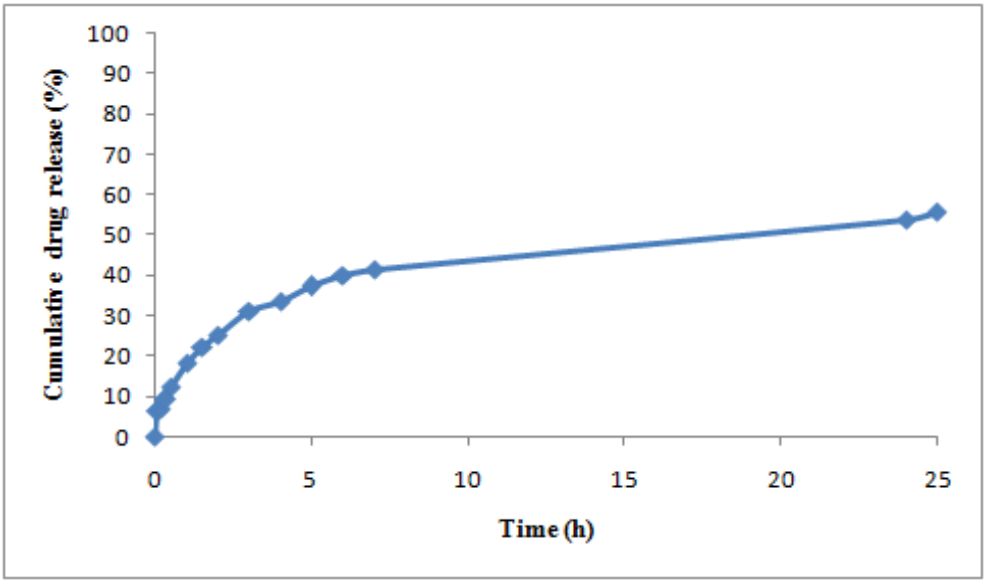

FIGURE 5- In vitro release profile of optimized formulation

The dissolution data were fitted to various kinetic equations and mechanism of drug release investigated. Equations (7-10) bellow are Zero order, First order, Higuchi and Korsmeyer-Peppas model, respectively.

$$Q_t = K_0 t \quad Q_t = K_0 t \tag{7}$$

$$\ln Q_t = \ln Q_0 - K_1 t \quad \ln Q_t = \ln Q_0 - K_1 t \tag{8}$$

$$Q_t = K_h t^{1/2} \quad Q_t = K_h t^{1/2} \tag{9}$$

$$\frac{Q_t}{Q_\infty} = K_p t^n \quad \frac{Q_t}{Q_\infty} = K_p t^n \tag{10}$$

Where, Q_t is the percentage of drug released at time t, Q_0 is the initial amount of drug present in the formulation, K_0, K_1, K_h and K_p are the constants, Qt/Q∞ is the fractional drug release at time t and n is the diffusional exponent characterizing the transport mechanism. The criteria for selecting the most appropriate model were based on the regression coefficient (R^2) which was determined from the slope of the following plots: Cumulative percent drug release vs. Time (Zero order kinetic model), Log cumulative of percent drug remaining vs. Time (First order kinetic model), Cumulative percent of drug release vs Square root of Time (Higuchi model), Log cumulative percent drug release vs. Log time (Korsmeyer-Peppas model). In Korsmeyer-Peppas model, first 60% of drug release was fitted and the release exponent "n" was calculated which is indicative of drug release mechanism. According to Korsmeyer theory, if $n < 0.45$ then the drug release follows Fickian diffusion mechanism, for $0.45 < n < 0.89$ it follows Anomalous (non-Fickian) diffusion and $n > 0.89$ for Super Case II release mechanism (Lokhande*et al*, 2013).

TABLE VI - Release kinetics data for optimized nanoparticles

Model	Zero order	First order	Higuchi	Korsmeyer-Peppas	
R^2	0.72	0.47	0.91	0.97	n = 0.42

The results of table VI show that the optimized nanoparticles follow a korsmeyer-Peppas release model (R^2=0.97). The n value (0.42) is lower than 0.45 indicating that the release follows a fickian diffusion mechanism.

CONCLUSIONS

The application of CCD is a useful tool for optimizing DC-loaded EC nanoparticles prepared by the emulsion solvent evaporation technique. The optimized nanoparticles obtained displayed an average particle size of 226.83 nm with a norrow polydispersity index (0.271), an EE of 49.09 % and a slow and prolonged drug release over a period of 24 hours by fickian diffusion mechanism governed by a Korsmeyer-Peppas release kinetics type. Ethylcellulose nanoparticles of Diclofenac sodium can be of significant practical use for a sustaining drug release and decreasing side effects.

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