Original Research Article

Development and optimization of enteric coated mucoadhesive microspheres of duloxetine hydrochloride using 3^2 full factorial design

Anupama Setia, Sahil Kansal, Naveen Goyal[1]

Department of Pharmaceutics, Rajendra Institute of Technology and Sciences, Sirsa, [1]Roorkee College of Pharmacy, Roorkee, Uttarakhand, India

Abstract

Background: Microspheres constitute an important part of oral drug delivery system by virtue of their small size and efficient carrier capacity. However, the success of these microspheres is limited due to their short residence time at the site of absorption. **Objective:** The objective of the present study was to formulate and systematically evaluate *in vitro* performance of enteric coated mucoadhesive microspheres of duloxetine hydrochloride (DLX), an acid labile drug. **Materials and Methods:** DLX microspheres were prepared by simple emulsification phase separation technique using chitosan as carrier and glutaraldehyde as a cross-linking agent. Microspheres prepared were coated with eudragit L-100 using an oil-in-oil solvent evaporation method. Eudragit L-100was used as enteric coating polymer with the aim to release the drug in small intestine The microspheres prepared were characterized by particle size, entrapment efficiency, swelling index (SI), mucoadhesion time, *in vitro* drug release and surface morphology. A 3^2 full factorial design was employed to study the effect of independent variables polymer-to-drug ratio (X_1) and stirring speed (X_2) on dependent variables, particle size, entrapment efficiency, SI, *in vitro* mucoadhesion and drug release up to 24 h (t_{24}). **Results:** Microspheres formed were discrete, spherical and free flowing. The microspheres exhibited good mucoadhesive property and also showed high percentage entrapment efficiency. The microspheres were able to sustain the drug release up to 24 h. **Conclusion:** Thus, the prepared enteric coated mucoadhesive microspheres may prove to be a potential controlled release formulation of DLX for oral administration.

Key words: *Chitosan, duloxetine hydrochloride, enteric coated microspheres, factorial design, mucoadhesive*

INTRODUCTION

Duloxetine hydrochloride (DLX) is a serotonin norepinephrine reuptake inhibitor. The claimed mechanism of action of the drug is based on the specific inhibition of both serotonin and norepinephrine reuptake while it weakly inhibits dopamine reuptake and has no significant affinity for histaminergic, dopaminergic cholinergic or adrenergic receptors.[1] DLX is also prescribed for diabetic peripheral neuropathy, a painful nerve disorder associated with diabetes that affects the hands, legs and feet as well as urinary incontinence.[2] Duloxetine is newer and preferable antidepressant because of its favorable pharmacodynamic features viz. dual inhibition, tolerability, safety, faster recovery, fewer side effects and low affinity for other neuronal receptors.[3] However, the drug is found to be acid labile, which results its degradation in gastric environment; thus, necessitating the need to develop an enteric coated system. Further, a steady state plasma concentration of drug is desired for effective treatment.

Enteric coated formulations are suitable vehicles to modify the release of drug or active pharmaceutical ingredient at specific target areas within the gastrointestinal tract. Enteric coating is an effective way to protect the drug against gastric environment and prevent the release of the encapsulated particles or the drug before reaching the target site.

Optimization using factorial designs is a highly efficient and systematic tool that shortens the time required for the development of pharmaceutical dosage forms and helps in improvement of

Address for correspondence:
Dr. Anupama Setia,
Rajendra Institute of Technology and Sciences,
Sirsa, Haryana India.
E-mail: setia.ani123@gmail.com

research and development work. Factorial designs are one of the major application of optimization where all the factors are studied in all possible combinations are considered as the most efficient in estimating the influence of individual variables and their interactions using minimum experiments.[4] The application of factorial design in pharmaceutical formulation development has played a key role in understanding the relationship between the independent variables and the responses to them.[5] The independent variables can be controlled, whereas responses are totally dependent. The contour plot is generated, which gives a visual representation of the values of the responses and this also helps the process of optimization by providing an empirical model equation for the response as a function of the different variables.[6-9]

Microspheres constitute an important part of oral drug delivery system by virtue of their small size and efficient carrier capacity. Microspheres are the carrier linked drug delivery system in which particle size ranges from 1 to 1,000 µm in diameter having a core of drug and entirely outer layers of polymers as a coating material. However, the success of these microspheres is limited due to their short residence time at the site of absorption. It would therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membrane.[10] This can be achieved by coupling bioadhesion characteristics to microspheres and developing mucoadhesive microspheres. Mucoadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site.

The objective of the present study was designed to develop a once a day controlled release formulation of DLX for oral administration. The formulation was so designed that release of the drug was targeted in the intestinal pH and were evaluated for particle size, particle shape and drug entrapment efficiency, percentage swelling index (SI), mucoadhesion time and *in vitro* drug release.

MATERIALS

DLX was a generous gift from Hetero Drugs Ltd. (Hyderabad, India). Chitosan was obtained as gift samples from Central Institute of Fisheries Technology, Cochin. All excipients and solvents used were of analytical grade.

METHODS

Preparation of microspheres

Microspheres formulations using chitosan as a mucoadhesive as well as carrier polymer were prepared using the simple emulsification phase separation technique as per earlier described method. [11-13] Briefly, a solution of chitosan (1% w/v) was prepared in glacial acetic acid (1% v/v). 5ml of methanolic solution of drug (1% w/v) was added to the desired volume of polymer solution as per Table 1. The resultant mixture was further added to 50 ml of light liquid paraffin containing 0.1 ml of span 80 as surfactant under constant stirring (2,000 rpm) using a three blade propeller stirrer to form a w/o emulsion. This procedure was followed by the addition of 0.5 ml of glutaraldehyde, a cross linking agent (25% v/v) drop wise with stirring at the same speed. Stirring was continued at the same speed for next 5 min and then stirring speed was reduced to different speeds according to the factorial design [Table 1]. Glutaraldehyde (0.25 ml) was further added twice to the mixture, once after 1 h and then after 2 h, respectively, with continuous stirring. Stirring was stopped after 1 h of the final addition of glutaraldehyde. The microspheres so obtained were separated by centrifugation and washed with petroleum ether several times to remove liquid paraffin. The microspheres were suspended in 5% w/v sodium bisulfite solution and stirred at the same speed for 15 min to remove residual glutaraldehyde. Finally, the microspheres were washed with distilled water and dried. Further microspheres (100 mg) were taken for enteric coating and dispersed in 5 ml of an organic solvent (acetone: Ethanol; 2:1) containing eudragit L-100 (500 mg). This organic phase was then poured into 70 ml of liquid paraffin containing span 80.[14,15] The system was maintained under stirring at 1000 rpm at room temperature for 3 h to allow the evaporation of the organic solvent. Finally, the enteric coated microspheres were collected, rinsed with *n*-hexane and air dried. All batches were enteric coated using the same procedure.

Optimization of enteric coated microspheres using 3^2 full factorial design

Response surface methodology (RSM) is characteristically employed to relate a response variable to the levels of the input

Table 1: 3^2 full factorial design layout

Batch code	Variable levels in coded form		Drug: Polymer	Actual quantity of drug and polymer		Stirring speed
	X_1	X_2		Drug (mg)	Chitosan (mg)	
CH-5	−1	−1	1:1	50	50	500
CH-6	−1	0	1:1	50	50	1000
CH-7	−1	1	1:1	50	50	1500
CH-8	0	−1	1:2	50	100	500
CH-9	0	0	1:2	50	100	1000
CH-10	0	1	1:2	50	100	1500
CH-11	1	−1	1:4	50	200	500
CH-12	1	0	1:4	50	200	1000
CH-13	1	1	1:4	50	200	1500

variables and to generate a design matrix to choose the optimal formulations. A statistical model, which consists of interactive and polynomial terms was utilized to evaluate the responses.[16] The responses were analyzed using analysis of variance (ANOVA) and the individual response parameters were evaluated using F test and polynomial equation was generated for each response using multiple linear regression analysis.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \qquad ...(1)$$

Where, Y is the dependent variable, b_0 is the arithmetic mean response of the nine runs and b_1 is the estimated coefficient for the factor X_1. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity. A 3^2 full factorial design was employed to study the effect of independent variables, i.e., drug polymer ratio (X_1) and the stirring speed (X_2) on dependent variables particle size, drug entrapment efficiency, mucoadhesion time, SI and drug released up to 24 h (t_{24}). Levels and factors for the factorial design are given in Table 2. Contour and RSM plots for each response were generated using the DESIGN EXPERT (STAT-EASE) demo version software 8.0.7.

Particle size of microspheres
The particle size of the enteric coated microspheres was determined by using optical microscopy method. Approximately, 100 microspheres were counted for particle size using a calibrated optical microscope (Labomed CX RIII, Ambala, India).

Drug entrapment efficiency
Microspheres (50 mg) were crushed in a glass mortar and pestle, and the powdered microspheres were suspended in 10 ml of phosphate buffer (pH 7.4). After 24 h, the solution was filtered and the filtrate was analyzed for the drug content. The drug entrapment efficiency was calculated using the following formula:

$$\text{Entrapment efficiency:} \frac{\text{Parctical durg content}}{\text{Theoretical durg content}} \times 100$$

SI
The equilibrium swelling studies are carried out by method as described earlier.[17] A known weight (100 mg) of microspheres with the drug was placed in 500 ml of phosphate buffer solution (pH 6.8) and allowed to swell for the required period of time at 37 ± 0.5°C using the United State Pharmacopoeia (USP) dissolution apparatus with the dissolution basket assembly at 50 rpm. To ensure complete equilibration, samples were allowed to swell for 24 h. The excess surface adhered liquid drops were

removed by blotting with soft tissue papers and the swollen microspheres were weighed to accuracy of 0.01 mg using an electronic microbalance. The microspheres were then dried in an oven at 60° C for 5 h until there was no change in the dried mass of the samples and the SI was then calculated from the formula:

$$SI = \frac{W_e - W_o}{W_o}$$

Where, W_o is the initial weight of the dry microparticles and W_e is the weight of the swollen microparticles at equilibrium swelling in the media. Each experiment was repeated 3 times and the average value/SD was taken as the SI value.

Mucoadhesion time
The mucoadhesion property of microspheres formulations was determined according to the earlier described method. [18] A 5 cm long piece of freshly cut pig intestine was obtained from a local abattoir within 1 h of killing of animal and was cleaned by washing with isotonic saline solution. An accurate weight of microspheres was placed on the mucosal surface, which was attached to a polyethylene plate that was fixed at an angle of 40° relative to the horizontal plane. Phosphate buffer (pH 6.8) warmed at 37 ± 1°C was passed at a rate of 5 ml/min over the tissue. The time required for detaching all the microspheres from mucosal surface of the pig intestine was recorded by visual inspection.

In vitro drug release studies
In vitro drug release profile of the drug from enteric coated microspheres, the dissolution test was carried out according to USP 23 method for modified release formulations (method A). [19] Dissolution studies were carried out using USP apparatus type-II i.e., paddle type at 50 rpm and at a temperature of 37 ± 0.5°C. Initial studies were carried out in 325 ml of 0.1 N HCl (pH 1.2) for 2 h. The pH of the dissolution media was adjusted to pH 6.8 by the addition of 125 ml of 0.2 M trisodium orthophosphate. The pH was adjusted with the aid of 2 N HCl or 2N NaOH. The dissolution was continued in phosphate buffer till 24 h. Samples were withdrawn at a predetermined time intervals and replaced with fresh media. Samples were filtered and then analyzed using ultraviolet-visible spectrophotometer at λ_{max} of 288 nm.

Data fitting
To determine the kinetics of drug release of DLX from enteric coated microspheres the released data was plotted for cumulative drug release versus time (zero order), log cumulative release versus time (first order), cumulative release versus sqrt time (Higuchi) and log cumulative release versus log time (Korsmeyer and peppas model) the curves obtained were regressed, the values of R^2 for various kinetic models were tested to describe the drug release from the microspheres.

Surface morphology
The surface morphology of microspheres was examined by scanning electron microscopy (SEM). The microspheres were mounted on metal stubs using double-sided tape and coated

Table 2: Coded levels of factors

Variables level	Low (−1)	Medium (0)	High (+1)
Drug-to-polymer ratio (X_1)	1:1	1:2	1:4
Stirring speed (X_2) rpm	500	1000	1500

with a 150 Å layer of gold under vacuum. Stubs were visualized under SEM.

Stability analysis of microspheres

In the present work, stability studies of optimized formulation (loaded with Duloxetine HCl) was carried out after storing the formulations at 4 ± 1°C and 25 ± 2°C/60 ± 5% relative humidity for 30 days in screw capped amber colored glass bottles. After every 15 days, the formulations were evaluated for % drug remaining and physical changes. The initial drug content was considered as 100%.

RESULTS AND DISCUSSION

The pH sensitive or enteric coated mucoadhesive microspheres of DLX were prepared by emulsification method. Chitosan was taken as the polymer for mucoadhesion, eudragit L100 was the polymer for enteric coating and glutaraldehyde was used as a cross-linking agent. Further 3^2 full factorial design was employed to study the effect of independent variables (drug polymer ratio [X_1] and stirring speed [X_2]) on dependent

variables particle size, drug entrapment efficiency, *in vitro* mucoadhesion time, SI and *in vitro* drug release up to 24 h. The results [Table 3] clearly indicate that all dependent variables are significantly affected by the independent variables. The polynomial equations (equation 2-6) for each response with their high magnitude of the coefficients and mathematical sign indicate about the fit of the model.

Factorial equation for particle size

The model proposes the following polynomial equation

$$Y = + 30.78 + 12.25X_1 - 4.06 X_2 - 0.29 X_1 X_2 - 3.26 X_1^2 - 0.055 X_2^2 \qquad ...(2)$$

The Model F-value of 253.75 [Table 4] implies the model is significant. There is only a 0.04% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" <0.0500 indicate model terms are significant. In this case A, B, A^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting

Table 3: Characteristics of prepared duloxetine hydrochloride loaded chitosan microspheres

Batch code	Particle size (μm) ± SD	Drug entrapment efficiency (%)	Mucoadhesion time (h)	% age swelling	t_{24}
CH-1	28.45 ± 0.64	74.09 ± 0.48	2.52 ± 0.25	148.27 ± 5.72	89.38 ± 2.38
CH-2	24.83 ± 0.92	76.19 ± 0.93	3.10 ± 0.32	213.74 ± 7.47	90.26 ± 2.64
CH-3	19.36 ± 0.49	71.28 ± 0.78	2.58 ± 0.18	316.29 ± 5.18	83.88 ± 1.94
CH-4	41.47 ± 0.54	78.49 ± 0.68	4.12 ± 0.21	192.58 ± 8.29	92.23 ± 2.49
CH-5	38.21 ± 0.72	83.96 ± 0.84	4.32 ± 0.35	253.63 ± 7.81	93.98 ± 1.83
CH-6	30.76 ± 0.58	72.49 ± 0.73	4.18 ± 0.19	329.46 ± 9.27	86.93 ± 2.38
CH-7	52.89 ± 0.43	69.81 ± 0.56	3.56 ± 0.24	216.49 ± 7.89	80.22 ± 2.64
CH-8	47.23 ± 0.74	74.16 ± 0.91	4.18 ± 0.15	268.54 ± 10.63	88.74 ± 1.94
CH-9	42.19 ± 0.69	65.62 ± 0.47	4.07 ± 0.24	339.21 ± 9.62	84.73 ± 2.49

All values are expressed as mean±SD, *n*=3

Table 4: ANOVA of dependent variables

| Dependent variables	Source of variation	Sum of squares	Degree of freedom	Mean square	*F* value	*P* value
Particle size	Regression	1001.31	5	200.26	253.75	0.0004
	Residuals	2.37	3	0.79		
	Total	1003.68	8			
	r^2	0.9976				
Drug entrapment efficiency	Regression	210.94	5	42.19	11.82	0.0345
	Residuals	10.71	3	3,57		
	Total	221.65	8			
	r^2	0.9517				
Mucoadhesion time	Regression	4.08	5	0.82	44.20	0.0052
	Residuals	0.055	3	0.018		
	Total	4.14	8			
	r^2	0.9866				
Swelling index	Regression	34888.85	5	6977.77	160.93	0.0008
	Residuals	130.08	3	43.36		
	Total	35018.92	8			
	r^2	0.9963				
t_{24}	Regression	148.75	5	29.75	21.33	0.0150
	Residuals	4.18	3	1.39		
	Total	152.93	8			
	r^2	0.9726				

ANOVA: Analysis of variance
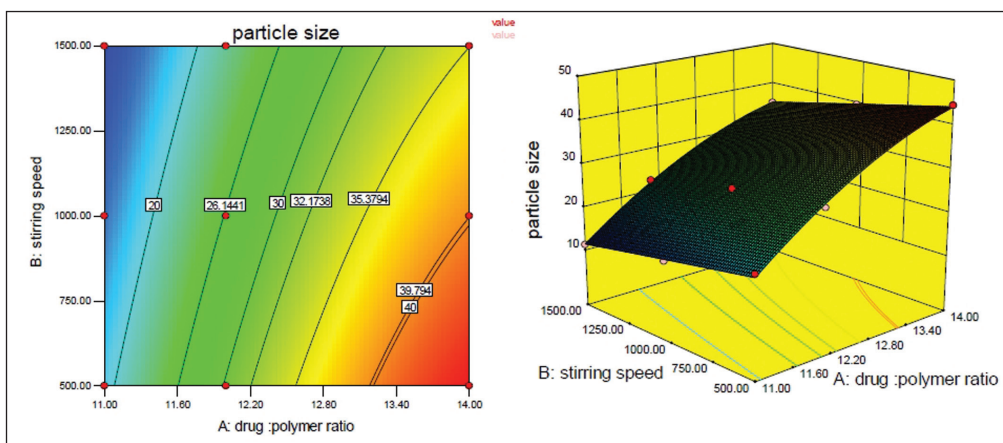
Figure 1: Contour and response surface methodology plot for study the effect of amount of polymer and stirring speed on particle size
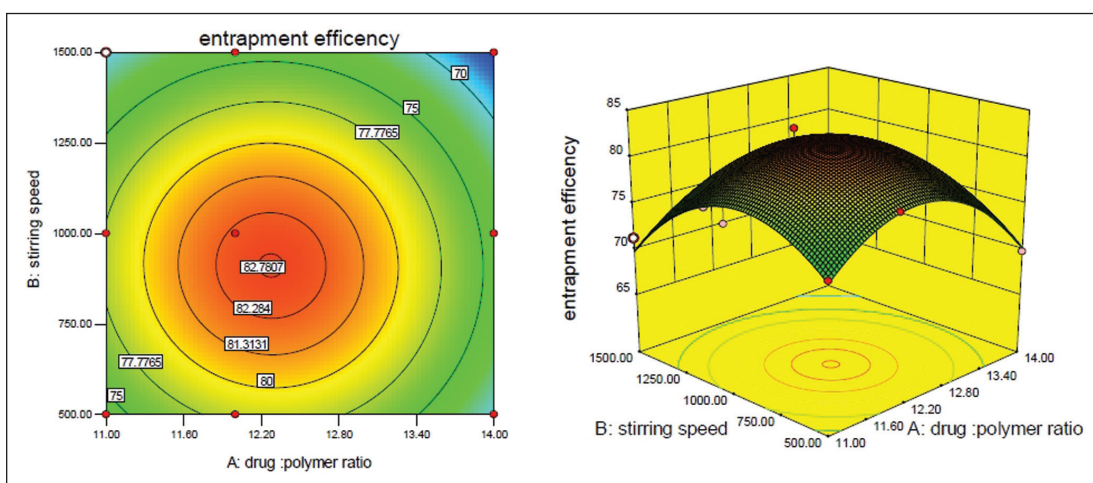

Figure 2: Contour and response surface methodology plot for study the effect of amount of polymer and stirring speed on entrapment efficiency

those required to support hierarchy), model reduction may improve your model.

The "Pred R-Squared" of 0.9778 is in reasonable agreement with the "Adj R-Squared" of 0.9937. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 44.987 indicates an adequate signal. This model can be used to navigate the design space [Table 4].

Results of the polynomial equation indicate that the effect of X_1 (drug polymer ratio) is positive and more significant than X_2 (stirring speed) i.e., as the drug polymer ratio was increased there was an increase in the polymer concentration, which lead to increased particle size, whereas as with the increase in stirring speed particle size was decreased. Figure 1 shows the contour and response surface plot showing the effect of independent variables on the particle size.

Factorial equation for entrapment efficiency
The model proposes the following polynomial equation for percentage drug entrapment:

$$\text{Entrapment efficiency} = +82.47 - 2.00X_1 + 2.19X_2 - 0.20X_1X_2 - 6.51X_1^2 - 6.14X_2^2 \quad ...(3)$$

The Model F-value of 11.82 implies the model is significant. There is only a 3.45% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" <0.0500 indicate model terms are significant. In this case X_1^2, X_2^2 are significant model terms. The "Pred R-Squared" of 0.6113 is not as close to the "Adj R-Squared" of 0.8712 as one might normally expect. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 11.007 indicates an adequate signal. This model can be used to navigate the design space [Table 4].

Figure 2 shows the contour and response surface plot giving the effect of independent variables on the entrapment efficiency. Both variables have significant efffect up to a level afterwards with the increase in both parameters the entrapment efficiency reduced.

Factorial equation for mucoadhesion time

$$\text{Mucoadhesion time} = +\, 4.78 + 0.60X_1 + 0.12X_2 + 0.12X_1X_2 - 1.21X_1^2 - 0.36X_2^2 \qquad ...(4)$$

The Model F-value of 44.20 implies the model is significant. There is only a 0.52% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" <0.0500 indicate model terms are significant. In this case, X_1, X_1^2, X_2^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Pred R-Squared" of 0.9010 is in reasonable agreement with the "Adj R-Squared" of 0.9643.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. A ratio of 16.561 indicates an adequate signal. This model can be used to navigate the design space [Table 4].

The results of the equation indicated that the both drug polymer ratio as well as stirring speed showed a positive effect on the mucoadhesion time up to a level thereafter with an increase in drug: Polymer as well as stirring speed mucoadhesion time decreased. The magnitude of the factor X_1 (drug polymer concentration) was higher than the X_2 (stirring speed) showing more significant effect on the mucoadhesion time. The effect of independent variables on the mucoadhesion was shown in the contour and response surface plot [Figure 3].

Factorial equation for SI

$$\text{SI} = +255.64 + 22.58A + 71.27B \qquad ...(5)$$

The Model F-value of 73.73 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case, X_1, X_2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support

hierarchy), model reduction may improve your model. The "Pred R-Squared" of 0.9086 is in reasonable agreement with the "Adj R-Squared" of 0.9479. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. A ratio of 21.523 indicates an adequate signal. This model can be used to navigate the design space [Table 4].

The swelling behavior of mucoadhesive polymers is a part of mechanisms, which are responsible for their adhesive and cohesive properties, stability during disintegration, and drug release. The swelling of the formulation increased with an increase in polymer concentration. The results of the equation indicate that the both drug polymer ratio as well as stirring speed showed a positive effect on the SI i.e., with an increase in drug polymer ratio and stirring speed there was improved SI but higher magnitude of the X_2 than X_1 indicated more significant effect of stirring speed on SI. Figure 4 shows the contour and response surface plot giving the effect of independent variables on the SI.

Factorial equation for t24

$$t_{24} = +\, 95.01 - 1.64X_1 - 0.27X_2 + 2.51X_1X_2 - 5.96X_1^2 - 4.27X_2^2 \qquad ...(6)$$

The Model F-value of 8.20 implies there is a 5.68% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" <0.0500 indicate model terms are significant. In this case, X_1, X_1^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Pred R-Squared" of 0.0906 is not as close to the "Adj R-Squared" of 0.8182 as one might normally expect. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. A ratio of 8.734 indicates an adequate signal. This model can be used to navigate the design space [Table 4].
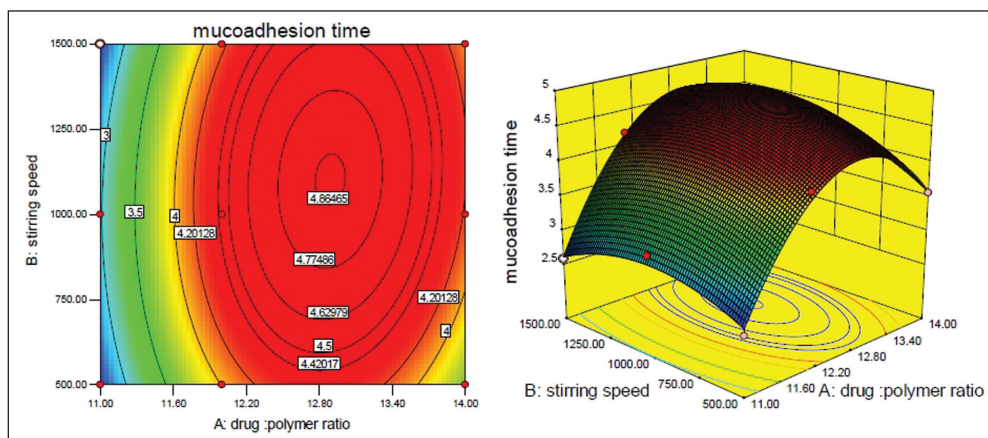
Figure 3: Contour and response surface methodology plot for study the effect of amount of polymer and stirring speed on mucoadhesion time
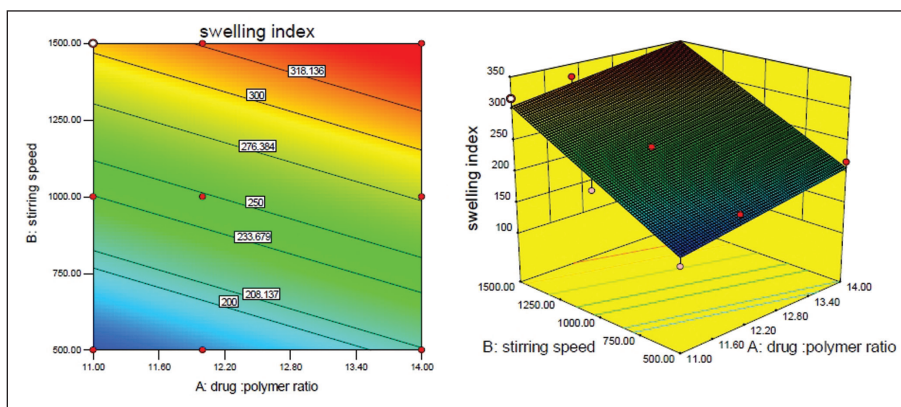
Figure 4: Contour and response surface methodology plot for study the effect of amount of polymer and stirring speed on swelling index
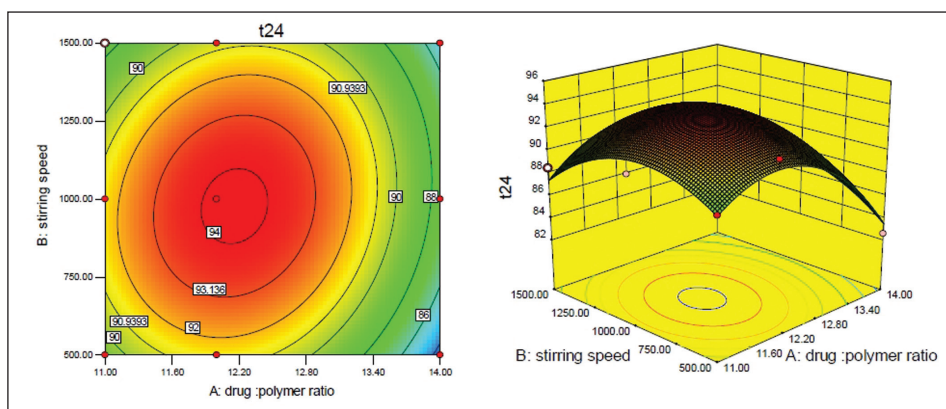

Figure 5: Contour and response surface methodology plot for study the effect of amount of polymer and stirring speed on t_{24}
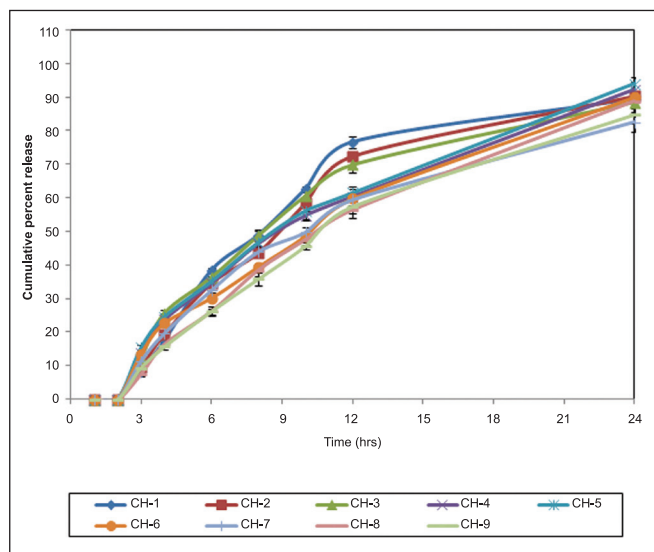

Figure 6: Dissolution profiles of prepared duloxetine hydrochloride loaded chitosan microspheres

The results of the equation showed that X_1 (drug polymer ratio) has a negative effect on the drug release as shown by the negative coefficient as compared to the stirring speed which showed a positive effect on the drug release after 24 h as shown by the positive coefficient. Lesser magnitude of both factors imply that both the factors lead to increase in t_{24} up to a level, whereas with further increase in the drug polymer ratio or stirring speed lead to decrease in the t_{24}. Figure 5 shows the contour and response surface plot giving the effect of independent variables on the drug realeased after 24 h.

Figure 6 shows the cumulative drug release of Duloxetine HCl from various enteric coated mucoadhesive microspheres. *In-vitro* release profiles of all batches were carried out in 0.1 N HCl for first 2 h and further in phosphate buffer (pH = 6.8). There was the absence of drug release in initial 2 h of dissolution studies, which was mainly because of the enteric coating of the microspheres. It was found that with the increase in drug polymer ratio; there was an increase in the drug release whereas release was decreased with the increase in stirring speed, which was in concordant with the entrapment efficiency, which was maximum for the CH-5 batch, which also gave the maximum drug release in 24 h.

Formulation optimization

A numerical optimization technique using the desirability approach was employed to develop an optimized formulation with the desired responses. For the optimization of enteric coated microspheres of DLX constraints were fixed for all factors and responses [Table 5].
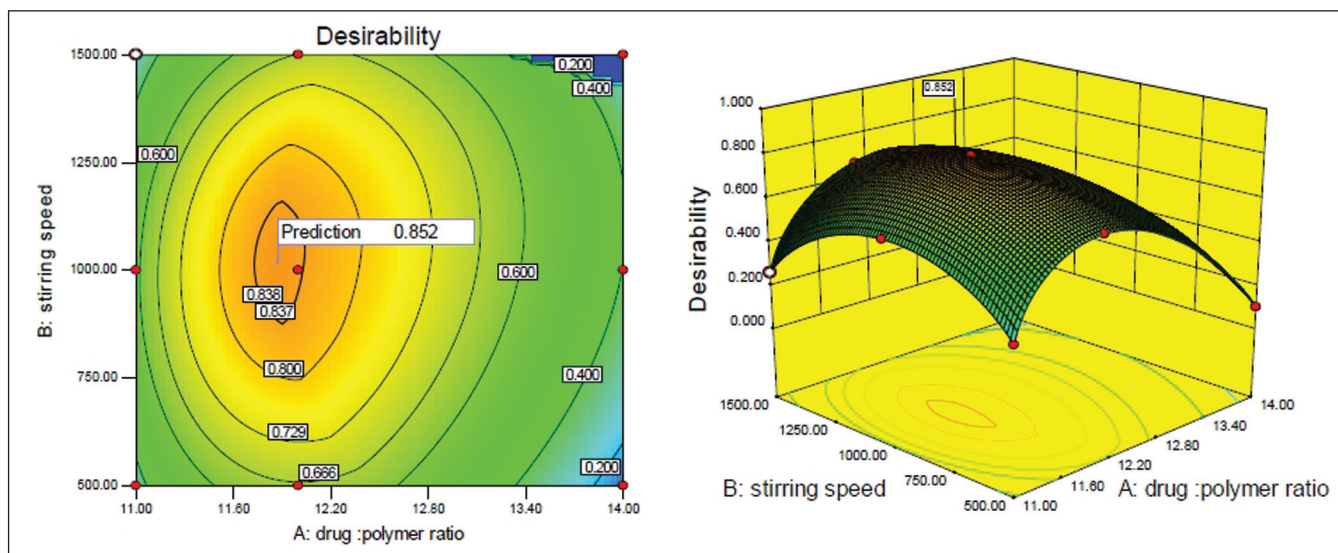
Figure 7: Contour and response surface methodology desirability plot for optimum results
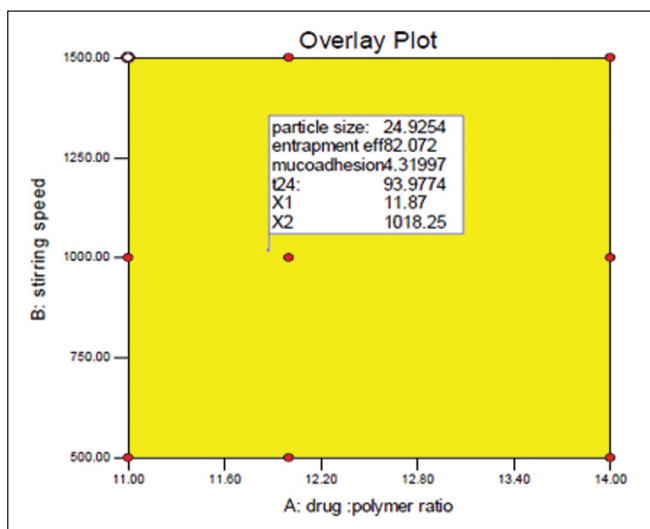

Figure 8: Overlay plot for optimum results

Figure 9: Scanning electron microscope photograph of duloxetine hydrochloride loaded chitosan microspheres

Table 5: Constraints for responses

Name	Goal	Lower limit	Upper limit
Particle size	Minimize	11.23	44.37
Entrapment efficiency	Maximize	65.62	83.96
Mucoadhesion time	Maximize	2.52	4.32
Swelling index	In range	148.27	339.21
t_{24}	Maximize	80.22	93.98

Constraints were set according to the formulation of enteric coated microspheres using the minimum amount of excipients, which would give desired response values i.e., minimum particle size, maximum entrapment efficiency, *in vitro* mucoadhesion, SI within a range and maximum drug release at 24 h (t_{24}).

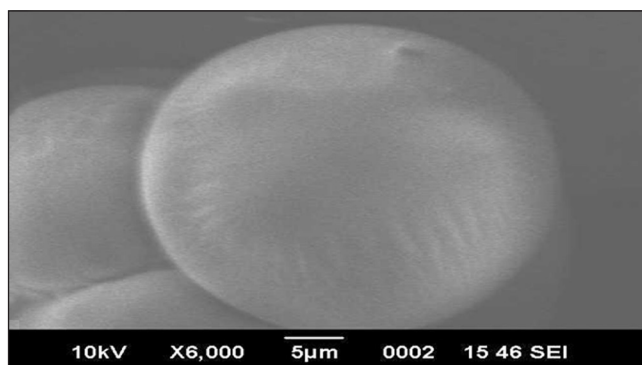In optimization [Figure 7] maximum desirability of 0.875 indicated optimum formulation was achieved at 1:18 drug

polymer ratio and stirring speed of 1002, which was nearest to the batch CH-5. Over lay plot [Figure 8] of the desirability give the details of the optimized batch giving the optimum results of the optimized batch, which were very closer to the results obtained by the CH-5 batch. Thus, it can be concluded that batch CH-5 may be considered as the optimized batch.

Surface morphology of microspheres

The SEM photographs of optimized batch of enteric coated microspheres (CH-5) [Figure 9] showed microspheres had a smooth surface and were spherical in shape. Smoothness of the surface indicates the uniformity of the coating.

Data analysis

The release kinetics of the CH-4, CH-5, CH-6 and CH-8 followed Higuchi model drug release mechanism and other batches followed first order release mechanism [Table 6]. Higuchi model is applicable to the system with drug dispersed in uniform swellable polymer matrix as with water soluble drugs. Higuchi model also called as diffusion release because it follows diffusion release mechanism having controlled and sustained release

Table 6: Kinetics of drug release of all formulations

Batch code	Zero order release	First order release	Higuchi's release	Korsmeyer-peppas release		Best fit model
	R^2	R^2	R^2	Slope (*n*)	R^2	
CH-1	0.834	0.966	0.935	0.707	0.819	First order
CH-2	0.877	0.987	0.959	0.690	0.782	First order
CH-3	0.856	0.990	0.957	0.652	0.827	First order
CH-4	0.918	0.985	0.986	0.686	0.719	Higuchi's release
CH-5	0.918	0.977	0.986	0.740	0.696	Higuchi's release
CH-6	0.941	0.984	0.989	0.616	0.713	Higuchi's release
CH-7	0.898	0.995	0.978	0.516	0.823	First order
CH-8	0.953	0.986	0.987	0.590	0.706	Higuchi's release
CH-9	0.946	0.994	0.985	0.526	0.745	First order

Table 7: Stability study of microspheres (CH-5) at different temperature with variable effect on % drug remaining and physical characteristic

Time (days)	% drug remained		Physical changes (size and color)	
	4±1°C	25 ± 2°C	4 ± 1°C	25 ± 2°C
0	100.00±0.2	100.00±0.3	—	—
15	099.86±0.3	099.79±0.2	No change	No change
30	099.62±0.2	099.51±0.3	No change	No change

All values are expressed as mean±SD, *n*=3

through diffusion mechanism. Higuchi model involved the more dissolution of the drug inside the matrix than the release of drug outside the formulation.

Stability studies of microspheres

Stability studies should include testing of those attributes of the drug product that are susceptible to change during storage and that are likely to influence quality, safety or efficacy. Effect of storage temperature on percentage drug remaining and physical changes (size and color) of microspheres were observed. Results of storage stability were evaluated using one-way ANOVA. The initial drug content was taken as 100% and compared with the formulations at 15 days interval. These results [Table 7] suggest that all microspheres formulations were stable at 4°C and at 25°C and the entire drug was retained inside. The results of stability study [Table 7] showed that there was no change observed in physical characteristic (size and color) of all the microspheres formulation after 30 days. These results suggested that all microspheres formulations were stable until 30th day at $4 \pm 1°C$ and $25 \pm 2°C$.

CONCLUSION

The results of a 3^2 full factorial design revealed that the polymer to-drug ratio and stirring speed significantly affected the dependent variables percentage drug entrapment efficiency and particle size. The microspheres of the best batch exhibited a high mucoadhesion time 4.32 h, 83.96% drug entrapment efficiency and SI of 253.63%. The *in vitro* release studies indicate that the mucoadhesive microspheres of duloxetine could sustain the release of the drug for more than 24 h.

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