

# FORMULATION AND EVALUATION OF NASAL MUCOADHESIVE MICROPARTICLES OF DILTIAZEM HYDROCHLORIDE

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**ABSTRACT:** The purpose of this research work was to formulate and evaluate nasal mucoadhesive microparticles of diltiazem hydrochloride. The microparticles were prepared by using modified emulsion solvent evaporation technique. From the preliminary trials it was found that the amount of ethyl cellulose and hydroxy propyl methyl cellulose affected the characteristics of microparticles. A 3<sup>2</sup> full factorial design was employed to optimized preliminary batch. The prepared microparticles were evaluated for different parameters like production yield, encapsulation efficiency, surface morphology, particle size and drug release behavior. In vitro mucoadhesive tests and ex-vivo studies using goat nasal mucosa were performed. The best batches were used for further study of drug permeation through nasal mucosa.

**KEYWORDS:** Mucoadhesive microparticles, diltiazem, factorial design, nasal mucosa

## INTRODUCTION

The nasal route of administration, which is in the focus of this work, has received a great deal of attention in recent years as a convenient and reliable method not only for local but also for systemic administration of drugs. Drugs have been shown to reach the CNS from the nasal cavity by a direct transport across the olfactory region situated at the loft of the nasal cavity. It is the only site in the human body where the nervous system is in direct contact with the surrounding environment. The nasal cavity offers a number of unique advantages such as easy accessibility, good permeability especially for lipophilic, low molecular weight drugs, avoidance of harsh environmental conditions and hepatic first pass metabolism, potential direct delivery to the brain.<sup>1-4</sup>

Mucoadhesive drug delivery systems have been used to improve and enhance drug bioavailability because the systems can contact with the absorption surface and prolong residence time resulting in a better absorption. Also reduces frequency of drug administration due to reduction in mucociliary clearance. Several polymers, particularly hydrophilic polymers containing numerous hydrogen bond (H-bond) forming groups (i.e. hydroxyl, carboxyl, amine and amide groups) have been investigated for mucoadhesive properties.<sup>5-7</sup>

Microparticles are one of the important novel drug delivery system. Microparticles are a polymeric device allows for slow, controlled and predictable drug release over a period of time and hence reduces overall amount

of drug needed. Microparticles, in general, have the potential to be used for targeted and controlled release drug delivery, but coupling of mucoadhesive properties to microparticles has additional advantages, e.g. 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.<sup>8-9</sup>

Diltiazem hydrochloride is a calcium ion cellular influx inhibitor (slow channel blocker or calcium antagonist). Diltiazem produces its antihypertensive effect primarily by relaxation of vascular smooth muscle and the resultant decrease in peripheral vascular resistance. Diltiazem is subject to an extensive first-pass effect, giving an absolute bioavailability of about 40%. Intranasal administration allows transport of drugs to the brain circumventing BBB, thus providing unique features and better option to target drugs to brain. The objective of this work is to improve nasal bioavailability of diltiazem hydrochloride by increasing its nasal retention time.<sup>10-15</sup>

## MATERIALS AND METHODS

### Materials

Diltiazem hydrochloride was obtained as gift sample from Dr. Reddy's Laboratory, Hyderabad. Ethyl cellulose, hydroxy propyl methyl cellulose and carbopol 934 were obtained from Loba Chemie Pvt Ltd. The other solvents like liquid paraffin (light), acetone, methylene

dichloride, methanol, tween 80 and petroleum ether were of Analytical Research (AR) Grade and obtained from S D Fine Chemicals, Mumbai.

### Preparation of microparticles

Microparticles were prepared by modified emulsion solvent evaporation technique. Drug (250 mg) was dissolved in mixture of methanol and dichloromethane (1:1) to form 0.025% w/v solution to which 350 mg of hydroxy propyl methyl cellulose was dispersed to form polymer solution. In this solution 75 mg of carbopol was added. Ethyl cellulose was dissolved in acetone separately to form 0.035%w/v solution. Both the polymer solutions were mixed properly. This dispersion was then added in liquid paraffin containing tween 80 with the help of syringe and continuous stirring was carried out at 2000 rpm on mechanical stirrer. Stirring was done for 2 hrs. The microparticles obtained were filtered and washed with petroleum ether to remove the traces of oil.

### Characterization of microparticles

#### Production yield

The total amount of microparticles obtained were weighed and the percentage yield calculated taking into consideration the weight of drug and polymer using the formula<sup>16</sup>

$$\% \text{ of production yield} = \frac{W_1 \times 100}{W_2}$$

Where  $W_1$  = wt of dried microparticles

$W_2$  = sum of initial dry wt of starting material.

#### Particle size analysis

Particle size of the microparticles was determined by optical microscopy. About 100 of microparticles were used for the study and the mean particle size was determined.<sup>16</sup>

### Scanning electron microscopy (SEM) of microparticles

SEM of microparticles was recorded using Scanning Electron Microscope (Jeol, JSM 5610 LV, Japan) with a 10 kV accelerating voltage.<sup>16</sup>

#### Drug entrapment efficiency

Weighed quantity of microparticles were crushed and suspended in methanol to extract the drug from microparticles. After 24 h, the filtrate was diluted with phosphate buffer pH 6.4 and final solution was assayed spectrophotometrically at 236 nm for drug content. Blank solution was prepared by same procedure without formulation.<sup>17</sup>

$$\text{Entrapment efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

### Mucoadhesive testing

A 1x1 cm piece of goat nasal mucosa was tied onto a glass slide using thread. Microparticles were spread (~50) onto the wet rinsed tissue specimen and the prepared glass slide was hung on one of the grooves of a USP tablet disintegration test apparatus. The disintegration test apparatus was operated where by tissue specimen was given regular up and down movements in the beaker of the disintegration apparatus containing phosphate buffer pH 6.4. Time required for complete washing of microparticles was noted.<sup>18</sup>

### In vitro drug release study

The drug release was studied using USP type II apparatus (Electrolab, TDT-06T, India) at  $37 \pm 0.50^\circ\text{C}$  and at 100 rpm using 400 ml of phosphate buffer pH 6.4 as a dissolution medium. One ml of the sample solution was withdrawn at predetermined time intervals, filtered, diluted suitably and analyzed spectrophotometrically. Equal amount of the fresh dissolution medium was replaced immediately after withdrawal of the test sample. Percentage drug dissolved at different time intervals was calculated using the Lambert-Beer's equation. The result was obtained in triplicate and the average value reported.<sup>19,20</sup>

### In vitro drug diffusion study

The drug diffusion from different formulation was determined using treated cellophane membrane and Keshary-chien diffusion cell. The treated cellophane membrane was fixed between the donor and receptor compartment of the diffusion cell to support the microparticles. 50 mg of microparticles were placed on cellophane membrane in the donor compartment contained phosphate buffer (pH 6.4). About 1 ml of sample was withdrawn and analyzed spectrophotometrically at 236 nm for drug content with sufficient dilution.<sup>20</sup>

### Drug permeation study through nasal mucosa of goat

Drug permeation through nasal mucosa was studied for pure drug as well as optimized formulation (BD 4). In this the nasal mucosa was separated properly from nasal bone and fixed between the donor and receptor compartment of the diffusion cell. The amount of drug in the receptor compartment containing phosphate buffer (pH 6.4) was determined spectrophotometrically at 236 nm from aliquots withdrawn at various time intervals.<sup>21</sup>

### Drug release pattern from microspheres

In order to understand the mechanism and kinetics of drug release, the results of the *in vitro* drug release study were fitted with various kinetic equations like zero order, first order and Higuchi model. In order to define a model which will represent a better fit for the formulation, drug release data was further analyzed by Peppas equation. These studies were performed by using PCP disso V3 software.

### Multiple regression analysis for 3<sup>2</sup> factorial design

The responses obtained from 3<sup>2</sup> factorial design analyses were subjected to multiple regression analysis. The polynomial equations determined using the form;

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 + b_{11} X_1^2 + b_{22} X_2^2$$

### RESULTS AND DISCUSSION

The mucoadhesive microparticles were prepared by modified emulsion solvent evaporation technique. Different ratios of ethyl cellulose and hydroxy propyl methyl cellulose were tried. Also varied amount of carbopol was used. From the preliminary study best batch was selected. On the basis of this study a 3<sup>2</sup> full factorial design was employed to study the effect of independent variables (i.e. amt of ethyl cellulose [X<sub>1</sub>] and amt of hydroxy propyl methyl cellulose [X<sub>2</sub>]) on dependant variables drug release, drug diffusion, drug entrapment efficiency and mucoadhesion time.

% production yield, particle size, drug entrapment efficiency and mucoadhesive strength of different batches were studied. From the factorial batches it was found that the production yield of BD 4 and BD 8 found to be 98.37% and 41.02% respectively. From this it may be concluded that lower yields for the microparticles were due to EC inducement this corroborates the finding of A Martinac *et al*,<sup>16</sup>. The mean particle size of the microparticles was found in the range of 31.25 ± 2.5 to 60.0 ± 3.5 μm. It has been mentioned in literature that the approximate range for nasal mucoadhesive microparticles should be between 10 to 100 μm<sup>8</sup>. Abd El-Hameed *et al*, 1997 have reported that as the speed of the stirrer increases the particle size decreases<sup>22</sup>, thus in this study the stirring speed was kept constant at 2000 rpm. It was observed that any further increase in speed causes breaking of the droplets resulting in non-uniform microparticles. The % entrapment efficiency of the microparticles was found to be in the range of 38.4 - 61.8%. With increasing amount of hydroxy propyl methyl cellulose the entrapment efficiency and mucoadhesive strength of microparticles were increased. (Table 1 and 2)

The SEM of blank microparticles and drug loaded microparticles was done. The pores at the surface of blank microparticles may be due to the rapid evaporation of solvent. During the solvent evaporation process, crust that is first formed on the surface of the droplets prevents the evaporation of the solvent causing the building up of the vapor pressure as a result small eruptions, opening are formed. Surface indentations could be attributed to the subsequent shrinking of the microspheres after solid crust

is formed<sup>16</sup>. The surface characteristics of drug loaded microparticles indicate a relatively different topography suggestive of surface drug adsorption. (Figure 1 and 2)

In vitro drug release study as well as drug diffusion study was done. The *in-vitro* drug release for BD1 and BD7 were 74.98% and 64.84% in 5 hrs respectively, from these results it is evident that as the amount of hydrophobic polymer (EC) increases, drug release decreases. As reported by Anand Srivastava *et al*., the increase in the amount of EC may increase the density of the polymer matrix at higher concentration which may result in an increased diffusion path length and hence a decrease in the overall drug release from the polymer matrix<sup>23</sup>. (Figure 3 and 4)

From the study one batch was selected for drug permeation through nasal mucosa of goat. BD 4 was selected as optimized batch which is having particle size of 31.25 ± 2.5 μm and % entrapment efficiency of 51.75 ± 0.057% and mucoadhesive strength of 286 ± 2.34 min. Batch BD 4 also showed good result of % drug release (89.11%) as well as % drug diffusion (87.16%) this might be due to the fact that smaller particles offered more surface area to release the drug Permeation of pure drug as well as BD 4 was done. From the study it was found that permeation of pure drug is faster as compare to the factorial batch. 92.04% of pure drug permeated through nasal mucosa in 2hrs while for BD 4 the amount of drug permeated in 5hours was 83.67%. Diltiazem is polar drug and it is highly soluble in aqueous environment thus pure drug rapidly dissolves and permeates faster while drug from microparticles permeate slowly as diffusion from the matrix of the polymer and dissolution is the rate determining steps for permeation. (Figure 5)

The data obtained from drug release study was used for kinetic study. The n values for BD1, BD2, BD6, BD7, BD8 and BD9 were found to be between 0 and 0.5, the mechanism of transport is Fickian indicating diffusion as the main mechanism for drug release. For batches BD3, BD4 and BD5 the n values are more than 0.5, the mechanism of drug release is Anomalous transport.<sup>24</sup>

Factorial equations for four responses as per the coefficients obtained were as follows (Table 3)

$$Y_1 = 83.66 - 5.9417 X_1 + 7.2733 X_2 - 12.6283 X_1 X_2$$

$$Y_2 = 57.067 - 7.345 X_2 - 4.8836 X_1 X_2 + 10.0205 X_2 X_2$$

$$Y_3 = 38.05 + 2.5444 X_1 - 4.405 X_2 - 2.5863 X_1 X_2 + 6.6644 X_1 X_1 + 6.9063 X_2 X_2$$

$$Y_4 = 302.33 + 33.33 X_1$$

The RSM obtained for the relationship between independent variables EC (X<sub>1</sub>), HPMC (X<sub>2</sub>) and the responses Y<sub>1</sub>, Y<sub>2</sub>, Y<sub>3</sub> and Y<sub>4</sub> support and substantiate earlier discussions (Figure 6)

Table 1 - 3<sup>2</sup> full factorial design layout

Batch Code	Variable levels in coded form		% Production yield	Mean particle size ( $\mu\text{m}$ )	% Entrapment efficiency	Mucoadhesion time ( min )
	X <sub>1</sub>	X <sub>2</sub>				
BD 1	-1	-1	63.3	46.25 $\pm$ 4.3	47.87 $\pm$ 0.7	305 $\pm$ 2.23
BD 2	-1	0	95.14	50 $\pm$ 2.5	41.75 $\pm$ 1.14	339 $\pm$ 2
BD 3	-1	+1	96.84	43.75 $\pm$ 3.12	46.83 $\pm$ 0.57	356 $\pm$ 2.34
BD 4	0	-1	98.37	31.25 $\pm$ 2.5	51.75 $\pm$ 0.057	286 $\pm$ 2.34
BD 5	0	0	97.36	53.75 $\pm$ 6.08	38.4 $\pm$ 1.19	326 $\pm$ 2.34
BD 6	0	+1	66.6	53.75 $\pm$ 6.08	41.7 $\pm$ 0.35	309 $\pm$ 1.87
BD 7	+1	-1	60	60.0 $\pm$ 3.5	61.8 $\pm$ 2.34	229 $\pm$ 1.87
BD 8	+1	0	41.02	38.75 $\pm$ 3.08	44.75 $\pm$ 2.47	256 $\pm$ 2.34
BD 9	+1	+1	63	46.25 $\pm$ 3.16	46.46 $\pm$ 2.48	315 $\pm$ 2.23

Table 2 - Variables and their levels

Variables	Levels		
	Lower (-1)	Middle(0)	Upper(+1)
X <sub>1</sub> -Amount of ethyl cellulose	250 mg	275 mg	300 mg
X <sub>2</sub> -Amount of HPMC	325 mg	350 mg	375 mg

Table 3 – Summary of results of regression analysis

Responses	Coefficient						R <sup>2</sup>
	b <sub>0</sub>	b <sub>1</sub>	b <sub>2</sub>	b <sub>12</sub>	b <sub>11</sub>	b <sub>22</sub>	
% drug released at time 4 hrs (Y <sub>1</sub> )	83.66	-5.942	7.273	-	-12.628	-	0.8462
% drug diffused at time 4 hrs (Y <sub>2</sub> )	57.067	-	-7.345	-4.884	-	10.021	0.9567
% entrapment efficiency(Y <sub>3</sub> )	38.05	2.544	-4.405	-2.586	6.664	6.906	0.9849
Mucoadhesive time (Y <sub>4</sub> )	302.33	33.33	-	-	-	-	0.5213

Figure 1 – Dummy microparticles

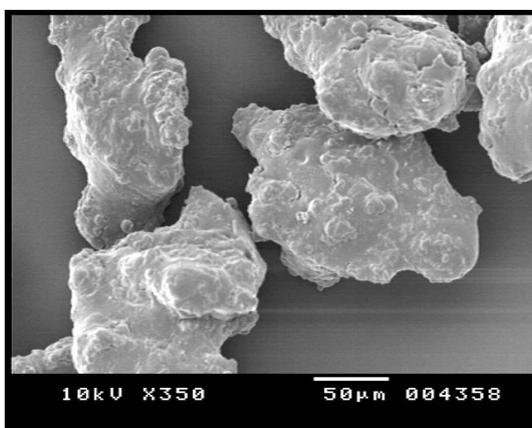


Figure 2 – Drug loaded microparticles

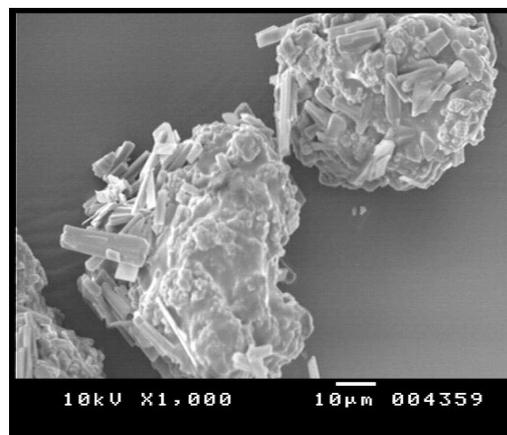


Figure 3 – % drug release from different factorial batches

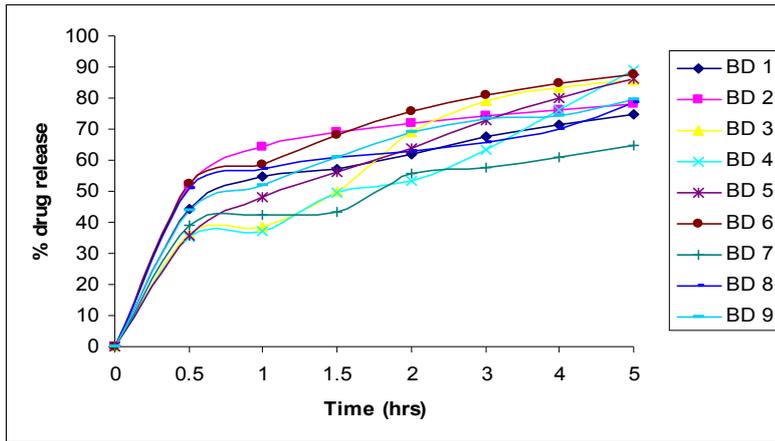


Figure 4 - % drug diffused from different factorial batches

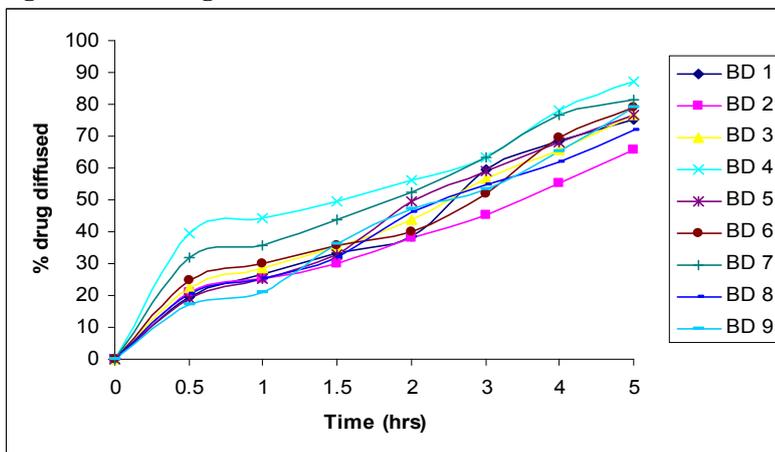


Figure 5 – Ex-vivo permeation of diltiazem through goat nasal mucosa

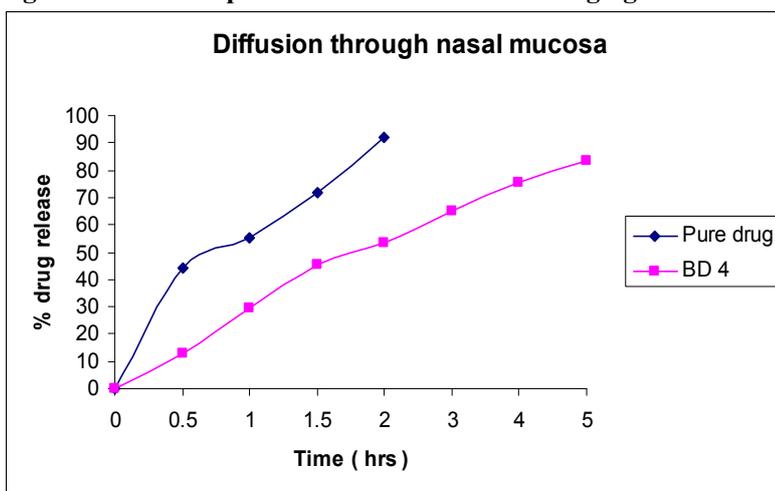
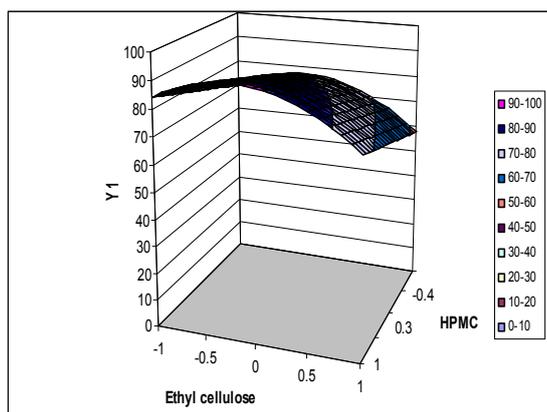
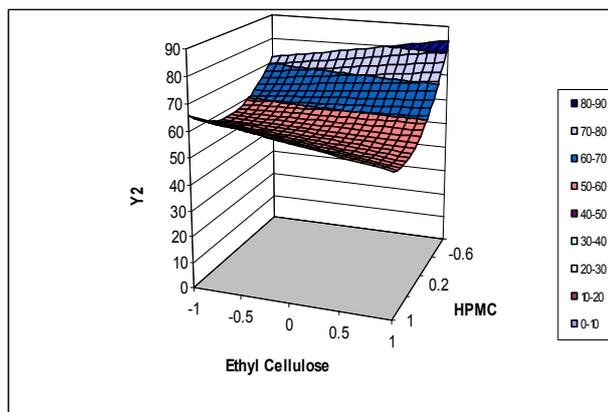


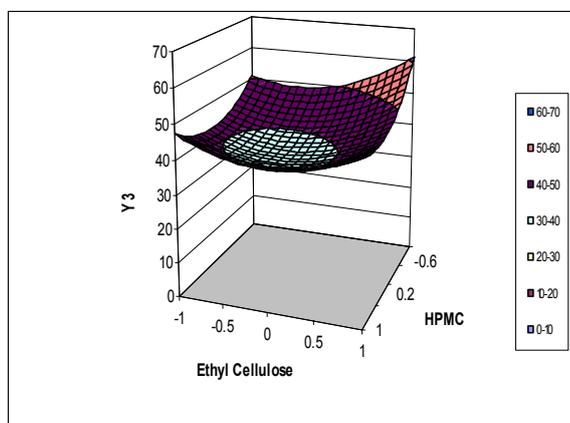
Figure 6 - Response surface plots showing effect of EC and HPMC on different variables



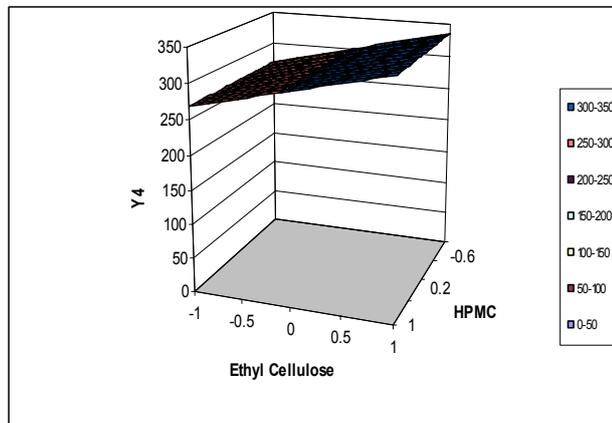
a. % drug release



b. % drug diffusion



c. % entrapment efficiency



d. mucoadhesion time

## CONCLUSION

These results indicate that the microparticles have potential to deliver diltiazem following intranasal administration. Its possibility to avoid first pass metabolism of diltiazem may ultimately show improvement of bioavailability than oral dosage. This may be due to increase in residence time.

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