Acta Pharmaceutica Sciencia
52: 401-410 (2010)

Design and optimization of mucoadhesive hydrophilic matrix tablet containing atenolol using central composite design

Mahendra Nakarani[1]*, Dhaval J. Patel[2], Dipen R. Bhimani[2], Jayvadan K. Patel[3], Subhash S. Vaghani[4]

[1]Unison Pharmaceuticals Limited, Ahmedabad, Gujarat, India.
[2]S.K. Patel College of Pharmacy, Edu.&Res., Ganpat University, Gujarat, India.
[3]Nootan College of Pharmacy, Visnagar, Gujarat, India.
[4]Smt. R. B. Patel Mahila Pharmacy College, Atkot, Gujarat, India

Abstract

The current study involves development of oral mucoadhesive hydrophilic matrices of atenolol, and optimization of their in vitro drug release profile and *ex vivo* mucoadhesion against goat intestinal mucosa. A 3^2 central composite design (CCD) was employed to systematically optimize the drug delivery formulations containing two polymers, viz., chitosan-652 and hydroxypropyl methylcellulose K100MCR (HPMC K100MCR). Response surface plots and contour plots were drawn, and optimum formulations were selected by feasibility and grid searches. Both the polymers had significant effect on the mucoadhesive strength of the tablets, measured as force of detachment against goat intestinal mucosa. Validation of the formulation optimization study carried out using six confirmatory runs, indicated a very high degree of prognostic ability. The study successfully undertook the development of an optimized once-a-day formulation of atenolol with excellent mucoadhesive and controlled release characteristics.

Keywords: atenolol, experimental design, mucoadhesive, response surface methodology, central composite design.

Introduction

Oral controlled release systems continue to be the most popular ones among all the drug delivery systems. Over the last two decades mucoadhesion has become of interest for its potential to optimize localized drug delivery, by retaining a dosage form at the site of action (e.g. within the gastrointestinal tract) or systemic delivery, by retaining a formulation in intimate contact with the absorption site (e.g. the nasal cavity) (Smart 2005).

Atenolol, a β-blocker, is prescribed widely in diverse cardiovascular diseases *viz.* hypertension, angina pectoris, arrhythmias and myocardial infarction (Hoffman 2001). The drug is also frequently indicated in the prophylactic treatment of migraine. Administration of conventional tablets of atenolol has been reported to exhibit fluctuations in the plasma drug levels, resulting

***Corresponding author:** nakmahen@yahoo.com

either in manifestation of side effects or reduction in drug concentration at the receptor site (Sastry et al. 1997, Vaithiyalingam et al. 2001). Accordingly, studies have been reported on regulation of drug release by formulating its diverse controlled release systems like hydrophilic matrices, osmotic pumps and transdermal drug delivery systems (Perez-Marcos et al. 1991, Vazques et al. 1996, Rouge et al. 1998, Sastry et al. 1998).

The objective of the current study was to develop mucoadhesive tablet formulations of atenolol and optimize their mucoadhesive and drug release characteristics employing the benefits of the response surface methodology with design of experiments methodology. Based on the principal of design of experiments, CCD was employed to investigate the effect of two independent factors. Design of experiments encompasses the use of various types of experimental designs, generation of polynomial equations and responses over the experimental domain to determine the optimum formulations.

Materials and methods

Materials

Atenolol was obtained as a gift sample from Alembic Pharmaceutical Ltd., Baroda, India. Chitosan-652 was obtained as a gift sample from DKSH, Bombay, India. HPMC K100MCR was obtained as a gift sample from Colorcon Asia Ltd, Goa, India. All other chemicals were of analytical reagent grade and were procured from local suppliers.

Preparation of atenolol mucoadhesive hydrophilic matrix tablets

Each ingredient was weighed accurately and then passed from 40#. Required amount of drug was initially mixed with chitosan-652, HPMC-K100MCR, DCP with the help of mortar and pestle. The sieved powder blend was hand granulated using 2% polyvinylpyrrolidone K-30 (PVP K-30) in isopropyl alcohol (IPA) solution. The resulting powder dough was passed through 20#. The resultant wet granules were dried at 60°C for 10-15 min. The resulting dry granules were blended with 2% sodium lauryl sulphate (SLS), 1% magnesium stearate (mg-stearate) and 1% talc for 15 min. The granules were compressed using 9mm standard concave punches (Table 1). Weight variation, friability, hardness, thickness and assay were performed for tablets. Optimization of core tablet was carried out by measuring mucoadhesive strength and in vitro dissolution studies.

Table 1. Composition of hydrophilic matrix tablet of atenolol

Ingredient	Concentration (mg/tablet)	Composition (%)
Atenolol	50	20
Chitosan-652	25-75	10-30
HPMC K100 MCR	50-100	20-40
Sodium lauryl sulphate	5.0	2
PVP K-30 (2% in IPA)	12.5	5
Dibasic calcium Phosphate	Q.S.	Q.S.
Talc	2.5	1
Mg Stearate	2.5	1

*Q.S indicate as quantity sufficient for preparation of hydrophilic matrix tablet of atenolol

Experimental design

A central composite design was used in order to investigate the joint influence of formulation variables. Central composite design with $\alpha = 1$ was employed as per the standard protocol (Singh et al. 2005). The concentration of chitosan-652 (X_1) and HPMC K100MCR (X_2) were selected as the independent variables

factors, studied at 3 levels each. Percentage of drug release after 8 h (Q_8), percentage of drug release after 18 h (Q_{18}) and mucoadhesive strength were taken as the dependent variables (response; Y). In this design, 2 factors are evaluated, each at 3 levels, and experimental trials are performed at all 13 possible combinations (Table 2). All other formulation variables and processing variables were kept invariant throughout the study.

Tablet assay and physical evaluation

The tablets were assayed for drug content using methanol as the extracting solvent, and the samples were analyzed spectrophotometrically (Shimadzu 1601, Japan) at 275 nm. Tablets were also evaluated for hardness (n = 6) using a Monsanto type hardness tester (Campbell, India), friability (n = 6) using a Roche friabilator (Tropical Lab Equipments, India), weight variation (n = 10) using an Electronic balance (Mettler, Switzerland), and thickness (n = 10) using a Vernier calipers (Baker Gauges Ltd., India).

Table 2. Central composite design lay out

Trial no.	Coded factor level	
	X_1	X_2
A	-1	-1
B	-1	0
C	-1	1
D	0	-1
E	0	0
F	0	1
G	1	-1
H	1	0
I	1	1
J	0	0
K	0	0
L	0	0
M	0	0

Translation of coded level in actual units:			
Coded level	-1	0	+1
X_1: Chitosan-652 (mg)	25	50	75
X_2: HPMC K100MCR (mg)	50	75	100

In vitro drug release study

Dissolution studies were performed for all the formulation combinations, in triplicate, employing USP-XXIV paddle method and pH 6.8 phosphate buffer solution (PBS) as the dissolution medium at 50 rpm and 37±0.5°C. A 5 mL aliquot of the sample was withdrawn periodically at suitable time intervals and the volume replaced with an equivalent amount of the same dissolution medium. The samples were analyzed spectrophotometrically at 275 nm (Shimadzu 1601, Japan).

In vitro mucoadhesion study

Mucoadhesion studies were conducted using a modification of the assembly described earlier, with goat intestinal mucosa as the model membrane. The mucosa was kept frozen in pH 6.8 phosphate buffer (PBS) and thawed to room temperature before use. The mucosal membrane was excised by removing the underlying connective and adipose tissue and was equilibrated at 37±1°C for 30 min in buffer (PBS pH 6.8) before the mucoadhesion evaluation study. The tablet was lowered onto the mucosa under a constant weight of 5 g for a total contact period of 1 min. Mucoadhesive strength was assessed in terms of the weight in grams required to detach the tablet from the membrane.

Optimization data analysis

Various RSM (Response surface methodology) computations for the current optimization study were performed employing Design expert software (Version 7.0.1, Stat-Ease Inc, Minneapolis, USA).

Polynomial models including interaction and quadratic terms were generated for all the response variables using multiple linear regression analysis (MLRA) approach. Also, the 3-D response surface graphs and 2-D contour plots were constructed in MS-Excel environment using the output files generated by the Design expert software.

Validation and selection optimization model

Six optimum checkpoints were selected by intensive grid search, performed over the entire experimental domain, to validate the chosen experimental design and polynomial equations. The formulations corresponding to these checkpoints were prepared and evaluated for various response properties. Subsequently, the resultant experimental data of response properties were quantitatively compared with that of the predicted values. Also, linear regression plots between observed and predicted values of the response properties were drawn using MS-Excel, forcing the line through origin.

Results and Discussions

Selection of polymers and suitable experimental design

The polymers, chitosan-652 and HPMC K100MCR, were selected owing to their excellent bioadhesive strength, release rate controlling ability, non-toxicity, non-irritancy, stability at gastro-intestinal pH and compatibility with the drug (Singh et al. 2009). Chitosan is a polycationic polymer, a positively charged hydrogel is formed in an acidic environment that could develop additional molecular attractive forces by electrostatic interaction with negatively charged mucosal surfaces or the negatively charged sialic acid groups of the mucus network. Also, it is a liner polyamine in which the amino groups are readily available to interact with negative surface. HPMC K100MCR is long chained, nonionic polymer and so its mucoadhesion is attributable to the formation of physical bonds or hydrogen bonding with the mucus components. HPMC K100MCR possesses larger number of hydroxyl groups that are responsible for adhesion. Successful use of the polymer combination of an ionic (polycationic polymer, chitosan-652) and a nonionic polymer (like HPMC K100MCR) is known to provide the formulation with controlled drug release along with desired mucoadhesive properties (Singh et al. 2009).

A CCD for two factors at three levels with $\alpha = 1$, equivalent to 3^2 factorial design, was chosen as the experimental design. This is an effective second-order experimental design associated with a minimum of experiments to estimate the influence of individual variables (main effects) and their second-order effects. Further, this design has an added advantage of determining the quadratic response surface, not estimable using a factorial design at two levels (Singh et al. 2006).

Tablet assay and physical evaluation

The content of drug in various formulations varied between 98.9% and 100.9% (*m/m*) (mean ± SD = 99.8 ± 0.6 %). Tablet weights varied between 260.5 to 240.3 mg (mean 250.4 ±1.75 mg), thickness between 2.20 to 2.35 mm (mean 2.25±0.19 mm), hardness between 5.8 to 7.5 kg cm^{-2} (mean 6.65±1.28 Kg cm^{-2}), and friability ranged between 0.3% to 0.5% (mean 0.4±0.13%). Thus, all physical parameters of the compressed matrices were within the permissible limits of USP.

In vitro dissolution study

Table 3 enlists various dissolution parameters computed for all the atenolol mucoadhesive formulations. Total amount of atenolol released from all the formulations until 24 h ranged between 93.86 and 100.08% indicating almost complete drug release from all the formulations. Rate of drug release (until 18 h) tended to decrease with increase in the concentration of HPMC K100MCR. This is in agreement with literature findings that the viscosity of the gel layer around the tablet increases with increase in the hydrogel concentration, thus limiting the release of active ingredient (Singh et al. 2006). With further increase in polymer amount (HPMC K100MCR), thicker gel forms inhibiting water penetration more strongly, resulting in significant reduction in the values of Q_{18} indicating slower drug release. At high levels of both the polymers, a significant fraction of the drug (~16.8%) remained unreleased until 18 h, which can eventually lead to significant reduction in the extent of bioavailability. As evident from the diverse nature of dissolution profiles (Fig. 1), the influence of polymer levels seems to be vital in regulating the drug release. Dissolution profiles (Fig. 1) of all the formulations portray an initial burst release of the drug, characteristic of most hydrophilic matrices (Singh et al. 2009). This could be attributed to the dissolution of drug present initially at the surface of the matrices and availability of higher amount of unreleased drug present in the dosage form. However, the formulations showed little burst effect at higher polymer levels, compared to lower polymer levels. The values of $t_{50\%}$ enhanced markedly from 4.51 h, observed at low levels of both the polymers, to as high as 9.15 h, observed at high levels of both the polymers. This finding indicated considerable release-retarding potential of the polymers for atenolol.

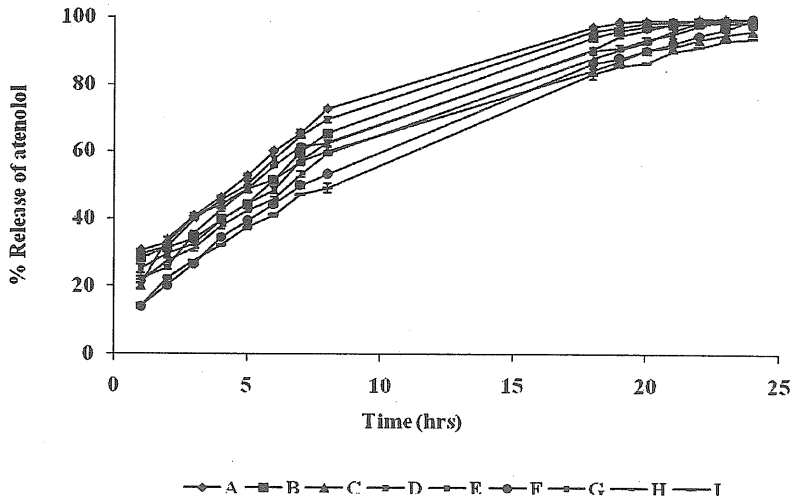


Figure 1. Dissolution profiles of various mucoadhesive tablet formulations (A to F9) of atenolol prepared as per the experimental design (n = 3).

Table 3. Dissolution parameters of various mucoadhesive formulations prepared as per the experimental design

Formulation code	Formulation composition		% Release after 8 h (Q_8)	% Release after 18 h (Q_{18})	% Release after 24 h (Q_{24})	$t_{50\%}$ (h)
	Chitosan-652 (mg)	HPMC K100 MCR (mg)				
A	25	50	72.76	97.20	99.40	4.51
B	25	75	65.55	94.16	98.64	6.30
C	25	100	60.16	84.28	96.16	6.72
D	50	50	69.56	95.96	99.60	7.52
E	50	75	62.60	90.16	98.56	7.85
F	50	100	55.28	86.48	99.60	8.38
G	100	50	62.44	90.56	100.08	8.52
H	100	75	59.28	88.00	99.76	9.15
I	100	100	49.20	83.20	93.86	11.2
J	50	50	62.00	90.00	99.30	7.23
K	50	50	61.90	90.10	99.70	7.38
L	50	50	63.30	89.90	98.70	7.41
M	50	50	63.00	91.20	98.60	7.48

In vitro mucoadhesion study

Figure 2 shows the bar chart depicting significant variation in the values of bioadhesive strength, obtained using different ratios of polymers. Maximum bioadhesive strength, therefore, was seen at the highest levels of the 2 polymers. The hydrogels are known to swell readily, when they come in contact with hydrated mucous membrane. Water sorption reduces the glass transition temperature below ambient conditions, and hydrogels become progressively rubbery due to uncoiling of polymer chains and subsequent increased mobility of the polymer chains. This glass-rubbery transition provides hydrogels plasticization resulting in a large adhesive surface for maximum contact with mucin and flexibility to the polymer chains for interpenetration with mucin (Singh et al. 2006).

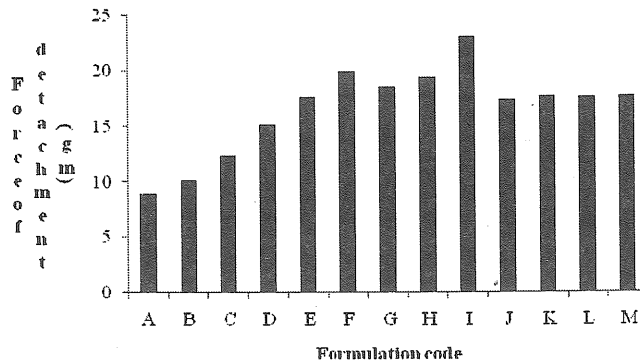


Figure. 2. Bar diagram showing bioadhesive strength determined as the force of detachment of mucoadhesive tablet formulations (A to M) of atenolol prepared as per central composite design.

Optimization data analysis

In order to investigate the factors systematically, a central composite design was employed. As shown in equation 1, a statistical model incorporating interactive and polynomial terms was used to evaluate the responses.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_1 X_2^2 + \beta_7 X_1^2 X_2 \quad (1)$$

Seven coefficients (b_1 to b_7) were calculated representing b_0 as the intercept, and b_3 to b_7 various quadratic and interaction terms.

Mathematical relationships generated using MLRA for the studied response variables are expressed as equations.

$$\text{Mucoadhesive strength} = 17.59+3.90X_1+2.00X_2+0.05X_1X_2-3.23X_1^2+0.06X_2^2 \quad (2)$$

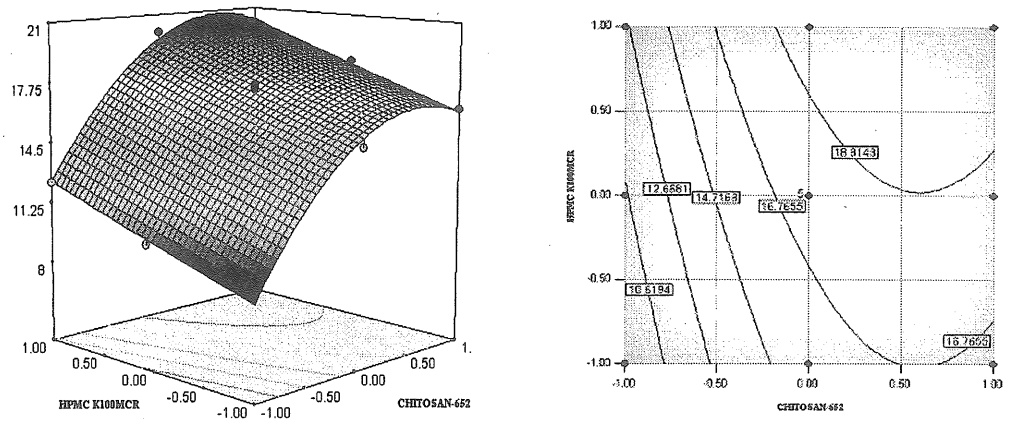
$$Q_8 = 62.54-3.75X_1-7.53X_2-1.43X_1X_2-0.09X_1^2-0.04X_2^2 \quad (3)$$

$$Q_{18} = 90.72-2.35X_1-5X_2+1.42X_1X_2-0.72X_1^2-0.57X_2^2 \quad (4)$$

Where, Q_8 = Release after 8 h; Q_{18} = Release after 18 h.

The polynomial equations comprise the coefficients for intercept, first-order main effects, interaction terms, and higher order effects. The sign and magnitude of the main effects signify the relative influence of each factor on the response. The values obtained for main effects of each factor in equations 2, 3 and 4 reveal that chitosan-652, individually, has rather more pronounced effect on the values of force of detachment,Q_8 and Q_{18} respectively.

Fig. 3a to 5a portray the 3-dimensional response surface plots, while Fig. 3b to 5b are the corresponding contour plots for the studied response properties *viz*, mucoadhesive strength, percentage of drug release after 8hrs (Q_8), and percentage of drug release after 18hrs (Q_{18}). Fig. 3a shows a nearly linear ascending pattern for the values of bioadhesive strength, as the content of either polymer is increased, the effect being much more prominent with chitosan-652 than with HPMC K100MCR. Maximum bioadhesive strength is observable at the highest levels of polymers, viz., chitosan-652 and HPMC K100MCR. Nearly vertical contour lines (Fig. 3b) corroborate the markedly significant influence of chitosan-652 on mucoadhesive strength.



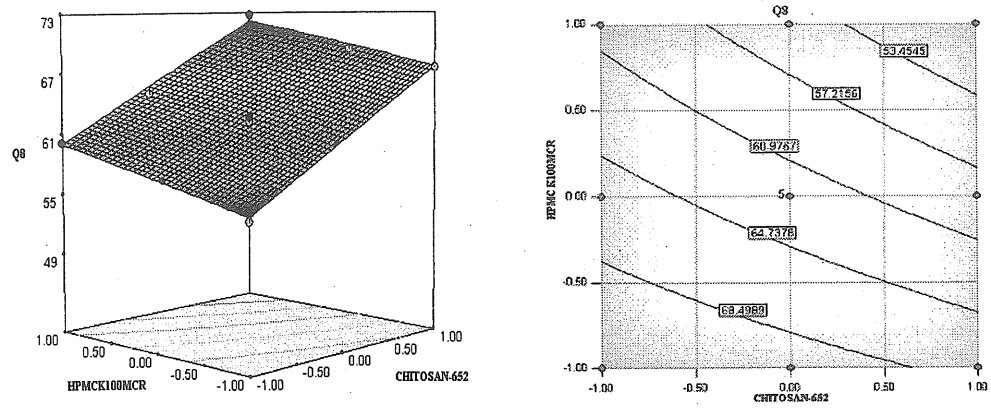
(3a) (3b)

Figure 3. a) Response surface plot showing the influence of chitosan-652 and HPMC K100MCR on the value of mucoadhesive strength of mucoadhesive tablet formulations of atenolol, b) the corresponding contour plot.

Fig. 4a and 4b reveal a sharp decline in the value of Q_8 with an increase in the amount of each of the polymers, *i.e.*, chitosan-652 and HPMC K100MCR, the influence of chitosan-652 being much more pronounced. Fig. 4a and 4b reveal a sharp decline in the value of Q_8 with an increase

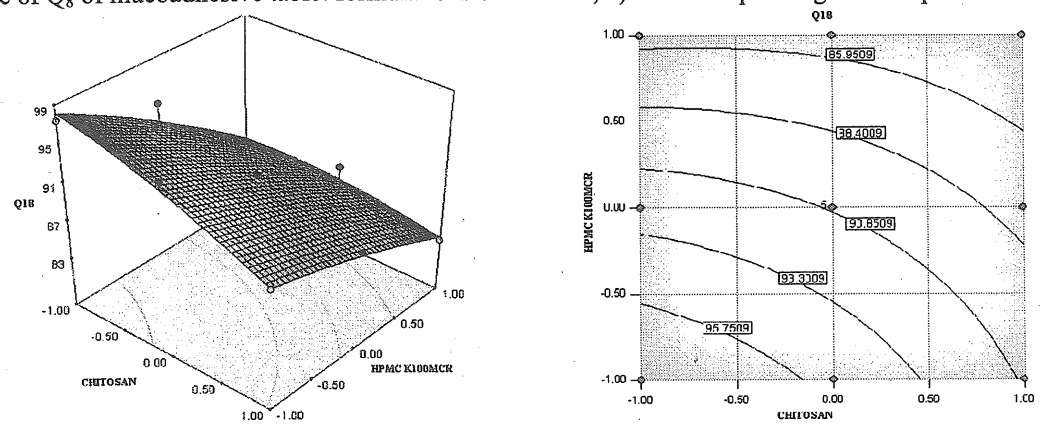
in the amount of each of the polymers, *i.e.*, chitosan-652 and HPMC K100MCR, the influence of chitosan-652 being much more pronounced.

Fig. 5a and 5b also exhibit that Q_{18} vary in a nonlinear manner, but in a descending pattern with an increase in the amount of each polymer. Except at high level of chitosan-652, this declining trend was observed until intermediate levels of HPMC K100MCR, after which a near plateau was discernible (i.e., the drug release values did not decrease appreciably). The contour plot (Fig. 5b) shows that HPMC K100MCR has a comparatively greater influence on the response variable than chitosan-652.



(4a) (4b)

Figure 4. a) Response surface plot showing the influence of chitosan-652 and HPMCK100MCR on the value of Q_8 of mucoadhesive tablet formulations of atenolol, b) the corresponding contour plot.



(5a) (5b)

Figure 5. a) Response surface plot showing the influence of chitosan-652 and HPMCK100MCR on the value of Q_{18} of mucoadhesive tablet formulations of atenolol, b) the corresponding contour plot.

Validation and selection optimization model

Upon comparison of the observed responses with those of the anticipated ones (Table 4), the prediction error varied between –1.60% and 1.51 %. Linear correlation plots drawn between the predicted and observed responses after forcing the line through the origin, also demonstrated high values of R^2 (0.886, 0.824 and 0.841 respectively for mucoadhesive strength, and Q_{18})

indicating goodness of fit. Upon "trading off" various response variables, the following maximizing criteria were adopted: $Q_8 \geq 50\%$, $Q_{18} \geq 85\%$ and mucoadhesive strength required is maximum level. Upon comprehensive evaluation of feasibility search and subsequently exhaustive grid searches, the formulation composition with polymer levels of chitosan-652 (50 mg) and HPMC K100MCR (100 mg) fulfilled maximum requisites of an optimum formulation because of better regulation of release rate and higher mucoadhesive strength.

Table 4. Comparison of the experimental results with the predicted responses

Composition					
X_1	X_2	Response variable	experiment value	predicted value	percentage error*
-0.15	1	Mucoadhesive Strength (g)	18.71	18.99	-1.47
		Q_8	55.7	55.73	-0.05
		Q_{18}	84.4	85.77	-1.60
1	0.41	Mucoadhesive Strength (g)	19.02	19.10	-0.42
		Q_8	55.08	55.01	0.13
		Q_{18}	84.87	86.08	-1.41
1	0.4	Mucoadhesive Strength (g)	19.27	19.08	1.00
		Q_8	54.21	54.10	0.20
		Q_{18}	85.6	86.12	-0.60
1	0.43	Mucoadhesive Strength (g)	19.44	19.15	1.51
		Q_8	54.54	54.83	-0.53
		Q_{18}	84.84	86.00	-1.35
1	0.35	Mucoadhesive Strength (g)	18.71	18.98	-1.42
		Q_8	55.86	55.55	0.56
		Q_{18}	86.57	86.32	0.29
1	0.46	Mucoadhesive Strength (g)	19.48	19.21	1.41
		Q_8	53.84	54.56	-1.32
		Q_{18}	84.84	85.88	-1.21

The formulation F showed Q_8 as 53.28%, Q_{18} as 86.48%, and mucoadhesive strength as 20 g. The said formulation, however, released the drug completely (i.e, 99.6% drug in 24 h). Ultimate composition of atenolol mucoadhesive tablet was given in Table 5.

Table 5. Composition of optimized formulation of atenolol mucoadhesive tablet

Ingredient	Amount (mg/tablet)	Amount (%/tablet)
Atenolol	50	20
Chitosan-652	50	20
HPMC K100 MCR	100	40
SLS	5.0	2
PVPK-30 (2% in isopropyl alcohol)	12.5	5
Dibasic calcium phosphate	27.5	11
Talc	2.5	1
Magnesium stearate	2.5	1
Total weight	250	100

Conclusion

In the present investigation, an attempt was made to development and optimization of a once-a-day formulation of atenolol hydrophilic matrix tablet with high regulation of the release rate and bioadhesive strength. For the formulation of the oral mucoadhesive tablet, chitosan-652 and HPMC K100MCR and their combinations were used in varying concentrations. High mucoadhesive strength of the formulation is likely to increase its gastrointestinal residence time, and improve the extent of bioavailability. Suitable balancing between the levels of two polymers (chitosan-652 and HPMC K100MCR) is significant in terms of control in drug release and adequate bioadhesion. The study successfully undertook the development of an optimized formulation of atenolol with excellent bioadhesive and controlled release characteristics using CCD.

References

Hoffman, B.B. (2001). Catecholamines, sympathomimetics drugs, and adrenergic receptor antagonists. In: Hardman JG, Limbird LE, editors. Goodman Gilman's: The Pharmacological Basis of Therapeutics. 10th ed. McGraw Hill. New York, pp 199-248.

Perez-Marcos, B., Iglesias, R. and Gomez-Amoza, J.L. (1991). Mechanical and drug release properties of atenolol-carbopol hydrophilic matrix tablets. *J. Control. Release* 17: 267-276.

Rouge, N., Allemann, E., Gex-Fabry, M., Balant, L., Cole, E.T., Buri, P. and Doelker, E. (1998). Comparative pharmacokinetic study of a floating multiple-unit capsule, a high-density multiple-unit capsule and an immediate-release tablet containing 25 mg atenolol. *Pharm. Acta. Helv.* 73: 81-87.

Sastry, S.V. and Khan, M.A. (1998). Aqueous based polymeric dispersion: Plackett-Burman design for screening of formulation variables of atenolol gastrointestinal therapeutic system. *Pharm. Acta. Helv.* 73: 105-112.

Sastry, S.V., Reddy, I.K. and Khan, M.A. (1997). Atenolol gastrointestinal therapeutic system: Optimization of formulation variables using response surface methodology. *J. Control. Release* 45: 121-130.

Singh, B., Chakkal, S. and Ahuja, N. (2006). Formulation and optimization of controlled release mucoadhesive tablets of atenolol using response surface methodology. *AAPS PharmSciTech.* 7: 19-28.

Singh, B., Kumar, R. and Ahuja, N. (2005). Optimizing drug delivery systems using systematic design of experiment. Part I: Fundamental aspects. *Crit. Rev. Ther. Drug. Carrier Syst.* 105: 22-27.

Singh, B., Mehta, G., Kumar, R., Bhatia, A., Ahuja, N. and Katare, O.P. (2005). Design, development and optimization of nimesulide-loaded liposomal systems for topical application. *Curr. Drug Deliv.* 2: 143-153.

Singh, B., Pahuja, S., Kapil, R. and Ahuja, N. (2009). Formulation development of oral controlled release tablets of hydralazine: Optimization of drug release and bioadhesive characteristics, *Acta Pharm.* 59: 1-13.

Smart, J.D. (2005). The basics and underlying mechanisms of mucoadhesion. *Adv. Drug Deliv. Rev.* 57: 1556-1568.

Vaithiyalingam, S.R., Sastry, S.V., Dehon, R.H., Reddy, I.K. and Khan, M.A. (2001). Long-term stability characterization of a controlled release gastrointestinal therapeutic system coated with a cellulose acetate pseudolatex. *Pharmazie* 56: 66-69.

Vazques, M.J., Casalderrey, M., Duro, R., Gomez-Amoza, J.L. Martinez-Pacheco, R., Souto, C. and Concheiro, A. (1996). Atenolol release from hydrophilic matrix tablets with hydroxypropyl methylcellulose (HPMC) mixtures as gelling agent: Effects of the viscosity of the HPMC mixture. *Eur. J. Pharm. Sci.* 4:39-48.

Received: 06.02.2010
Accepted: 23.05.2010