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Der Pharmacia Lettre, 2016, 8 (10):206-212
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Formulation and optimization of Mucoadhesive Galantamine Loaded Nanoparticles

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ABSTRACT

Optimization is used in formulation design to make process fabrication as perfect and effective as possible. Optimization is needed to explore and define ranges for formulation and processing parameters to produce 'the best fit model' under a given set of restrictions. Nanopaticulate formulations were designed and developed for anti Alzheimer's drug galantamine hydrobromide. Chitosan and thiolated chitosan were used for fabrication of nanoparticles by ionic gelation method with tripolyphosphate (TPP) as cross-linking agent. The influence of various factors i.e. Polymer concentration, cross linking agent concentration and stirrer speed were investigated by Box-Behnken Design for optimization. Effect of the selected factors were studied on the responses measured i.e. Particle size, drug entrapment efficiency and mucoadhesive potential.

Keywords: Optimization, Nanoparticles, Box-Behnken Design, Chitosan, Thiolated chitosan

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INTRODUCTION

Nanofabrication is critical process depends upon various factors and in order to achieve desired parameters, various optimization techniques are used. Out of various optimization techniques, Response Surface Methodology (RSM), combines mathematical and statistical techniques for constructing models, which are being widely utilized for formulation optimizations. By the help of optimization techniques, all formulation factors are evaluated in all possible combinations with minimum number of experimental runs. All the dependent variables (responses) are correlated with the independent variables with the empirical model equation. The 3D response surface plots, contour plots and cube plots are generated with the help of optimization method and helps in finding of interactions among the process variables.

Galantamine is a reversible competitive inhibitor of cholinesterase's, the enzyme responsible for inactivating acetylcholine and indicated for the treatment of mild to moderate Alzheimer's type dementia [1]. Chitosan and its modified derivatives, have been diversely employed in drug delivery due biodegradability, biocompatibility and also used for food applications. More recently, chitosan nanoparticles have attracted much attention due to versatile physiochemical properties like high drug loading capacity, better mucoadhesive and adsorption performance [2]. Thiolation of chitosan also improves the mucoadhesive potential as it tightly adheres to mucosal epithlium through covalent bonding with mucin gylcoprotiens via thiol-disulfide linkage [3]. Several techniques have been developed to prepare chitosan nanoparticles, such as ionotropic gelation, microemulsion, emulsification solvent diffusion,

polyelectrolyte complex, emulsification cross-linking, complex coacervation and solvent evaporation method [4]. The unique structural feature of chitosan is the presence of the primary amine at the C-2 position of the glucosamine residues. Due to its polycationic nature and it get easily interacts with negatively charged multivalent ions such as tripolyphosphate (TPP) which enable nanoparticle formulation via both physical and chemical cross-linking [5, 6]. For pharmaceutical applications, physical cross-linking is more promising since it is reversible and also avoids potential toxicity of the reagents. In present investigation chitosan and thiolated chitosan were used to fabricate galantamine nanoparticle by ionic gelation method and optimized by Box- Behnken Design.

MATERIALS AND METHODS

Galantamine hydrobromide (GT) was gifted by Ranbaxy Pharmaceuticals Ltd. (Gurgaon), India. Chitosan (CH) was obtained as gift sample from CIFT, Kochi, India. Sodium tripoly phosphate (TPP) and mannitol was purchased from Hi Media lab Pvt. Ltd. (Mumbai) and all other chemicals used are of analytical grade.

Table: I Design layout of galantamine nanoparticles

	Coded Levels of Numeric factors			Categoric factors
Formulation Batch	**X1**	**X2**	**X3**	**Polymer type**
1	0	-1	-1	Chitosan
2	1	-1	0	Thiolated chitosan
3	0	1	-1	Chitosan
4	-1	0	-1	Chitosan
5	-1	-1	0	Thiolated chitosan
6	0	0	0	Thiolated chitosan
7	-1	0	1	Chitosan
8	-1	1	0	Chitosan
9	-1	-1	0	Chitosan
10	-1	0	-1	Thiolated chitosan
11	0	0	0	Chitosan
12	0	0	0	Thiolated chitosan
13	0	0	0	Thiolated chitosan
14	1	-1	0	Chitosan
15	0	0	0	Chitosan
16	0	-1	-1	Chitosan
17	1	1	0	Thiolated chitosan
18	1	0	-1	Thiolated chitosan
19	1	0	-1	Chitosan
20	-1	0	1	Thiolated chitosan
21	1	1	0	Chitosan
22	-1	1	0	Thiolated chitosan
23	1	0	1	Thiolated chitosan
24	0	0	0	Chitosan
25	0	0	0	Thiolated chitosan
26	0	1	1	Thiolated chitosan
27	0	-1	1	Thiolated chitosan
28	0	0	0	Chitosan
29	0	0	0	Thiolated chitosan
30	0	-1	1	Chitosan
31	0	1	-1	Thiolated chitosan
32	0	0	0	Chitosan
33	0	1	1	Chitosan
34	1	0	1	Chitosan

Translation of Coded levels in actual units	**Minimum**	**Medium**	**Maximum**
Coded Levels of Numeric factors			
X1 : Drug Polymer ratio	-1	0	1
X2 : Stirrer Speed	-1	0	1
X3 : Polymer TPP Ratio	-1	0	1
Categoric Factor : Polymer type			
Chitosan (%)	0.1	0.2	0.3
Thiolated Chitosan (%)	0.1	0.2	0.3
Cross linking agent			
Sodium tripolyphosphate (TPP) %	0.03	0.06	0.1

Experimental design and preparation of galantamine nanoparticles
Chitosan (CH) and thiolated chitosan (TCH), nanoparticles (NPs) incorporating galantamine were fabricated by modified ionic gelation method using TPP as cross linking agent.

In this method 0.1% chitosan in 2% acetic acid solution was prepared and pH was adjusted to 5.6 using aqueous solution of sodium hydroxide. Nanoparticles were obtained as result of the drop wise addition of TPP solution to the aqueous solution of polymer (TCH/CH) by continuous stirring, TCH/CH to TPP weight ratio used is 3:1. The drug was dissolved in polymeric solution before cross linking. As a result of ionic cross linking, a slight milky turbid solution was obtained, which was further stirred for half an hour. The resultant nanoparticles were separated by centrifugation at 13,000 rpm for 1h at 4◦C. The pellets was redispersed in water and lyophilized by using 2% D - mannitol as cryoprotectant.

The experimental design layout is summarized in Table I, for the formulation of polymeric nanoparticles was generated by Box-Behnken Design. The effects of selected three numeric factors (drug polymer ratio, stirrer speed and drug TPP ratio) and one categoric factor that is polymer type (chitosan and thiolated chitosan), each at three level on the three responses (particle size, drug entrapment efficiency and mucoadhesive potential) were evaluated in order to create optimum design space. The software Design Expert version 9.0.6.2 (Stat-ease Inc.Minneapolis, MN) was employed for statistical analysis of the obtained data.

Characterization of formulated nanoparticles
Measurement of particle size, zeta potential and polydispersity index
Average particle size (Z-average), polydispersity index (PDI) and zeta potential of the prepared nanoparticles were determined by dynamic light scattering analysis using Zetasizer (Nano ZS 90, Malvern Instruments, U.K.) All the measurements were carried out by dispersing the nanoparticles in appropriate volume of deionised water at 25 ◦C.

Percent drug entrapment efficiency (%DEE)
The supernatant of formulations after centrifugation were collected and filtered. The amount of drug present was determined by UV spectrophotometer (Varian Cary-5000, Netherland) at 289nm. The Percentage drug entrapment efficiency (DEE) was calculated using formula:

$$\%\ DEE = \frac{The\ amount\ of\ drug\ (W) - Free\ drug\ in\ supernatant(w)}{Total\ amount\ of\ drug\ (W)} \times 100$$

Mucoadhesive potential
Chitosan and TCH discs were prepared by direct compression using single punch hydraulic press (K- Imaya Engineers) at the pressure of 10 tons for 10s having 13 mm diameter with flat surface carrying 200 mg of nanoparticle formulation. Nasal tissue from upper respiratory tract of the nasal cavity of goat was obtained from animals immediately after slaughter at local slaughterhouse. The tissues were washed with deionized water and placed in normal saline solution at 4 ^{o}C for further studies.

Mucoadhesive potential of the polymeric discs was carried out using a texture analyzer (TA XT2, Stable Microsystems, U.K.). A disc was attached to the cylindrical probe (20 mm diameter) by double sided adhesive tape. The tissue was equilibrated for 15 min at 37.0 ± 0.5 ^{0}C before placing on to the holder stage of texture analyzer. The probe attached to disc was loaded with 5 kg weight cell. The test speed was settled at 1mm/s and probe was moved downwards, touches the tissue for 30s and afterwards the probe was subsequently withdrawn. The maximum force required to separate the probe from the tissue (i.e. maximum detachment force; F_{max}) was detected directly from texture analyzer and was used to compare the mucoadhesive potential of the polymeric nanoparticles.

RESULTS AND DISCUSSION

Box- Behnken Design for Optimization
The values of all responses measured (particle size, drug entrapment efficiency (% DEE) and mucoadhesive potential) are shown in table II. Particle size of all the formulation were found to be in the range of 114.7 nm to 565 nm, % DEE varied from 65.9% to 90.6% and mucoadhesive potential was observed in the range of 0.4N to 5.2N.
Quadratic surface model was found to be best fit model for the responses particle size and DEE and linear model for the response mucoadhesive potential with p values for X_1, X_2 and X_3 as 0.0189, 0.0047and 0.0001and F values as

7.30, 5.90 and 23.16 respectively. The adequate precision is the measure of signal to noise ratio and its value being more than 4 for all these selected response variables and hence model can be used to navigate the optimum design space. The reliability of regression models was also established from the high R^2 for the particle size, drug entrapment efficiency and mucoadhesive potential (0.8084, 0.8641 and 0.7616) values and their similar adjusted R^2 values (0.7960, 0.7758 and 0.7287) respectively.

The following polynomial coded equations were generated for GT nanoparticles

Particle Size = +275.92* A +100.43+49.44* B +74.03* C +18.88* D +33.02* AB +20.48* CD -12.66A^2+56.54* B^2 -5.47C^2

%DEE = +83.49 -5.83* A -5.83* B - 4.99* C -1.34* D -1.77* AB - 0.92* AC -1.71* BC -1.06* CD +1.72*BD-1.38*CD-0.20*A^2-5.04*B^2+0.46*C^2

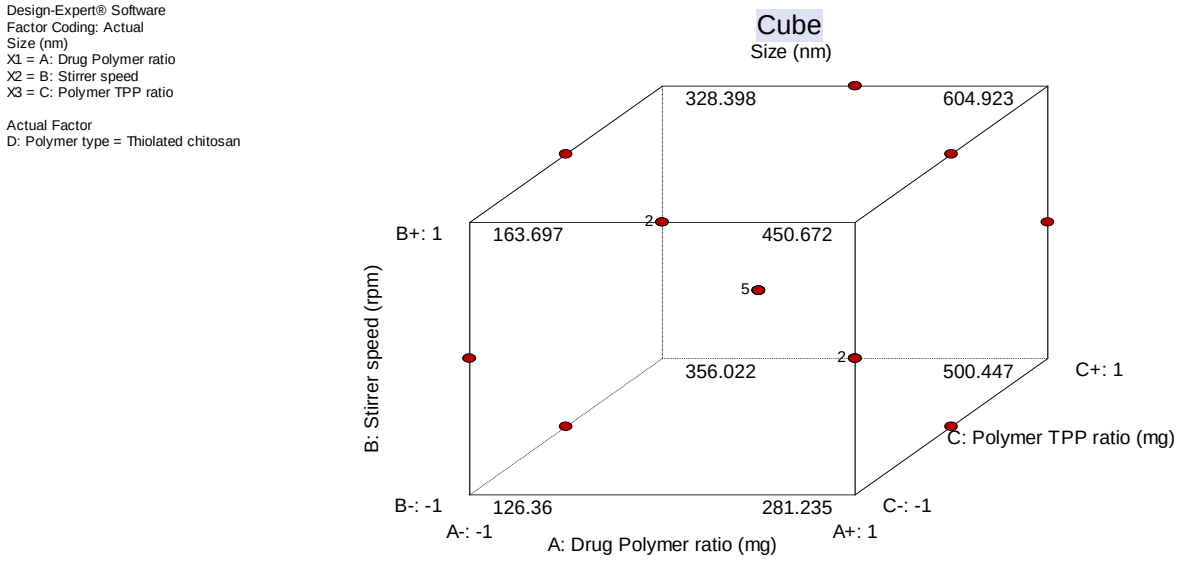
Mucoadhesive potential = +0.52+0.085*A-0.069* B+0.11*C +0.58* D

Where, A= Drug polymer ratio
B = Stirrer speed
C = Polymer TPP ratio
D = Polymer type

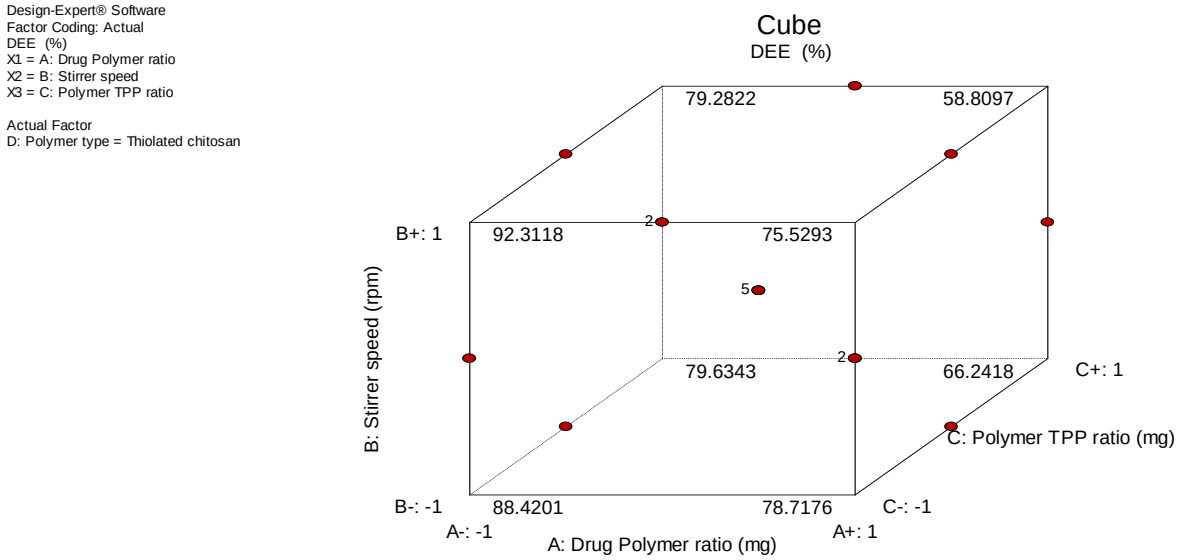
Table II: Responses obtained from the Box- Behnkan Design for formulation optimization

Batch No.	Partice Size (nm)	DEE (%)	Mucoahesive potential (N)
1	156.7	89.9	1.3
2	408.2	68.0	4.1
3	309.0	83.1	1.0
4	114.7	90.6	0.8
5	202.1	87.9	3.0
6	248.3	84.2	3.2
7	216.9	85.1	0.9
8	226.4	86.1	0.6
9	215.8	85.1	0.8
10	149.3	90.0	2.8
11	361.5	79.3	0.5
12	232.5	84.9	3.9
13	244.7	85.9	1.6
14	350.0	81.0	0.9
15	306.1	82.3	0.4
16	175.0	87.9	1.2
17	575.0	65.9	2.8
18	308.0	83.9	1.3
19	297.5	84.8	1.4
20	292.7	84.9	5.2
21	505.2	67.0	1.6
22	249.3	85.0	2.4
23	465.0	69.7	3.9
24	265.0	84.0	0.8
25	314.0	83.6	4.0
26	464.2	66.9	3.9
27	498.0	69.0	3.2
28	262.0	84.6	1.0
29	306.2	81.1	2.9
30	261.6	84.1	1.4
31	299.1	83.8	2.9
32	217.0	85.0	1.2
33	427.0	70.0	0.9
34	365.2	81.1	1.2

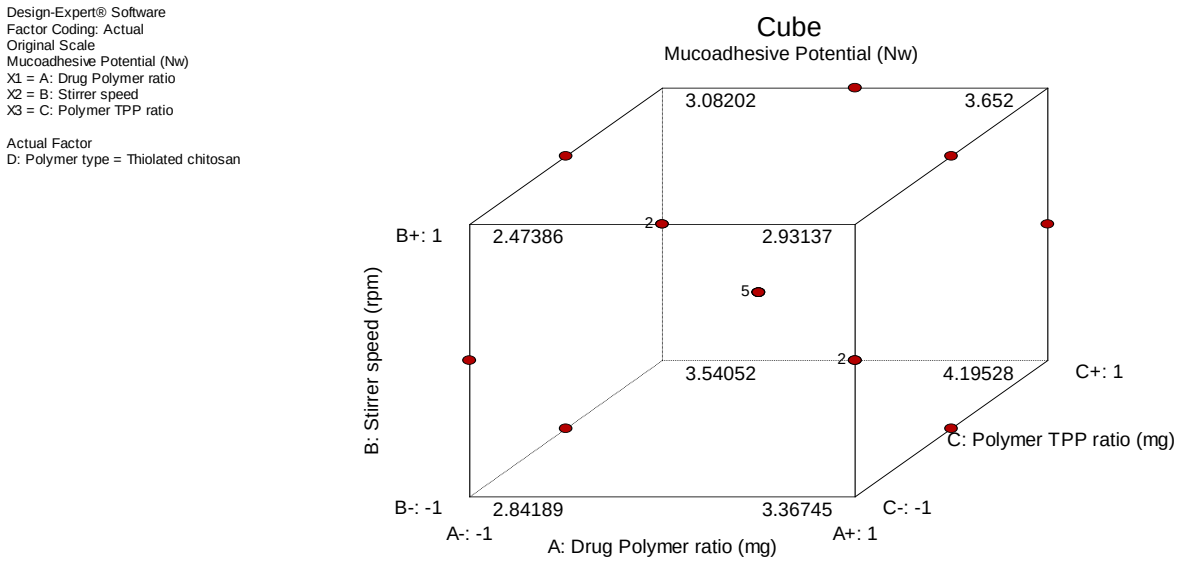
(a)


Design-Expert® Software
Factor Coding: Actual
Size (nm)
X1 = A: Drug Polymer ratio
X2 = B: Stirrer speed
X3 = C: Polymer TPP ratio
Actual Factor
D: Polymer type = Thiolated chitosan
Cube
Size (nm)
328.398
604.923
B+: 1
163.697
2
450.672
5
356.022
2
500.447
C+: 1
B: Stirrer speed (rpm)
C: Polymer TPP ratio (mg)
B-: -1
126.36
281.235
C-: -1
A-: -1
A+: 1
A: Drug Polymer ratio (mg)

(b)


Design-Expert® Software
Factor Coding: Actual
DEE (%)
X1 = A: Drug Polymer ratio
X2 = B: Stirrer speed
X3 = C: Polymer TPP ratio
Actual Factor
D: Polymer type = Thiolated chitosan
Cube
DEE (%)
79.2822
58.8097
B+: 1
92.3118
2
75.5293
5
79.6343
2
66.2418
C+: 1
B: Stirrer speed (rpm)
C: Polymer TPP ratio (mg)
B-: -1
88.4201
78.7176
C-: -1
A-: -1
A+: 1
A: Drug Polymer ratio (mg)

(c)



(d)

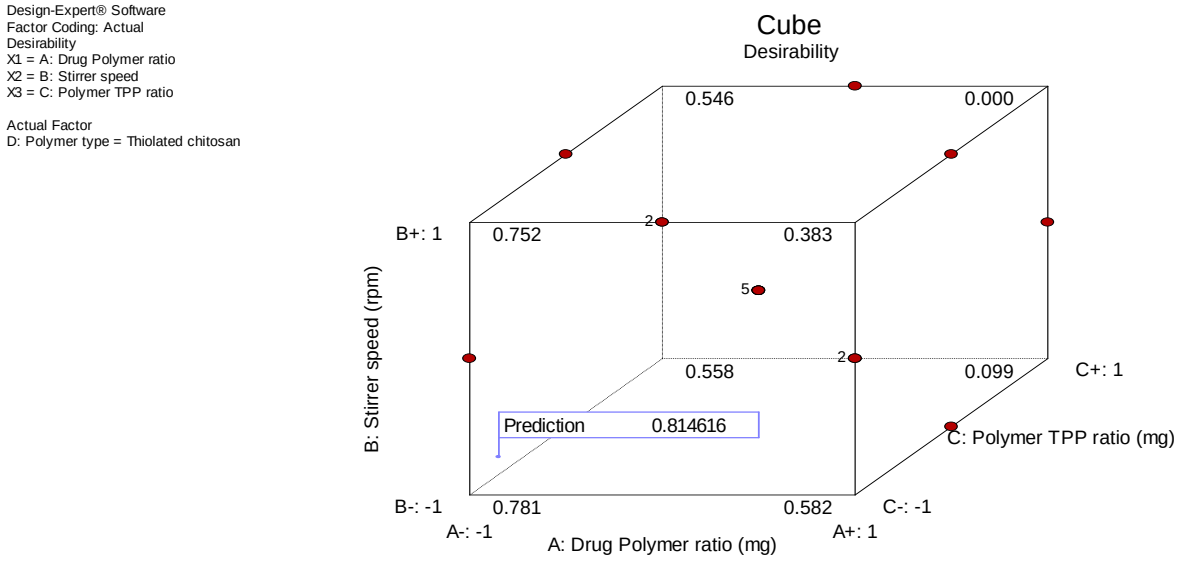


Figure: I Cube plots showing the combined effects numeric variables on particle size (a), %DEE (b), Mucoadhesive potential (c) and desirability (d)

The cube plots, highlighting the effects selected numeric variables on the three responses measured have been shown in Figure (I). A design space with desirability of 0.8146 was generated from the numerical optimization. The optimum formulation parameters, as suggested by the software were 0.01% thiolated chitosan, 1000 rpm stirrer speed and 0.06% sodium triploy phosphate as the crosslinking agent. The optimized formulation was fabricated and evaluated which yielded the responses X1, X2 and X3 as 177.37nm, 90.54% and 2.8N respectively. The numerical

optimization was validated by comparing the predicted and actual values of responses which revealed a statistically insignificant percent prediction error of 4.79, 3.45 and 3.49 for X1, X2 and X3 respectively.

It was observed that the particle size was increases with increase in the polymer concentration. At stirrer speed around 1000 rpm (medium), minimum particle size was obtained and at higher speed i.e. 1500 rpm and at lower speed i.e. 500 rpm the particle size increases. This behavior can be attributed to the fact that low stirring speed were not provide sufficient attrition on nanoparticles whereas high stirring speed may have resulted in to higher charge on particles which ultimately resulted in high agglomeration. Particle size was found to be increased at higher polymer TPP ratio as increase in TPP concentration causes higher cross linking with polymer.

It was observed that DEE increased with decrease in drug polymer ratio as higher amount of polymer can entrap drug in nanoformulation which may leach out in to the solution. Optimum stirring speed was prerequisite for high DEE as at lower speed (500 rpm) and higher (1500 rpm), the DEE decrease from more than 85% to less than 70%. Increase in TPP concentration, increases the cross linking due to which more drug get entrapped in the particles.

Mucoadhesive potential mainly depends upon the type of polymer, with thiolated chitosan mucoadhesive potential was found be maximum (5.2 N) and on the other hand with chitosan maximum mucoadhesive potential was found to be (1.3). Increase in the polymer concentration and cross linking agent (TPP), slightly increases the mucoadhesive potential and increase in stirrer speed cause little decrease in the mucoadhesive potential.

The solution obtained from numerical optimization was prepared and evaluated. A zeta potential was found to be +27.6 mV, revealed adequate electrostatic and steric stability of the optimized nanoparticles and positive value of zeta potential is due to the polycationic nature of the polymer. Poly dispersity index (PDI) was found to 0.32, suggesting higher monodispersity of the nanoparticles in medium.

CONCLUSION

The present study elaborated the optimization of galantamine hydrobromide encapsulated chitosan and thiolated chitosan nanoparticles by Box-Behnken Design to yield smaller particle size with maximum drug encapsulation and mucoadhesive potential for efficient nose to brain drug delivery. Particle size mainly depends on the polymer concentration and amount of crosslinking agent. Optimization process suggested that the thiolated chitosan is better mucoadhesive polymer as compared to chitosan and can be utilized for the fabrication of mucoadhesive nanoparticles for galantamine hydrobromide in the treatment of Alzheimer's.

Acknowledgment
The author is highly thankful to the Chairman, Department of Pharmaceutical Sciences, for providing the necessary facilities.

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