ORIGINAL ARTICLE

Preparation and evaluation of spray-dried mucoadhesive microspheres for intranasal delivery of prochlorperazine using factorial design

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The purpose of present research work was to develop spray-dried mucoadhesive microspheres of prochlorperazine (PCPZ) for intranasal administration with an aim to avoid first-pass metabolism and to improve therapeutic effectiveness. A 2^3 factorial design was employed with amount of polymer, feed flow rate and volume of gluteraldehyde as independent variables while particle size of the microspheres and percentage drug entrapment efficiency as dependent variables. The microspheres were evaluated for drug loading, surface morphology, degree of swelling, *in-vitro* mucoadhesion, drug release, histopathology and stability studies. Particle size of all batches was found to be in the range of 7.32–15.67 μm. The percentage entrapment efficiency was found to be in the range between 84.90 and 96.21. *In-vitro* mucoadhesion was performed by adhesion number using goat nasal mucosa and was observed in a range from 76.25 to 87.72. The optimum formulation was selected based on the criteria of attaining the minimum value of particle size with substantial entrapment efficiency. Scanning electron microscopy analysis of the microspheres revealed that the microspheres were nearly smooth and spherical. *In-vitro* diffusion studies showed non-Fickian drug release. The Fourier transform infrared spectrophotometer spectra revealed no interaction between drug and excipients. Optimum formulation was found to be nonirritant in histopathology study carried out on goat nasal mucosa. The prepared microspheres were found to be stable over a period of 3 months even after stored at 40°C. In conclusion, PCPZ loaded mucoadhesive chitosan microspheres were reported for the first time, being suitable for intranasal delivery for the treatment of nausea and vomiting.

Key words: *Chitosan, factorial design, nasal drug delivery, prochlorperazine, spray drying*

INTRODUCTION

In the recent years, the nasal route has gained tremendous attention as an expedient and reliable method for the systemic drug delivery by many pharmaceutical scientists and clinicians due to its enormous potential utility for drug delivery. It offers an attractive substitute for drugs that have limited oral bioavailability, are demolished by gastrointestinal fluids or are extremely liable to hepatic first pass metabolism. Intranasal drug delivery is also an ideal substitute for the parenteral route for systemic drug delivery due to noninvasive, essentially painless, does not require sterile preparation, and is easily and readily administered by the patient or a physician.[1,2]

The nasal cavity as a site for systemic absorption of drugs has benefits for instance relatively large surface area (due to numerous microvilli), porous endothelial membrane, highly vascularised epithelial layer, improved blood flow, evading of first-pass metabolism due to lack of gastric and pancreatic enzymatic activity, neutral pH of the nasal mucus and ready accessibility.[3] However, the nasal route has limitations like mucociliary clearance, low permeability etc. In

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Access this article online

Quick Response Code:

Website: www.asiapharmaceutics.info

DOI: 10.4103/0973-8398.160314

order to conquer these limitations, two main approaches have been employed: Amendment of the permeability of the nasal membrane using absorption enhancers (such as surfactants, cyclodextrins, bile salts and phospholipids), which can support the absorption of poorly absorbable drugs and the utilisation of mucoadhesive systems such as bioadhesive liquid formulations, microspheres, powders and liquid gelling formulations that diminish the mucociliary clearance of the drug formulation and thus enhance the contact time between the drug and the site of absorption.[4,5]

Amongst the various approaches obtainable to increase the intranasal delivery of drugs, the mucoadhesive microparticle drug delivery system is a smart concept in that the drug can capture within particles to be released at nasal mucosal surface, where the particles are adhered owing to their mucoadhesiveness. Thus, this system has the capability to control the rate of drug clearance from the nasal cavity as well as to secure the drug from enzymatic degradation.[3,6]

Different techniques have been attempted by various researchers to formulate intranasal mucoadhesive microspheres, e.g., solvent evaporation, ionotropic gelation, emulsification-crosslinking, thermal crosslinking, precipitation coacervation and spray drying. Amongst all these various techniques, spray drying is most vital technique and used successfully to produce mucoadhesive microspheres. This technique is widely used owing to consistency, reproducibility and probable control on particle size and moisture content. This technique is used for thermolabile drug substances and also possible to maintain aseptic condition. Microspheres formed by this method have a very high drug loading. The properties of the spray-dried microspheres can be controlled by both the process and formulation parameters.[7]

Prochlorperazine (PCPZ), a piperazine phenothiazine, is commonly used to treat nausea and vomiting caused by radiation therapy, cancer chemotherapy, surgery and other conditions. It has also been used to relieve pain and nausea associated with acute migraine headaches. It is also used in the treatment of psychosis and manic phase of bipolar disorder.[8] It is well-absorbed from the gastrointestinal tract but is subject to considerable first-pass metabolism in the liver; its oral absolute bioavailability is approximately 12.5%.[9-11] Presently, PCPZ conventional dosage form available in the Indian market are given by oral, intravenous (i.v.) and rectal route (buccal tablets, and i.v. solutions and suppositories). PCPZ base is generally administered by the rectal route and PCPZ maleate by the oral or buccal routes while PCPZ edisylate and mesylate can be given orally or parenterally. The oral route of administration of PCPZ is impractical for patients who are vomiting (drug could be discharged by vomiting) or who have impaired gastric emptying. i.v. administration provides rapid effects to a patient, but the onset of effects is too quick to cause detrimental effects. In addition, it gives a local pain and may cause an unpredicted accident when it is not perfectly prepared.[12] Suppository formulations have also been used, but this approach has low patient acceptability.

Available conventional dosage forms of PCPZ meleate by oral, parenteral and rectal route suffering from several drawbacks. Hence, an attempt has been made to develop alternative drug delivery system that uses nasal mucoadhesive microsphere to improve rate and extent of absorption, to bypass hepatic first-pass metabolism and thereby, to improve drug bioavailability.

MATERIALS AND METHODS

Materials

Prochlorperazine maleate (PCPM) was a generous gift sample from Vaikunth Chemicals Ltd., (Ankleshwar, India). Chitosan with 85–90% degree of deacetylation was purchased from Balaji Drugs (Surat, India). Glutaraldehyde (GLA), glacial acetic acid and span 80® were obtained from S. D. Fine Chemicals (Mumbai, India). The rest of chemicals and reagents used in the study were of analytical grade.

Preparation of microspheres

Microspheres were prepared by spray drying method. Chitosan was dissolved in glacial acetic acid solution (1% v/v), and PCPM (250 mg) was dissolved in this solution. Different concentration of GLA as cross-linking agent was added to the former solution and stirred for 2 h on mechanical stirrer at 500 rpm. The total volume of solution used for each formulation was 300 ml. Microspheres were obtained by spraying the solution with spray-dryer (LU-222, Labultima, India) using a standard 0.7 mm nozzle. The process conditions were as follows: Inlet temperature 130–135°C, outlet temperature 80–90°C and aspirator speed 35–40%. After drying procedure, microspheres were harvested from the apparatus collector and weighed.

Experimental design

A 2^3 factorial design for three factors at two levels each was selected to optimise the varied response variables. Experimental trials were performed at all eight possible combinations. In this investigation, the three factors, amount of polymer (X_1), feed flow rate (X_2) and volume of GLA (X_3) were selected as independent variables. Two responses, particle size (Y_1) and % entrapment efficiency (Y_2), were measured for each trial and taken as dependent variables. The factorial design parameters with corresponding formulations are outlined in Table 1. All other formulation variables and processing variables were kept invariant throughout the study.

Optimisation data analysis and model-validation

ANOVA was used to establish the statistical validation of the polynomial equations generated by Design Expert® software (version 9.0.3, Stat-Ease Inc., Minneapolis, MN, USA). Fitting a multiple linear regression model to 2^3 factorial design gives a

Table 1: Experimental design and formulation composition by 23 factorial design

Formulation code	X_1	X_2	X_3
F1	1000 (+1)	1 (−1)	0.3 (−1)
F2	500 (−1)	3 (+1)	0.3 (−1)
F3	500 (−1)	1 (−1)	0.3 (−1)
F4	1000 (+1)	3 (+1)	0.9 (+1)
F5	1000 (+1)	3 (+1)	0.3 (−1)
F6	500 (−1)	1 (−1)	0.9 (+1)
F7	1000 (+1)	1 (−1)	0.9 (+1)
F8	500 (−1)	3 (+1)	0.9 (+1)

X_1: Amount of polymer (mg), X_2: Feed flow rate (ml/min), X_3: Volume of GLA (ml), +1 (high) and−1 (low): Levels used, actual (coded). GLA: Glutaraldehyde

predictor equation incorporating interactive and polynomial term to evaluate the responses (Equation 1):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 \quad (1)$$

Where, Y is the measured response associated with each factor level combination; b_0 is an intercept representing the arithmetic average of all quantitative outcomes of eight runs; b_1 to b_{123} are regression coefficients computed from the observed experimental values of Y and X_1, X_2 and X_3 are the coded levels of independent variables. The terms X_1X_2, X_2X_3 and X_1X_3 represent the interaction terms. The main effects (X_1, X_2 and X_3) represent the average result of changing one factor at a time from its low to high value. The interaction terms show how the response changes when two factors are changed simultaneously. The polynomial equation was used to draw conclusions after considering the magnitude of coefficients and the mathematical sign it carries that is, positive or negative. A positive sign signifies a synergistic effect, whereas a negative sign stands for an antagonistic effect.

In the model analysis, the responses (the particle size and % entrapment efficiency of the microspheres) of all model formulations were treated by Design Expert® software. The best fitting mathematical model was selected based on the comparisons of several statistical parameters including the coefficient of variation, the multiple correlation coefficient (R^2), adjusted multiple correlation coefficient (adjusted R^2) and the predicted residual sum of square (PRESS), provided by Design Expert® software. Among them, PRESS indicates how well the model fits the data and for the chosen model it should be small relative to the other models under consideration. Level of significance was considered at $P < 0.05$. Three-dimensional response surface plots and two-dimensional (2D) contour plots resulting from equations were obtained by the Design Expert® software. Subsequently, the desirability approach was used to generate the optimum settings for the formulations.[13,14]

Linear model: $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3$ (2)

2FI (interaction) model: $Y = b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23} \times {}_2X_3$ (3)

Characterisation of the microspheres

Production yield

The production yields of microspheres of various formulation batches were calculated using the weight of the final product after drying (practical mass) with respect to the initial total weight of the drug and polymer used for preparation of microspheres (theoretical mass) and percent production yields according to the Equation (4) mentioned below.[15]

$$\text{Production yield} = \frac{\text{Practical mass (microspheres)}}{\text{Theoretical mass (polymer + drug)}} \times 100 \quad (4)$$

Particle size analysis

The particle sizes of the microspheres of all trials were analysed using stereomicroscope which was calibrated using calibrated micrometres. The microscope was equipped with the software (Motic images plus 2) through a camera. A small amount of dry microspheres were suspended in water (10 ml). The suspension was ultrasonicated for 10 s. A small drop of suspension was placed on a clean glass slide and covered with the cover slip to form a specimen. The slide with specimen was observed under the microscope. An image was taken with the help of camera, and the particle size was determined using software. The magnification of the microscope used for observations was ×100. Size of around 100 particles was measured randomly for each batch on the different portions of the slide. The average particle size of the microspheres was expressed as the volume surface diameter (μm) and standard deviation (SD) σ was calculated for each batch of microspheres.[16]

Drug loading and entrapment efficiency

To determine the PCPM content in each microsphere formulation, accurately weighed samples of microspheres (5 mg) were dissolved in 10 ml phosphate buffer pH 6.4 solution with constant stirring overnight and sonicated before analysing the amount of PCPM. After filtering through a whatman filter paper, the filtrates were diluted suitably, and the PCPM content was measured spectrophotometrically at a wavelength of 255 nm on UV-spectrophotometer (Shimadzu UV1610, Japan). The percent drug loading and entrapment efficiency were calculated according to the Equations (5) and (6) respectively mentioned below.[17] These were determined by three separately prepared microspheres and were expressed as the mean ± SD.

$$\text{Drug loading}(\%) = \frac{M_{\text{actual}}}{\text{Weighed quantity of microspheres}} \times 100 \quad (5)$$

$$\text{Entrapment efficiency}\,(\%) = \frac{M_{actual}}{M_{theoretical}} \times 100 \quad (6)$$

Where M_{actual} is the actual PCPM content in the weighed quantity of powder of microspheres and $M_{theoretical}$ is the theoretical amount of drug in microspheres calculated from the quantity added in the spray-drying process.

Degree of swelling

The swelling ability of microspheres in physiological media (nasal simulated fluid) was determined by allowing the microspheres to swell in the phosphate buffer saline pH 6.4. To ensure the complete equilibrium, exactly weighed 100 mg of microspheres were allowed to swell in phosphate buffer saline of pH 6.4 for 24 h and washed thoroughly with deionised water. The degree of swelling was obtained using the following Equation (7).

$$\alpha = W_s - W_o/W_o \quad (7)$$

Where, α is the degree of swelling; W_o is the weight of microspheres before swelling and W_s is the weight of microspheres after swelling.[18,19]

In-vitro mucoadhesive strength determination

A freshly cut 2 cm^2 piece of goat nasal mucosa was obtained from the local abattoir and cleaned by washing with isotonic saline solution. Accurately weighed 100 mg of microspheres were placed on goat nasal mucosa which was fixed over polyethylene support. About 100 µl of simulated nasal electrolytes was placed on microspheres, and this plate was incubated for 20 min in desiccators at 90% relative humidity to allow the polymer to interact with the membrane. The support was then fixed at an angle of 45° relative to the horizontal plane. The nasal mucosa was thoroughly washed with phosphate buffer (pH 6.4) at the rate of 5 ml/min using a peristaltic pump. One hour after administration of microspheres, the concentration of the drug in the collected perfusate was determined by spectrophotometrically. The amount of microspheres corresponding to the amount of drug in the perfusate was determined. The amount of adhered microspheres was estimated as the difference between the amount of applied microspheres and the amount of flowed microspheres. The percent mucoadhesion was calculated using the following Equation (8).[20,21]

$$\text{In-vitro mucoadhesion}\,(\%) = \frac{\text{Amount of drug in washout liquid}}{\text{Actual amount of drug in applied microspheres}} \times 100 \quad (8)$$

In-vitro drug diffusion study

The *in-vitro* drug diffusion test of microspheres was performed using a glass-fabricated Franz diffusion cell apparatus, which consisted of donor and receptor compartments. A dialysis membrane (cut-off molecular weight: 12,000, Hi Media, India) was used to keep the microspheres (5 mg) on the donor side, which allowed free diffusion of PCPM to the receptor compartment containing 25 ml phosphate buffer solution pH 6.4 that was within the pH range in nasal cavity. The temperature was maintained at 37 ± 1°C using circulating water bath. The receptor compartment was stirred with a magnetic stirrer. At scheduled time intervals, aliquots (1 ml) were withdrawn from receptor compartments and replaced with the same volume of fresh prewarmed buffer solution. The samples were assayed spectrophotometrically at 255 nm. All experiments were carried out in triplicate, and average values were calculated.

In-vitro drug diffusion kinetics

To understand the drug release mechanisms, the results obtained were fitted in four kinetic models: zero order and first order kinetics, Higuchi and Korsemeyer-Peppas model. Criteria for selecting the most appropriate model were based on obtained R^2 values.[22,23]

Ex vivo permeation study

An *ex vivo* drug permeation study of the optimised batch of microspheres was performed using Franz diffusion cell across goat nasal mucosa as permeation barrier, obtained from the local abattoir within 1 h of sacrificing the animal. The nasal mucosa was carefully cut with a scalpel and mounted in the diffusion chamber with mucosal and serosal surfaces facing the donor and receiver compartments, respectively. Microspheres equivalent to 5 mg of PCPM were placed in the donor chamber containing 3 mL of simulated nasal fluid (aqueous solution containing 8.77 mg/mL NaCl, 2.98 mg/mL KCl and 0.59 mg/mL $CaCl_2$/L). Other experimental procedures and sample collections were performed in the same fashion as in the case of *in-vitro* drug diffusion studies. To assure optimal conditions for the viability of the tissues, this study was carried out in cell culture incubator.[15,24]

Scanning electron microscopy

Shape and surface morphology of the optimised formulations was studied by scanning electron microscopy (SEM 5610 LVs, JSM) operated at an accelerating voltage of 15 kV.

Fourier transform infrared spectrophotometer study

The Fourier transform infrared spectrophotometer (FTIR) study was carried out in order to find out the drug excipient compatibility. Samples (a) pure PCPM, (b) blank chitosan microspheres and (c) PCPM loaded microspheres were subjected to FTIR studies. The procedure consisted of dispersing 2–3 mg of samples with KBr and compressing into disc by applying a pressure for 5 min in a hydraulic press. The pellet was placed in the light path and the scanning range used was 4000–400/cm to obtain spectra. Disappearance of PCPM peaks or shifting of peak in any of the spectra was studied.

Histopathological examination of nasal mucosa

Goat nasal mucosa obtained from a local abattoir within 2 h of killing the animal was cleaned by washing with isotonic saline solution. After 8 h of applying the loaded microspheres, the nasal mucosa was fixed in 10% neutral carbonate buffered formalin solution routinely processed and embedded in paraffin. To assure optimal conditions for the viability of the tissues, the experiment was carried out in a cell culture incubator. Paraffin sections (7 mm) were cut on glass slides and stained with hematoxylin and eosin. Sections were examined under a digital optical microscope (Motic Instruments Inc., Canada), to detect any damage to the tissue during *in-vitro* permeation, by a pathologist blinded to the study.[25]

Stability studies

The optimised batch was subjected to short-term stability studies of 3 months as per ICH guidelines. The vials filled with microspheres were sealed with air tight rubber closures and kept under ambient temperature and moisture conditions (40°C and 75% RH) for a period of 3 months in a programmable environmental test chambers (Remi Instruments Ltd., Mumbai, India). Samples were analysed for the particle size and % entrapment efficiency at 1, 2 and 3 months interval.[14,26]

RESULTS AND DISCUSSION

Here, chitosan containing microspheres loaded with PCPM were prepared by spray drying method. In this method, drying of the feed and embedding of the drug takes place as single step, and thus it proved to be simple, easy and speedy. The obtained spray-dried microspheres appeared as yellow to brownish colour powder. The microspheres were found to be discrete, spherical and free flowing powder.

Formulation of microspheres

A 2^3 factorial design was utilised in the present study using Design Expert Software. In this design three factors were evaluated, each at two levels, and experimental trials were carried out at all eight possible combinations. The independent variables selected were the amount of polymer (X_1), feed flow rate (X_2) and volume of GLA (X_3). The dependent variables are particle size (Y_1) and % entrapment efficiency (Y_2) with constraints applied to the formulation of microspheres.

Optimisation data analysis and model-validation

Fitting of data to the model

The three factors with lower and upper design points in coded and uncoded values are shown in Table 1. The ranges of responses Y_1 and Y_2 were 7.32–15.67 μm and 84.90–96.21%, respectively. All the responses observed for eight formulations prepared were fitted to various models using Design Expert® software. 2FI model was selected for both the responses on the basis of the *P* values and low PRESS value indicating adequate fitting of the model. The values of R^2, adjusted R^2, predicted R^2 and PRESS value are given in Table 2. ANOVA was applied for estimating the significance of the model, at 5% significance level. The results of ANOVA presented in Table 3, for the dependent variables demonstrate that the model was significant for both the response variables.

Effect of formulation variables on particle size (Y_1): 2FI model was significant with model f-value of 20,444.56 ($P < 0.05$). The 2FI Equation (9) generated by software was as follows:

$$Y_1 = 11.34 + 2.14 X_1 + 1.08 X_2 - 0.97 X_3 - 0.23 X_1X_2 + 0.028 X_1X_3 - 0.41 X_2X_3 \quad (9)$$

The significance levels of the coefficient b_{13} were found to be $P = 0.1695$, so it was omitted from the full model to generate a reduced model. The coefficients b_1, b_2, b_3, b_{12} and b_{23} were found to be significant at $P < 0.05$; hence they were retained in the reduced model. The reduced model was tested in proportion to determine whether the coefficient b_{13} contributed significant information to the prediction of Y_1. The critical value of F for $\alpha = 0.05$ was equal to 161.45 (df = 1, 1). Since the calculated value ($F = 22.22$) was less than the critical value ($F = 161.45$), it may be concluded that the term b_{13} did not contribute significantly to the prediction of Y_1 and can be omitted to generate the reduced model (Equation 10).

$$Y_1 = 11.34 + 2.14 X_1 + 1.08 X_2 - 0.97 X_3 - 0.23 X_1X_2 - 0.41 X_2X_3 \quad (10)$$

Equation (10) reveals that X_1 and X_2 have agonistic effect and X_3 has antagonistic effect on the particle size.

Effect of formulation variables on % entrapment efficiency (Y_2): 2FI model was significant with model *F* value of 266.36 ($P < 0.05$). The Equation (11) for the full model generated by software was as follows:

$$Y_2 = 91.05 + 2.21 X_1 - 1.97 X_2 - 1.57 X_3 - 0.23 X_1X_2 - 0.23 X_1X_3 - 0.96 X_2X_3 \quad (11)$$

The significance level of the coefficient, b_{12} and b_{13} were found to be $P = 0.2292$, so this were omitted from the full model to generate a reduced model. The coefficients b_1, b_2, b_3, b_{12} and b_{23} were found to be significant at $P < 0.05$; hence they were retained in the reduced model. The reduced model was tested in proportion to determine whether the coefficient b_{12} and b_{13} contributed significant information to the prediction of Y_2. The critical value of F for $\alpha = 0.05$ was equal to 199.50 (df = 2, 1). Since the calculated value ($F = 7.13$) was less than the critical value ($F = 199.50$), it may be concluded that the term b_{12} and b_{13} did not contribute significantly to the prediction of Y_2 and can be omitted to generate the reduced model (Equation 12).

$$Y_2 = 91.05 + 2.21 X_1 - 1.97 X_2 - 1.57 X_3 - 0.96 X_2X_3 \quad (12)$$

Equation (12) reveals that X_1 has agonistic; whereas X_2 and X_3 have antagonistic effect on the % EE.

Contour plot and response surface analysis

Three-dimensional response surface plots generated by the Design Expert software are presented in Figure 1, while 2D contour plots are presented in Figure 2 for the studied responses that is, particle size and % entrapment efficiency. These figures depicts response surface, contour plots of the effects of amount of polymer (X_1) and feed flow rate (X_2) on particle size, which indicate a linear effect on particle size of the microspheres. The combined effect of feed flow rate (X_2) and volume of GLA (X_3) indicates a nonlinear effect on particle size of the microspheres. It was observed that effect of the amount of polymer (X_1) and volume of GLA (X_3) shows linear effect on particle size of the microspheres.

Response surface and contour plots of the effects of amount of polymer (X_1) and feed flow rate (X_2) on % entrapment efficiency shows a nonlinear effect. The feed flow rate (X_2) and volume of GLA (X_3) indicates antagonistic effect on % entrapment efficiency of the microspheres. It was observed that effect of the amount of polymer (X_1) and volume of GLA (X3) also shows nonlinear effect on % entrapment efficiency of the microspheres.

Selection of optimised formula

After generating the reduced model polynomial equations to relate the dependent and independent variables, the process was optimised for all three responses. The optimum formulation was selected based on the criteria of attaining the minimum value of particle size with substantial entrapment efficiency. The final optimal experimental parameters were calculated using the extensive grid search and feasibility search provided in the Design Expert software.

Validation of the 2^3 factorial design results

The result in Table 4 shows obtained and predicted values of both the responses Y_1 and Y_2 for all the formulations along with the % prediction error. It can be seen that in all cases there was a rational concurrence among the predicted and the experimental values, as prediction error was found to vary between −0.10% and +0.31%.

Table 2: Summary of results of regression analysis for responses Y_1 and Y_2

Models	R^2	Adjusted R^2	Predicted R^2	PRESS	SD	% CV
2FI model						
Response (Y_1)	1.0000	0.9999	0.9995	0.029	0.021	0.19
Response (Y_2)	0.9994	0.9956	0.9600	3.92	0.25	0.27

PRESS: Predicted residual sum of square, SD: Standard deviation, CV: Coefficient of variation

Table 3: Results of analysis of variance for measured response

Response	Model	df	SS	MS	*F*	Significance *F*
Y_1						
Regression	FM	6	55.20	9.20	20444.56	0.0054
	RM	5	55.19	11.04	3396.57	0.0003
Error	FM	1	0.00045	0.00045	-	
	RM	2	0.0065	0.00325	-	
Y_2						
Regression	FM	6	97.89	16.31	266.36	0.0469
	RM	4	97.02	24.26	78.57	0.0023
Error	FM	1	0.061	0.061	-	
	RM	3	0.93	0.31	-	

df: Degrees of freedom, SS: Sum of square; MS: Mean sum of square, *F*: Fischer's ratio

Table 4: The predicted and observed response variables of the chitosan microspheres

Formulation	Particle size (Y_1)			Percentage drug entrapment (Y_2)		
	Observed	Predicted	*Percentage error	Observed	Predicted	*Percentage error
F1	13.17	13.163	0.05	96.21	96.297	−0.09
F2	11.93	11.922	0.07	89.32	89.407	−0.10
F3	8.46	8.467	−0.08	91.04	90.953	0.10
F4	12.98	12.973	0.05	88.21	88.297	−0.10
F5	15.67	15.621	0.31	93.91	93.82	0.10
F6	7.32	7.313	0.10	90.12	90.207	−0.10
F7	12.11	12.118	−0.07	94.71	94.623	0.09
F8	9.1	9.107	−0.08	84.9	84.82	0.09

*Predicted error (%)=(Observed value−Predicted value)/Predicted value×100%

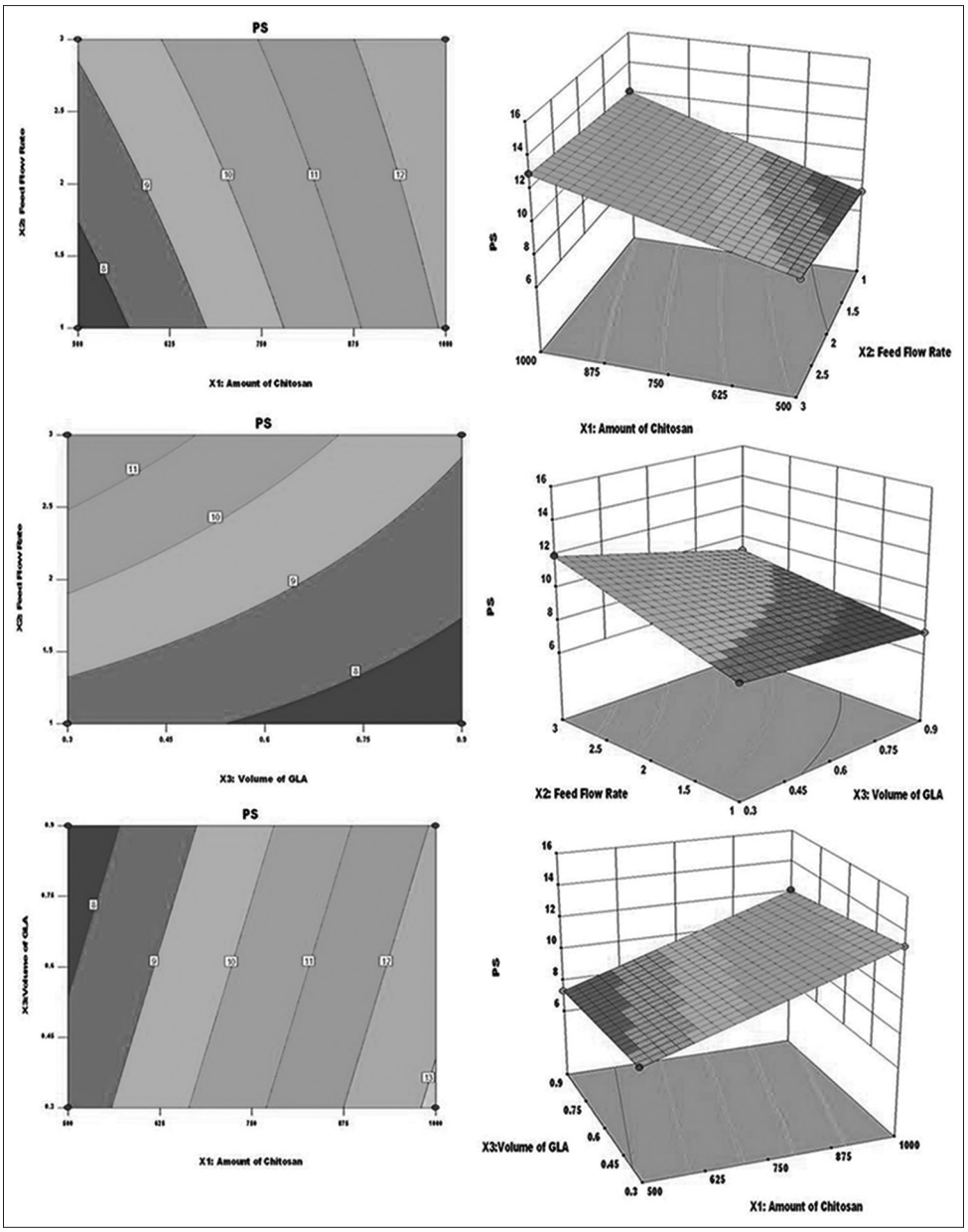

Figure 1: Response surface and contour plots showing influence of X_1 and X_2, X_2 and X_3 and X_1 and X_3 on particle size

Thus, it can be concluded that the equations describe adequately the influence of the chosen independent variables on the responses under study. This shows that the optimisation technique was suitable for optimizing the PCPM loaded microsphere. Therefore, the low magnitudes of error in the current study prove the high predictive aptitude of the optimisation method by factorial design.

Characterisation of microspheres

Production yield

The production yields of microspheres prepared by spray drying method were found to be in the range between 35.83 and 41.44% as shown in Table 5. It was found that production yield of microspheres prepared using higher amount of chitosan is greater. Low production yield was observed due to sticking of particles to side wall of drying chamber. Further, light weight particles and finer particles are exhausted by aspirator because spray dryer apparatus is not equipped with a trap to recuperate it. Hence, collectively extremely fewer amounts of microspheres are obtained using spray drying method.

Drug loading

Drug loading of prepared all batches ranged in between 46.66 ± 2.40% and 82.20 ± 2.62%. It was found to be conversely related with the amount of chitosan and declines with an increase in volume of GLA. The feed flow rate alone did not show any significant effect on drug loading.

Swelling index

The swelling index determination was carried out of all formulations to study clearance of the drug from the nasal

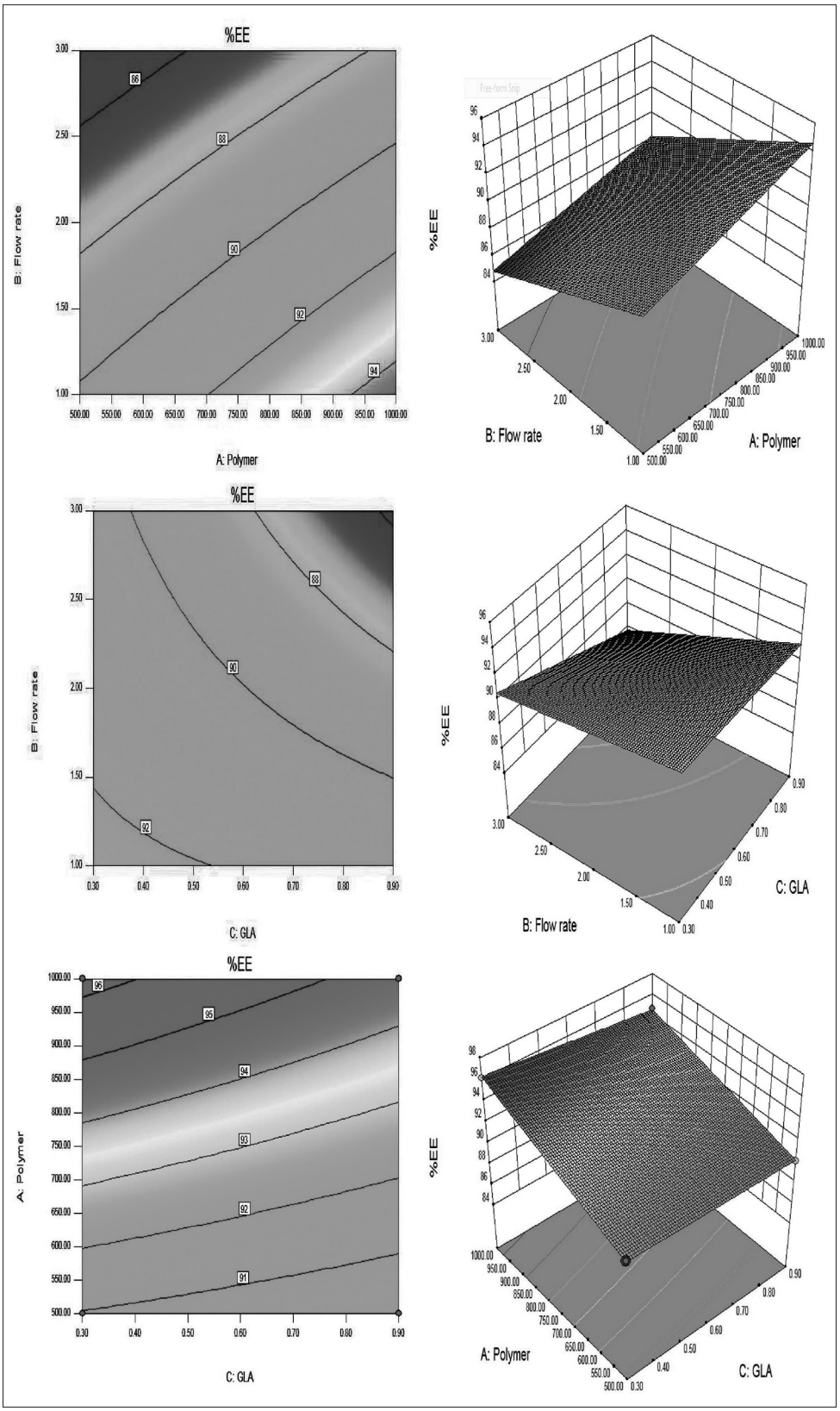

Figure 2: Response surface and contour plots showing influence of X_1 and X_2, X_2 and X_3 and X_1 and X_3 on % entrapment efficiency

cavity. The degree of swelling of all the formulations is shown in Table 5. It was shown that with an increase in the amount of chitosan, the degree of swelling also increases ranging from 0.79 ± 0.01 to 0.87 ± 0.02. It is suggested that when the microspheres are in contact with mucus layer, they swell rapidly and take up liquid from the mucus layer.

Hence, the epithelial cells loose water and shrink which opens the epithelial tight junction allowing drug to be absorbed. Increase in swelling index results in reduction in mucociliary clearance due to improved adhesion of microspheres with nasal mucosa. Longer residence time of microspheres assures enhanced drug release.

Mucoadhesive strength

Mucoadhesion studies were performed to ensure the adhesion of formulation to the mucosa for a prolonged period at the site of absorption. The results of the *in-vitro* mucoadhesion studies are shown in Table 5. The results indicated that amount of chitosan and volume of GLA was directly proportional to mucoadhesion strength. This could be attributed to the availability of a high amount of polymer for interaction with mucus. These results were same as that obtained by Genta *et al*., 1998.[27]

***In-vitro* and *ex vivo* drug diffusion study**

The drug release profile of the optimised batch (F6) of microspheres is shown in Figure 3. It was observed that cross-linked microspheres prepared with chitosan moderately sustained the drug release up to 8 h without any lag time. The drug release from microspheres was at slower rate due to the presence of cross-linking agent (GLA). Optimised formulation (F6) was further subjected to *ex vivo* permeation studies using the goat nasal mucosa. The percent drug permeated after 8 h was found to be 88.71%.

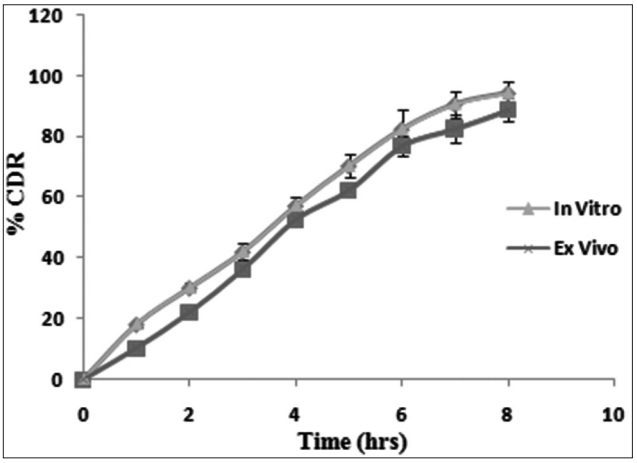

Figure 3: Comparison of *in vitro* and *ex vivo* drug release profiles of formulation F6

Drug release kinetic study

To analyse the mechanism for the release and release rate kinetics of the dosage form, the data obtained from *in-vitro* drug release from optimied formulation (F6) were fitted to models representation zero order, first order, Higuchi and Korsemeyer-Peppas. Based on highest regression values R the best fit model follows Korsemeyer-Peppas model release profile and the results of linear correlation coefficient (R^2) values are tabulated in Table 6. Further, the observed release exponent value ($n = 0.835$) is indicative of the fact that the drug release from the formulation follows non-Fickian transport mechanism.

Scanning electron microscopy

The optimised batch F6 was analysed by SEM for examining the shape and surface structure of the microspheres. The microspheres were found to be discrete and spherical in shape and had nearly smooth surfaces [Figure 4]. In addition, no free drug crystals were scrutinised on the surface of the microspheres. The obtained microspheres had no pores or crack on the surface, which would consequently slow clearance and good deposition pattern in the nasal cavity.

Fourier transform infrared spectrophotometer study

The FTIR spectrum of the pure PCPM, blank chitosan microspheres and PCPM loaded microspheres were compared to find any change in the frequency of functional group in microspheres with relevant functional group of the drug [Figure 5]. The spectral observations signified that the major FTIR absorption peaks viewed in the spectra of the drug were

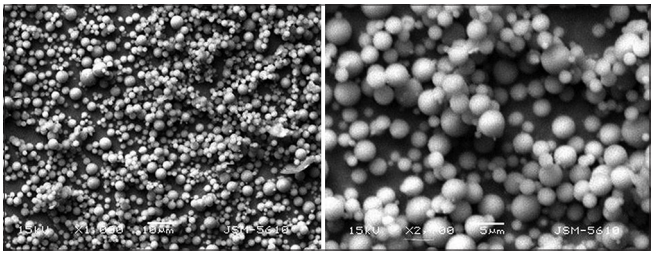

Figure 4: Scanning electron microscopy photomicrographs of optimised microsphere formulation (F6)

Table 5: Characteristics of PCPM loaded microspheres

Formulation code	Production yield* (%±SD)	Particle size† (µm±SD)	Drug loading* (%±SD)	Entrapment efficiency* (%±SD)	Swelling index* (%±SD)	Mucoadhesive strength* (%±SD)
F1	39.20±1.23	13.17±0.48	49.09±1.32	96.21±1.48	0.86±0.02	83.71±2.07
F2	36.22±1.43	11.93±0.73	82.20±2.62	89.32±0.98	0.82±0.03	77.61±3.46
F3	37.10±2.14	8.46±0.99	81.80±3.47	91.04±1.03	0.81±0.01	76.25±2.26
F4	37.81±1.87	12.98±0.66	46.66±2.40	88.21±1.30	0.85±0.02	87.72±1.22
F5	35.83±1.61	15.67±0.54	52.42±3.97	93.91±1.06	0.87±0.02	84.83±3.66
F6	38.26±1.92	7.32±0.85	78.52±1.56	90.12±0.97	0.79±0.01	79.90±2.74
F7	38.67±1.98	12.11±0.76	48.98±2.59	94.71±1.81	0.84±0.010	86.87±1.86
F8	41.44±2.36	9.10±0.69	68.29±1.84	84.9±1.02	0.80±0.014	80.62±4.96

*Values expressed as mean±SD, *n*=3, †Average of 100 particles±SD. SD: Standard deviation

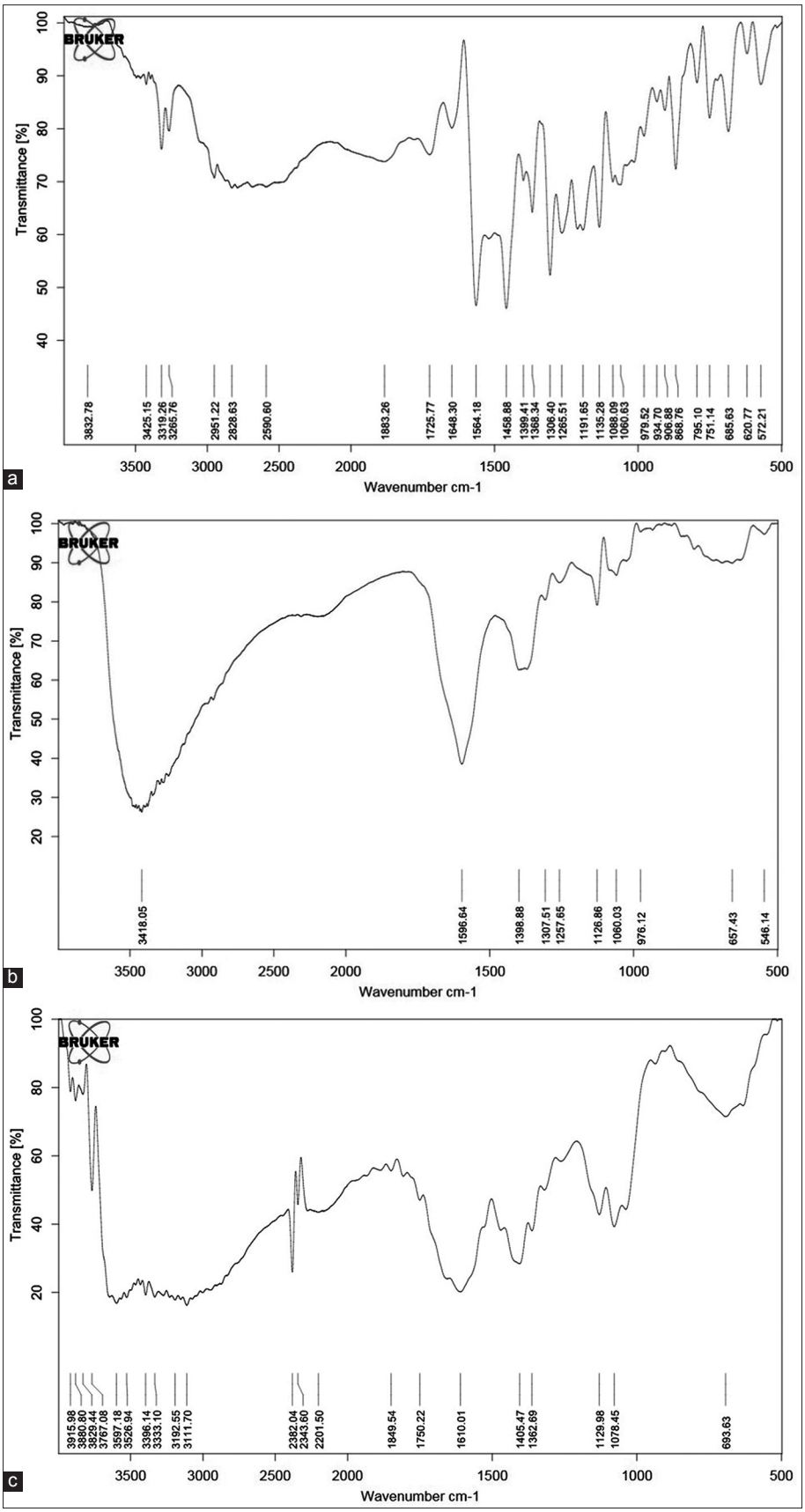

Figure 5: IR spectrum of (a) pure PCPM, (b) blank chitosan and (c) PCPM loaded chitosan microsphere

near to those in the spectra of the microspheres. It indicates that the method of preparation and processing parameters has not affected the drug stability.

Histopathology study

It is essential to check histological changes in the nasal mucosa caused by formulations before practically utilisation. Examination of tissue showed the manifestation of ciliated respiratory epithelium along with normal goblet cells [Figure 6]. On comparison of treated nasal mucosa with control, no severe signs of damage such as appearance of epithelial necrosis or sloughing of epithelial cells were detected on the integrity of nasal mucosa.

Stability study

The optimised formulation (F6) was evaluated at periodical intervals of time for 3 months accelerated storage conditions. The average particle size remained relatively unchanged with no significant change in % entrapment efficiency after 3 months [Table 7]. Hence, it can be concluded that the drug was retained within the microspheres and formulation was found to be stable throughout the stability period.

CONCLUSION

In the present study, chitosan-based mucoadhesive microspheres were prepared by spray drying method. Different variables such as the amount of chitosan, feed flow rate and volume of GLA were optimised by the factorial design. A 2^3 experimental design was employed to identify optimal formulation parameters for a microsphere preparation with the minimum value of particle size with substantial entrapment efficiency. From the mathematical models generated, an optimal formulation comprising of 500 mg chitosan, 1 ml/min feed flow rate and 0.9 ml GLA was identified to provide desired values for particle size (7.32 μm) and entrapment efficiency (90.12%). SEM analysis of the microspheres revealed that the microspheres were nearly smooth and spherical nature with ideal surface morphology. Particle size was in the range of 7.32–15.67 μm, which is considered to be ideal for nasal drug delivery. All batches showed good *in-vitro* mucoadhesion (76.25–87.72%). These properties make microspheres based on chitosan appropriate for the nasal administration; in fact the mucoadhesiveness might prolong the residence time of the formulation within the nasal cavity. Results of *in-vitro* kinetic study suggested that the mechanism of drug release from microspheres was diffusion and erosion controlled. The FTIR study proves the chemical stability of PCPM even after entrapment in microspheres. Results of histopathological study confirmed that the prepared PCPM-loaded microsphere system had no sign of lesions on the nasal mucosa. The result from the present study indicates that it is possible to achieve enhanced bioavailability of PCPM using chitosan microspheres. Prepared mucoadhesive Chitosan microsphere might be used as alternate to currently available marketed PCPM formulations. However, wide pharmacokinetic studies require to be established so as to explore the investigated nasal drug delivery formulation as a substitute.

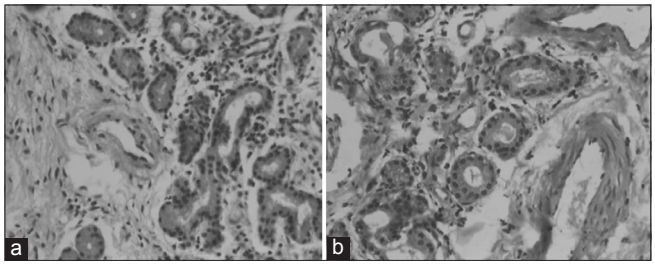

Figure 6: (a) Light photomicrograph of goat nasal mucosa, untreated control mucosa and (b) PCPM-loaded microspheres-treated mucosa

Table 6: Model fitting of the release profile of optimised formulation (F6)

Formulation code	R^2 value				
	Zero order	First order	Higuchi	Korsmayer-Peppas	
				R^2	*n*
Optimised batch (F6)	0.983	0.953	0.987	0.994	0.835

Table 7: Evaluation parameters for optimised batch (F6) during stability studies

Time (months)	Particle size† (μm±SD)	Entrapment efficiency* (%±SD)
0	7.32±0.85	90.12±0.97
1	7.81±0.60	89.76±0.91
2	8.17±0.43	89.04±0.62
3	8.75±0.71	88.55±0.79

*Values expressed as mean±SD, *n*=3, †Average of 100 particles±SD. SD: Standard deviation

ACKNOWLEDGMENTS

Authors are thankful to Vaikunth Chemicals Ltd. (Ankleshwar, Gujarat, India), for gifting sample of PCPZ. The authors are also grateful to Trustees, Pioneer Pharmacy Degree College (Vadodara, Gujarat, India), for providing the essential amenities to accomplish this work.

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How to cite this article: Shah SS, Gohil DY, Pandya DN, Meshram DB. Preparation and evaluation of spray-dried mucoadhesive microspheres for intranasal delivery of prochlorperazine using factorial design. Asian J Pharm 2015;9:178-89.

Source of Support: Nil. **Conflict of Interest:** None declared.