



Formulation Development and *In Vitro* Evaluation of Mucoadhesive Microspheres of Simvastatin for Nasal Delivery

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ABSTRACT

The purpose of present research work was to develop mucoadhesive microspheres of Simvastatin for nasal delivery with the aim to avoid hepatic first-pass metabolism, improve therapeutic efficacy and enhance residence time in the treatment of hypercholesterolemia and dyslipidemia. The microspheres were prepared by emulsification-crosslinking method to optimize parameters like external phase (heavy and light liquid paraffin in the ratio of 1:1), stirring rate (1200 rpm), dioctyl sodium sulfosuccinate concentration (DOSS) (0.2% w/v), drug: chitosan ratio (1:2), volume of cross linking agent glutaraldehyde (0.1 ml) and crosslinking time (2 h). A 2³ factorial design was employed with drug: polymer ratio, volume of cross linking agent and cross-linking time as independent variables while particle size of the microspheres and *in vitro* mucoadhesion were the dependent variables. The microspheres were evaluated for particle size and shape and surface morphology by SEM, drug loading, drug incorporation efficiency, *In vitro* mucoadhesion, and *In vitro* drug release study. Particle size was found to be in the range of 29.48 to 37.08 μm , which is favourable for intranasal absorption. The particle size was significantly increased when high concentration of chitosan was used. Aqueous to oil phase ratio, stirring rate and DOSS concentration also influenced the particle size of the microspheres. It was found that, as stirring rate was increased, the size of the microspheres was decreased. The volume of glutaraldehyde and crosslinking time had also affects drug release pattern. The percentage incorporation efficiency was found to be in the range between 83.63 to 94.95 %. *In vitro* mucoadhesion was performed by adhesion number using sheep nasal mucosa and was observed in a range from 75.35 \pm 1.44 to 89.92 \pm 1.01%. DSC and XRD results indicated a molecular level dispersion of simvastatin in the microspheres. *In vitro* release studies in pH 5.5 acetate buffer at 12 h was found to be 86.54%.

Keywords: Chitosan, Microspheres, Mucoadhesion, Nasal delivery, Simvastatin.

INTRODUCTION

The nasal route has gained tremendous attention for systemic drug delivery by many researchers within the last few decades due to its great potential utility for drug delivery. It offers an attractive alternative for drugs that have limited oral bioavailability, are destroyed by gastrointestinal fluids, or are highly susceptible to hepatic first pass or gut wall metabolism. Nasal drug delivery also offers the convenience and safety of being non invasive. However, the nasal route has limitations like mucociliary clearance, low permeability etc. Mucoadhesive preparations like microspheres have been developed to increase the contact time of the dosage form, thus enhance drug absorption and its bioavailability.¹⁻⁴

Simvastatin a anti hyperlipidaemic, HMG-COA reductase inhibitor is the drug of choice in the treatment of hypercholesterolemia and dyslipidemia. Simvastatin undergoes extensive first pass metabolism by oral route and thus exhibits only 5% oral bioavailability. The present investigation was aimed at avoidance of first pass metabolism of simvastatin by preparing chitosan microspheres for nasal administration.^{5,6} Mucoadhesive microparticle nasal delivery is an attractive concept in that the drug can entrapped inside particles to be released at nasal mucosal surface, where the particles are adhered due to their bio/ mucoadhesiveness. Extensive

works on microspheres using mucoadhesive polymers for drug like pentazocine⁷, FITC-dextran⁸ reported.

Various biodegradable materials have been used as carriers for microparticulate drug delivery systems. Recently, chitosan microspheres have received considerable attention due to its biodegradability, biocompatibility, high charge density, toxicity and mucoadhesive property. The gelling property of chitosan offers diverse uses including microencapsulation and controlled release of drugs via microparticulate systems.⁹ Different methods have been tried by various researchers to prepare chitosan microspheres, e.g. ionotropic gelation¹⁰, emulsification-crosslinking^{4,11}, thermal crosslinking¹², solvent evaporation¹³, spray drying¹⁴ and precipitation coacervation.³

In the present study, chitosan microspheres were prepared by emulsification-crosslinking method. The influence of various process and formulation parameters, namely, chitosan concentration, stirring rate, volume of GA (crosslinking agent), crosslinking time, aqueous to oil phase volume ratio and DOSS (dioctyl sodium sulfosuccinate) concentration on particle size distribution of chitosan microspheres cross linked with GA was investigated.

MATERIALS AND METHODS

Materials

Simvastatin USP was a gift sample from Aurobindo Pharma Ltd., Hyderabad. Chitosan was obtained from Research lab fine chem., Mumbai, Glutaraldehyde (GA) (25% aqueous solution) was obtained from Loba Chemie Pvt. Ltd., Mumbai, Dioctyl sodium sulfosuccinate (DOSS) was procured from Wilson Laboratories, Mumbai, India. Hexane was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. All other chemicals and reagents used in the study were of analytical grade.

Preparation of chitosan microspheres

Chitosan microspheres were prepared by simple w/o emulsification-cross linking process using liquid paraffin (heavy and light, 1:1) as external phase^{11,15}. Briefly, accurately weighed quantity of chitosan was dissolved in 2% aqueous acetic acid solution by continuously stirring until a homogeneous solution was obtained. The drug

was added in chitosan solution and the dispersion was added slowly through syringe to liquid paraffin (heavy and light, 1:1) containing 0.1% w/v of DOSS as stabilizer under constant stirring at 1200 rpm for 15 min using a Remi (RQG-126/D) high speed stirrer. To this W/O emulsion, appropriate quantities of glutaraldehyde (25% solution, as cross-linking agent) were added slowly and stirring was continued for 2 h. The hardened microspheres were separated by vacuum filtration and washed several times with hexane to remove oil. Finally, microspheres were washed with distilled water to remove unreacted glutaraldehyde. The microspheres were air dried for 24 h and then stored in vacuum desiccator until further use.

To investigate the effect of different formulation and process variables on particle size of chitosan microspheres, various batches of formulations were prepared by varying one parameter and keeping the others constant as given in Table 1.

Table 1: Process variables for prototype formulation of microspheres

Process variables	Constant parameters
Stirring speed A1 (600 rpm) A2 (800 rpm) A3 (1000 rpm) A4 (1200rpm)	Chitosan concentration (2% w/v) Aqueous: oil phase ratio (1:10) Surfactant concentration (0.2% w/v) Volume of glutaraldehyde (0.4 ml)
Chitosan concentration A5 (1%) A6 (2%) A7 (3%)	Stirring speed (1200 rpm) Aqueous: oil phase ratio (1:10) Surfactant concentration (0.2% w/v) Volume of glutaraldehyde (0.4 ml)
Surfactant concentration (DOSS) A8 (0.1%) A9 (0.2%) A10 (0.3%)	Stirring speed (1200 rpm) Chitosan concentration (2% w/v) Aqueous: oil phase ratio (1:10) Volume of glutaraldehyde (0.4 ml)
Aqueous: oil phase ratio A11 (1:5) A12 (1:10) A13 (1:15)	Stirring speed (1200 rpm) Chitosan concentration (2% w/v) Surfactant concentration (0.2% w/v) Volume of glutaraldehyde (0.4 ml)
Drug: polymer ratio D1 (1:1) D2 (1:2) D3 (1:3)	Stirring speed (1200 rpm) Chitosan concentration (2% w/v) Aqueous: oil phase ratio (1:5) Surfactant concentration (0.1% w/v) Volume of glutaraldehyde (0.4 ml)
Volume of cross-linking agent and crosslinking time D4 (0.4 mL/2h) D5 (0.2 mL/2h) D6 (0.1 mL/2h) D7 (0.1 mL/1h)	Stirring speed (1200 rpm) Chitosan concentration (2% w/v) Aqueous: oil phase ratio (1:5) Surfactant concentration (0.1% w/v)

Experimental design

Various batches of chitosan microspheres were prepared based on the 2³ factorial design. The independent variables were drug: polymer ratio (X1), concentration of cross linking agent glutaraldehyde (X2) and cross-linking

time (X3). *In vitro* mucoadhesion strength (Y1) and Particle size of the microspheres (Y2) were taken as response parameters as the dependent variables. Independent and dependent variables depicted on Table-2.



Microsphere Characterization

Morphology

All batches of microspheres were preliminarily checked for shape and size by optical microscopy at 10X

magnification. The samples were prepared by suspending a small amount of microspheres in paraffin oil. The surface morphology of the Simvastatin loaded microspheres was examined by scanning electron microscopy (JSM 6360A, Jeol, Japan).

Table 2: Formulation of the microspheres utilizing a 2³ factorial design

Batch No	X1	X2	X3	Percentage yield	Drug loading (%)	Y1* In vitro mucoadhesion (%)	Y2* Particle size (µm)	Incorporation efficiency (%)
F1	-	-	-	84.00	40.87	80.14±0.61	30.40±5.77	85.83
F2	+	-	-	84.37	26.8	83.33±0.48	30.86±4.62	90.45
F3	-	+	-	87.5	41.17	75.35±1.44	33.53±4.71	90.07
F4	+	+	-	86.25	26.71	79.69±0.29	34.40±5.52	92.17
F5	-	-	+	88.00	40.95	84.2±1.10	30.02±6.31	90.1
F6	+	-	+	89.37	26.55	88.08±0.80	29.48±5.82	94.95
F7	-	+	+	85.00	39.35	81.43±0.94	35.54±5.71	83.63
F8	+	+	+	85.62	25.35	89.92±1.01	37.08±4.7	86.85

* Values are expressed as mean±SD.

Table 3: Solution of optimized formulation as per design expert software (batch 01)

Drug: Polymer ratio	Volume of Glutaraldehyde	Cross linking time	Mucoadhesion strength	Particle size	Desirability
1:2	0.1	2	89.565	30.8475	0.894

Percentage yield

The practical percentage yield was calculated from the weight of dried microspheres recovered from each batch in relation to the sum of the initial weight of starting materials. The percentage yield was calculated using the following formula:

$$\% \text{ yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100 \quad \dots\dots(1.1)$$

Particle size analysis

A microscopic image analysis technique for determination of particle size was applied. The morphology and particle sizes were determined in a Motic DMW2-223 digital microscope equipped with a 1/399 CCD camera imaging accessory and computer controlled image analysis software (Motic images plus, 2.0 version). The microspheres were dispersed on a microscope slide. A microscopically field was scanned by video camera. The images of the scanned field are analyzed by the software. In all measurements at least 100 particles were examined for their mean particle diameter (µm). The average particle size was determined by using the Edmundson's equation.

$$D_{\text{mean}} = \sum nd / \sum n \dots\dots\dots(1.2)$$

Where, n: Number of microspheres observed, d: mean size range

Determination of incorporation efficiency

The Simvastatin loaded microspheres (50 mg) were crushed in glass mortar and pestle and the powdered microspheres were suspended in 20 mL methanol under stirring. The solution was filtered and analyzed for the drug content using UV visible spectrophotometer at 238 nm. The drug loading and % incorporation efficiency was calculated using following equations:

$$\text{Drug loading (\%)} = \frac{M_{\text{actual}}}{\text{Weighed quantity of powder of microspheres}} \times 100 \quad \dots\dots\dots(1.3)$$

$$\text{Incorporation efficiency (\%)} = \frac{M_{\text{actual}}}{M_{\text{Theoretical}}} \times 100 \quad \dots\dots\dots(1.4)$$

Where, M_{actual} is the actual Simvastatin content in weighed quantity of powder of microspheres and M_{theoretical} is the theoretical amount of Simvastatin in microspheres calculated from the quantity added in the cross emulsification process.¹⁶

Swelling studies

The equilibrium water uptake of the microspheres was determined by measuring the extent of swelling of the matrix in acetate buffer pH 5.5. To ensure complete equilibration, microspheres were allowed to swell for 12 h. The excess surface adhered liquid drops were removed by blotting with soft tissue papers and the swollen microspheres were weighed to electronic balance¹⁵. The microspheres were then dried in an oven at 60°C for 3 h until there was no change in the dried mass of the microspheres. The % equilibrium water uptake was calculated as:



% Water uptake = $\frac{\text{Weight of swollen microspheres} - \text{Weight of dry microsphere}}{\text{Weight of dry microsphere}} \times 100$ (1.5)

Measurement of *in vitro* mucoadhesion

The microspheres were placed on sheep nasal mucosa after fixing to the polyethylene support. The mucosa was then placed in the desiccator to maintain at >80% RH at room temperature for 30 min to allow the polymer to hydrate and to prevent drying of the mucus. The mucosa was then observed under a microscope and the number of particles attached to the particular area was counted. After 30 min, the polyethylene support was introduced into a plastic tube cut in circular manner and held in an inclined position at an angle of 45°. Mucosa was washed thoroughly at flow rate of 1 mL min⁻¹ for 5 min with acetate buffer pH 5.5. Tissue was again observed under a microscope to see the number of microspheres remaining in the same field area. The adhesion number was determined by the following equation:

$$Na = N/No \times 100 \quad \dots\dots(1.6)$$

Where, Na is adhesion number, NO is total number of particles in a particular area and N is number of particles attached to the mucosa after washing.^{4,15}

***In vitro* drug release study¹⁷**

In vitro release of drug from the microspheres was carried out in a modified Franz diffusion cell with dialysis membrane (cut-off 12,000-14,000 kDa). For preparation of artificial membrane, dialysis membrane was soaked in acetate buffer pH 5.5 for 24h before mounting on the diffusion cell. After a preincubation time of 20 min., accurately weighed of drug loaded microspheres equivalent to 10 mg simvastatin was placed in the donar compartment with 2 ml and 20 ml acetate buffer pH 5.5 was placed in donar and receptor compartment respectively. Temperature of receptor compartment was maintained at 37±1°C during the experiment. At set time interval, 1 ml sample were withdrawn from the receptor compartment; replacing the sampled volume with acetate buffer pH 5.5 after each sampling for period of 12h. Sample withdrawn were suitably diluted and analyzed using UV visible spectrophotometer at 238 nm.

Differential scanning calorimetry (DSC) analysis

DSC was performed on placebo microspheres, drug-loaded microspheres and plain simvastatin to determine the thermal behavior. It was also used to determine the existence of possible interaction between the polymer and drug. Samples were heated from 0°C to 350°C at the heating rate of 20°C/min in nitrogen atmosphere.

X-ray diffraction (X-RD) studies

The X-RD patterns of placebo microspheres, plain simvastatin and drug-loaded microspheres were recorded using an X-ray diffractometer (Bruker AXS D8 Advance). These studies are useful to investigate crystallinity of drug in the microspheres. The samples were mounted on a

sample holder and X-RD patterns were recorded in the range of 5-50° at the speed of 5°/min.

***Ex vivo* permeation study**

Fresh nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. A piece of nasal mucosa was mounted as flat sheet in a two chamber diffusion cell maintained at 37 ± 0.5°C with the mucosal side facing the donor compartment. Acetate buffer pH 5.5 was placed in the receptor compartment. After a pre-incubation time of 20 min, microspheres equivalent to 10 mg simvastatin was placed on the mucosal surface in the donor compartment. At predetermined time interval, 1 ml sample were withdrawn from the receiver compartment and replacing the sampled volume with acetate buffer pH 5.5 after each sampling for period of 12h. Sample withdrawn were suitably diluted and analyzed using UV visible spectrophotometer at 238 nm.

Histopathological study

Histopathological evaluation of tissue incubated in acetate buffer pH 5.5 after collection was compared with tissue incubated in the diffusion chamber with microspheres. Tissue was fixed in 10% buffered formalin, routinely processed and embedded in paraffin. Paraffin sections (7 µm) were cut on glass slides and stained with hematoxylin and eosin (HE). Sections were examined under a digital optical microscope (Motic), to detect any damage to the tissue during *ex vivo* permeation study.

Stability of the microspheres.

The microsphere batches of optimized formulation were stored in a stability chamber (Remi CHM-10S) at 40°C and 75% RH for 3 months and observed for particle size, drug content and *in vitro* drug release at 1 month intervals.

RESULTS AND DISCUSSION

Morphology

The microspheres obtained were of good morphological characteristics, spherical in shape, smooth surface without aggregation (Fig- 1a,b) and free flowing powders. The volume of GA and crosslinking time did not alter the surface morphology of the microspheres. However, one noticeable characteristic of the cross linked chitosan microspheres was their yellow to brownish color. The intensity of the color of the microspheres increased as the volume of GA and time of crosslinking increased.

Percentage yield

Yield of production was found in the range between 84.0-89.37% (Table-2). The low percentage yield in some formulations may be due to microspheres lost during the washing process. A 100% yield could not be achieved principally due to adhesion of microspheres to stirring rod and filtration assembly.



Particle size analysis

Average particle size of microspheres ranged from 29.48 ± 5.82 - 37.08 ± 4.7 μm , such particles are considered to be suitable for nasal administration (Fig-1C)

Effect of stirring rate

The mean particle size of the microspheres decreased with increasing stirring rate. When the stirring rate was increased from 600rpm to 1200 rpm, the particle size of microspheres was observed to decrease from 112-138.88 μm to 24.5-37 μm . At stirring rate of 600 rpm, along with the larger particle formation, sphericity and smoothness of the microspheres was affected. This may be due to less efficient shearing of the chitosan solution to form fine droplets at low stirring rate. At higher stirring rate of 1000 and 1200 rpm, smooth and spherical particles were

obtained with mean particle size of 37.5-61 μm and 24.5-37 μm respectively.

Effect of chitosan concentration

The particle size of the microspheres was found to be dependent on the concentration of chitosan. At lower concentration (1%), the viscosity of the solution is very less to have drop wise addition is not possible and microspheres obtained are irregular in shape. At higher concentration (3%), the chitosan solution was so viscous that it was difficult to pass through syringe and produced large droplet. At medium concentration (2%), the mean particle size was observed to be 21.1-41.3 μm and showed good sphericity and free flowing in nature. The mean particle size of microspheres was significantly increased when high concentration of chitosan (3%) was used.

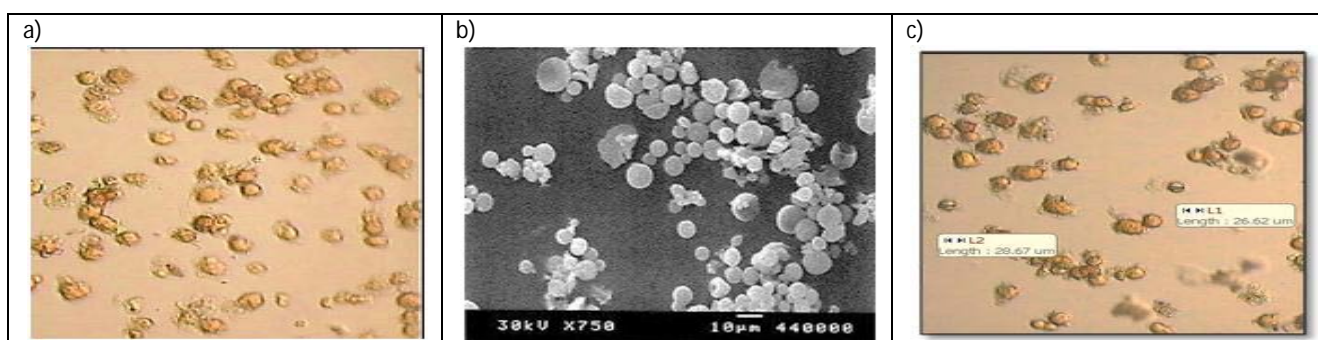


Figure 1: a) Photomicrograph of drug loaded chitosan microspheres (batch O1); b) SEM photograph of drug loaded chitosan microspheres (batch O1); c) Photomicrograph for particle size of microspheres measurement.

Effect of DOSS concentration

Microspheres fabricated with 0.1% (w/v) DOSS showed the lowest particle size but also aggregates formed while those prepared with 0.2% (w/v) showed particle size in the range i.e. 21.1-41.3 μm and spherical in shape. An increase in surfactant concentration led to the decreased emulsion droplet size during the formation of microparticles. Microspheres fabricated with 0.3% (w/v) DOSS showed particles are irregular in shape. Hence, optimized concentration of DOSS (0.2% (w/v)) was selected as stabilizing agent which forms a thin film around the droplets of chitosan to prevent coalescence and helps in droplet stabilization.

Effect of aqueous to oil phase ratio

The mean particle size of microspheres was increased with an increase in aqueous to oil phase ratio. When ratio was 1:5 and 1:10 was used, a slight increase in microsphere particle size was observed from 35.18 ± 5.65 μm and 37.47 ± 6.61 μm . At higher ratios Of 1:15 and a significant increase in the particle size of microspheres and aggregation of microspheres was also observed. From these results, it can be assumed that as the ratio of aqueous to oil phase was increased, the mean distance between the droplets of chitosan (aqueous phase) in oil phase will be decreased, which may result in increasing the chances of coalescence between the droplets. This may lead to aggregation of microspheres and increasing

the particle size. Another reason for increase in the particle size may be due to decrease in the shearing efficiency of the stirrer due to increased viscosity.

Effect of drug: polymer ratio

Designed by varying drug: polymer ratio i.e. 1:1, 1:2 and 1:3 and formulation procedure was used as described in procedure of preparation of chitosan microspheres. Three sets of formulations were prepared while keeping other process variables as per preliminary trials such as stirring speed (1200rpm), chitosan concentration (2%w/v), concentration of surfactant (0.1%w/v) and aqueous: oil phase ratio (1:5) and the amount of cross-linking agent (Glutaraldehyde) (0.4 ml) constant. The obtained microspheres were evaluated for physical appearance, particle size, drug loading and incorporation efficiency. The % incorporation efficiency of 1:1, 1:2 and 1:3 batches were found to be 91.86%, 93.45% and 94.11% respectively and drug loading was found to be 41.51%, 26.7% and 23.32% respectively and particles were spherical in shape and particle size in the range 31.29 ± 6.93 , 32.14 ± 6.82 and 30.24 ± 6.92 respectively. As drug: polymer ratio affects the drug content & % incorporation efficiency of microspheres. As drug to polymer ratio decreases % incorporation efficiency increases. In the above study, there is a slight increase in the % incorporation efficiency with decrease in drug to polymer ratio.

Effect of volume of GA (Glutaraldehyde) and crosslinking time

The microspheres prepared with varying the amount of cross-linking agent i.e. 0.4 ml, 0.2 ml, 0.1 ml and 0.1 ml respectively and crosslinking time viz. 2h, 2h, 2h and 1h respectively. As glutaraldehyde concentration & cross linking time affects the release of drug from microspheres, as the glutaraldehyde concentration and cross linking time increases, the drug release time extends and 0.1 ml glutaraldehyde with 2hr cross linking shows good drug release pattern i.e. at 12h- 89.17% drug release so it was selected for further studies

Drug loading and incorporation efficiency

The % incorporation efficiency was found to be in the range between 83.63 to 94.95. The % incorporation efficiency showed a dependence on drug loading, amount of cross-linking agent and time of cross-linking. The formulations loaded with higher amount of drug: polymer ratio exhibited higher incorporation efficiency (Table-2).

Swelling studies

The % equilibrium water uptake of the microspheres was found to be $119.76 \pm 5.36\%$. As the amount of GA was increased, the equilibrium water uptake was decreased. This may be due to the formation of a rigid network structure at higher concentration of crosslinking. Hence, the crosslinking of microspheres has a great influence on the equilibrium water uptake.

In vitro mucoadhesion

The results of *in vitro* mucoadhesion (Table-2) showed that all the batches of microspheres had satisfactory mucoadhesive properties ranging from 75.35 ± 1.44 - $89.92 \pm 1.01\%$ and could adequately adhere on nasal mucosa. The results also showed that, with increasing polymer ratio, higher mucoadhesion percentages were obtained. This could be attributed to the availability of a higher amount of polymer for interaction with mucus. Most of the studies showed that the prerequisite for a good mucoadhesion is the high flexibility of polymer backbone structure and of its polar functional groups. Such a flexibility of the polymer chains, however, is reduced if the polymer molecules are highly cross linked. Although highly cross-linked microspheres will absorb water, they are insoluble and will not form a liquid gel on the nasal epithelium but rather a more solid gel-like structure. The cross linked microspheres are more rigid as compared to non cross linked microspheres and availability of the less number of sites for mucoadhesion, results in reduction in the mucoadhesive properties. The formulation, F8, with highest mucoadhesion (89.92%) was considered to be the best.

In vitro drug release study

The *in vitro* release study of Simvastatin-loaded microspheres of optimized batch was carried out using modified Franz diffusion cell with dialysis membrane (cut-

off 12,000-14,000 kDa) and pH 5.5 acetate buffer for 12 h. The release pattern is shown in Figure 2.

As the polymer to drug ratio was increased the extent of drug release decreased. A significant decrease in the rate and extent of drug release is attributed to increase in density of polymer matrix that results in increased diffusion path length which the drug molecules have to traverse. The release of the drug has been controlled by swelling control mechanism. Additionally, the larger particle size at higher polymer concentration also restricted the total surface area resulting in slower release. As the volume of glutaraldehyde and crosslinking time decreases the %CDR increases. Also the batches containing 1:1 drug polymer ratio shown more drug release than 1:2 ratio, because of higher amount of polymer.

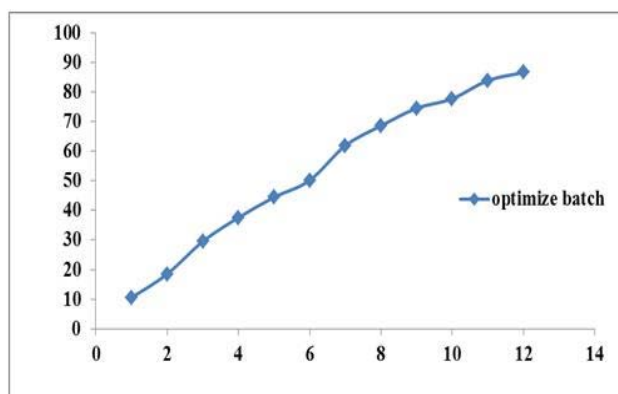


Figure 2: *In vitro* drug release study of optimized batches

Differential scanning calorimetry

DSC thermogram of Simvastatin, blank chitosan microspheres and Simvastatin loaded chitosan microspheres are displayed in Figure 3. Thermogram of Simvastatin showed a sharp peak at 139°C , indicating the melting of the drug. In the case of blank chitosan microspheres and Simvastatin loaded microspheres, no characteristic peak was observed at 139°C suggesting that Simvastatin is molecularly dispersed in the matrix.

X-ray diffraction (X-RD) studies

X-RD patterns recorded for plain Simvastatin (a), placebo microspheres (b) and Simvastatin loaded microspheres (c) are presented in Fig-4. Simvastatin peaks observed at 2θ (28.4° , 22.6° , 18.8° , 17.2° , 10.9° , and 9.3°) are due to the crystalline nature of Simvastatin. These peaks were not observed in the Simvastatin -loaded microspheres. This indicates that drug particles are dispersed at molecular level in the polymer matrices since no indication about the crystalline nature of the drug was observed in the drug loaded microspheres

Ex vivo permeation study

The permeation study was performed using sheep nasal mucosa. The permeation data of formulation from sheep nasal mucosa was found near to that obtained with dialysis membrane *in vitro*.

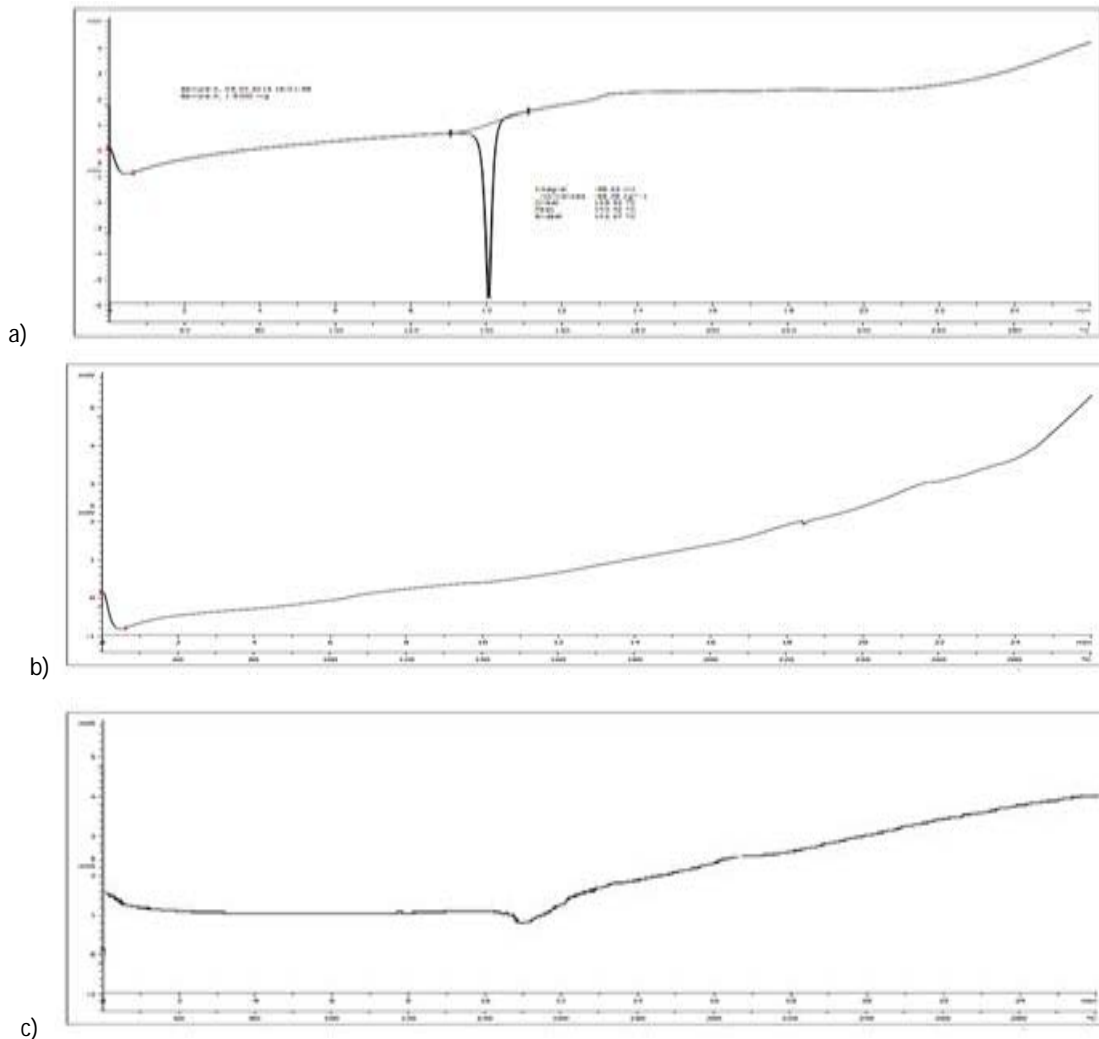


Figure 3: DSC thermograms of (a) Pure simvastatin, (b) Placebo microspheres and (c) Drug-loaded microspheres.

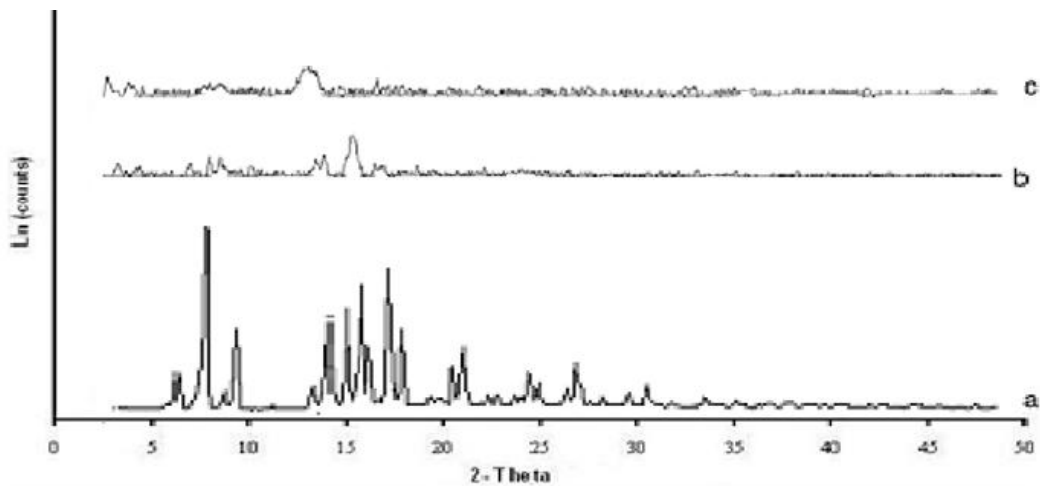


Figure 4: Powder X-ray diffraction patterns of (a) pure simvastatin, (b) placebo microspheres and (c) drug-loaded microspheres.

Histopathological study

It is necessary to examine histological changes in nasal mucosa caused by formulations, if it is to be considered for practical use. Histological studies shows control mucosa that is normal nasal mucosa stained with haematoxylin-eosin shows in Figure 5(a) and the effect of formulation on sheep nasal mucosa, 12 h after applying

the formulation shows in Figure 5(b). No change in mucosal structure was seen when treated with formulation as compared to the control.

Stability of the microspheres

Three stability batches of optimized formulations were evaluated at periodical intervals of time for 3 M accelerated storage conditions. The average particle size

remained relatively unchanged (29.56 ± 0.83) with no significant change in Drug loading (26.60 ± 0.31) *in vitro* drug release (86.72 ± 0.47) after 3 months.

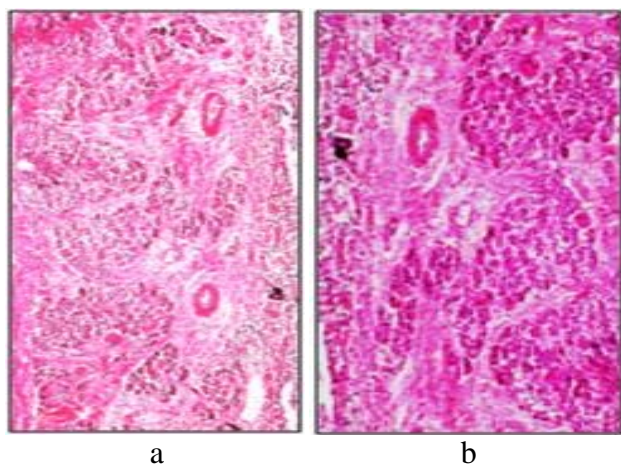


Figure: 5 a) Untreated nasal mucosa; **b)** Treated nasal mucosa

CONCLUSION

In the present study chitosan microspheres were prepared by emulsification-crosslinking method. Various variables such as the drug: polymer ratio, glutaraldehyde concentration and the cross-linking time were optimized 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 and maximum value of *in vitro* mucoadhesion. SEM confirmed the spherical nature and nearly smooth surfaces of the microspheres. Particle size was in the range of 29.48 ± 5.82 - 37.08 ± 4.7 μm which is considered to be favourable for intranasal absorption. All batches showed good *in vitro* mucoadhesion (75.35 ± 1.44 - $89.92 \pm 1.01\%$). Results of DSC and X-RD study indicated drug-polymer compatibility and a molecular level dispersion of simvastatin in the microspheres. Hence, the results of the present study clearly indicated promising potentials of chitosan microspheres for delivering simvastatin intranasally and could be viewed as a potential alternative to conventional dosage forms.

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