SECTION III
Preparation, optimization, evaluation and characterization of formulations

Chapter 5
Microspheres

5a. CHITOSAN GLUTAMATE MICRSOPHERES

5a.1.INTRODUCTION

Due to its bio-compatibility, there has been substantial number of studies on the biomedical used of chitosan as a drug carrier. Chitosan can be cross linked to various degrees to modulate drug release form the matrix. There are number of reports using glutaraldehyde as a crosslinking agent in the preparation of microspheres. Chitosan glutamate is proved to be suitable for nasal drug delivery systems due to its mucoadhesive potential, permeation enhancing effect and tolerability. Powder dosage form is suitable for enhancing the residence time in the nasal cavity as compared to solution. Mucoadhesive microspheres are one of the potential nasal drug delivery systems whereby many of the limitations of nasal drug delivery like lower residence time and degradability issues can be solved by modulation of microspheres. Therefore we attempted to formulate and optimize chitosan glutamate microspheres incorporating sumatriptan succinate for nasal drug delivery.

Factorial experimental design, multiple regression analysis, and desirability function have been proven to be a useful approach for the optimization of formulations. It was found from the preliminary studies that the amounts of chitosan glutamate, amount of crosslinking agent and drug loading had significant influence on the properties of microspheres. The objective of this chapter was to formulate and optimize mucoadhesive microsphere of chitosan glutamate incorporating sumatriptan succinate which can be used as the nasal drug delivery system for better treatment of migraine. Experimental design and desirability function were applied for the optimization. As part of the optimization process, the main effect, interaction effects, and quadratic effects of amounts of concentration of chitosan glutamate, drug loading and volume of crosslinking agent on drug entrapment, particle size, mucoadhesive strength and effective permeability were investigated

5a.2 EXPERIMENTAL

5a.2.1. Preparation of chitosan glutamate microspheres by emulsification crosslinking method

Chitosan glutamate microspheres were prepared using emulsification crosslinking technique (Jameela et al, 1998). .Designated amount of sumatriptan succinate was dissolved in the designated concentration of aqueous solution of chitosan glutamate (1.5%w/v, 2%w/v or 2.5% w/v) to get desired %drug loading (20%w/w, 30%w/w or 50%w/w). The prepared aqueous solution of drug and polymer was then added drop wise into 40 ml of soyabean oil (oil phase) containing 2% (w/w) Span 85 as an emulsifying agent. The aqueous phase was emulsified into the oily phase by stirring the system at 2500 rpm. Stirring was continued using Remi twin stage stirrer (Remi, India) for 15 minutes to obtain a w/o emulsion The chitosan glutamate in the internal phase of the w/o emulsion was cross linked using chemical crosslinking technique Cross linking of the chitosan glutamate in the internal phase of the w/o emulsion was done by adding a designated volume (7.5µl,16.25µl or 25µl)of glutaraldehyde. Stirring was continued for 1 hour and the microspheres formed were separated by centrifugation and washed with petroleum ether to remove soyabean oil followed by washing with acetone. Finally microspheres were dried at room temperature and stored in desiccator till further use. Each batch was prepared in triplicate.

5a.2.2. % Entrapment efficiency

To weighed amount of chitosan glutamate microspheres, 10 ml of 0.1N hydrochloric acid was added and allowed to stand fro 24 hours to degrade the microsphere matrix. The actual drug content was determined by measuring absorbance at 283 nm as described in chapter 3 using UV spectrophotometer (Shimadzu UV, 1601). Encapsulation efficiency was calculated from the ratio of actual to theoretical drug content and expressed as a percentage The analysis was performed three times for each batch of microspheres.

5a.2.3. Determination of residual glutaraldehyde

Accurately weighed amount of microspheres were taken in beaker in 10ml distilled water was added. The mixture was shaken well and filtered using a whatman filter paper. The filtrate was analyzed for the residual glutaraldehyde as per the method given in chapter 3.

5a.2.4. Particle size analysis

The particle size distributions of the prepared polymer microspheres were determined with a Malvern Hydro 2000SM particle size analyzer (Malvern Instruments, UK). Iso propyl alcohol containing a drop of tween 80 was used as a non-dissolving dispersion medium. The particle suspensions were sonicated in an ultrasonic bath for 10 min prior to analysis. In the measurement cell, the use of a magnetic stirrer facilitated the continuous flux of particles. The analysis was performed three times for each batch of microspheres.

5a.2.5. Evaluation of Mucoadhesive Strength

The mucoadhesive strength of the microspheres were determined by measuring the force required to detach the formulation from nasal mucosal tissue using a modified method described by Jones et al(Jones et al,2000). Briefly, nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. Tissues were immediately used after separation. At the time of testing a section of nasal tissue was secured, keeping the mucosal side out, to upper probe using a cyanoacrylate adhesive. Upper probe was attached to precalibrated force transducer SS12LA (BIOPAC systems, Inc., CA, USA) connected to the Biopac MP-30 data acquisition system. The surface area of each exposed mucosal membrane was 0 785cm^2. At room temperature, fixed amount of sample was uniformly sprayed on lower probe using double sided adhesive tape Upper probe was lowered until the tissue contacted the surface of the sample. Immediately, a force of 0.1 N was applied for 3 min to ensure intimate contact between the tissues and the samples The probe was then moved upwards at a constant speed of 0.15 mm/s and the force in terms detachment stress in dyne/cm^2, was determined from the weight required to detach the tissues from the surface of each formulation was determined as the peak value in the resultant force-time plot, using the following equation (Chang et al, 1985)

$$Detachmentstress\left(dyne/cm^2\right) = m.g/A$$

Where, m: the weight added in gram, g: acceleration due to gravity taken as 980 cm/sec^2, A area of tissue exposed.

5a.2.5a.Effect of initial contact time on mucoadhesive strength

Effect of varying contact time (1min, 2min, 3min, 5min and 10 min) was investigated for microsphere preparations to optimize initial contact time. In brief, formulations were

allowed to be in contact with mucosa for carrying contact times (1min, 2min, 3min, 5min and 10 min) and the bioadhesive force was determined as discussed above Contact time that resulted in maximum bioadhesive strength was selected as optimum contact time required for adequate adhesion.

5a.2.6. *In Vitro* Permeation Studies

In vitro permeation studies were done as described previously by many research groups (Ceschel et al, 2000 and Pisal et al, 2004).Nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse Tissue samples of 100-mm thickness were inserted in Franz diffusion cells displaying a permeation area of 0.785 cm^2. 20 ml of PBS pH 6 4 at 37°C was added to the acceptor chamber. To ensure oxygenation and agitation, a mixture of 95% O_2 and 5% CO_2 was bubbled through system. The temperature within the chambers was maintained at 37°C. After a preincubation time of 20 min, pure drug solution and microspheres equivalent to 2 5 mg of drug was placed in the donor chamber. At predetermined time points, 1ml samples were withdrawn from the acceptor compartment. The samples withdrawn were filtered and used for analysis. Blank samples (without sumatriptan) were also run simultaneously throughout the experiment to check for any interference. The amount of permeated drug was determined using a UV-Visible spectrophotometry by the method described in chapter 3. The viability of the tissue in the permeation study was investigated before and after the experiment by staining with trypan blue (0.1% solution in PBS). After a incubation time medium from both compartment was removed, tissues were washed and were examined by light microscopy for exclusion of the marker. Exclusion of the marker from the tissue cells was considered to be viable as described by Verena et al (Verena et al, 2004).

5a.2.6.1.Data Analyses of Permeation Studies

The effective permeability coefficients Peff (cm s^{-1}) under steady state conditions across excised mucosa can be calculated according using following equation

$$Peff = \left(\frac{dC}{dt}\right)_{ss} \frac{V}{AC_D}$$

where $(dC/dt)_{ss}$ (μg ml^{-1} s^{-1}) is the time dependent change of concentration in the steady-state, A(cm^2)is the permeation area, V(ml) the volume of the receiver compartment and C_D(ug ml^{-1}) the initial donor concentration (Lang et al,1996).

5a.2.7.Histopathological evaluation of mucosa

Histopathological evaluation of freshly excised tissue was compared with tissue mounted in the diffusion chamber during permeation studies of optimized batch of microspheres. Tissues were fixed in 10% buffered formalin. Routinely processed and embedded in paraffin. Paraffin sections cut on glass slides were stained with hematoxylin and eosin. Sections were examined under light microscope.

5a.2.8. Angle of repose

The angle of repose was measured from a heap carefully built up by dropping the microsphere samples through a glass funnel to the horizontal plate. Each time microspheres were transferred to the funnel and the diameter (D) and height (h) of the cone produced after pouring the microspheres was measured. Angle of repose was determined by following equation (Lachman et al. 1986).

$$\tan\theta = 2h/D$$

The results were averaged from three determinations.

5a.2.9. Morphology Analysis

Scanning electron microscopy (SEM) was used to observe the structure of particles. Microspheres were mounted on brass stubs using double-sided adhesive tape Stub was fixed into the sample holder and placed in vacuum chamber. The samples were analyzed using low vacuum (10^{-3} torr) by SEM (JSM 5610LV, JEOL, Oxford Instruments, UK) at 15kV for morphology studies.

5a.2.10. FTIR studies

FTIR spectral measurements were performed using a Shimadzu 8300 FTIR spectrometer. Microspheres were grounded with KBr and FTIR spectra were taken in the range 4500-500 cm^{-1}.

5a.2.11. Stability study

The optimized batch was subjected to stability studies. Formulation was stored in vials at room temperature for six months. The change in % entrapment efficiency and particle size was determined after 6 months.

5a.3. FACTORIAL DESIGN AND DESIRABILITY FUNCTION

Factorial design is a useful tool in order to characterize multivariable processes. It gives the possibility to separate the important factors from those, which are not, and identifying any possible interactions between them. In this study a 3^3 full factorial design was used to determine the effect of the concentration (%w/v) of chitosan glutamate, percentage drug loading(%w/w with respect to polymer) and volume of glutaraldehyde on the particle size,% drug entrapment, mucoadhesive strength and effective permeability across sheep nasal mucosal membrane. Before the application of the design a number of preliminary trials were conducted to determine the conditions at which the microspheres were produced with desired particle size and drug entrapment. The levels of the factors were also determined by this procedure. The factors and their levels are shown in Table 5a.1. Three independent factors, the concentration of chitosan glutamate (A), the percentage drug loading (B), and the volume of glutaraldehyde (C), were set at three different levels. High and low levels of each factor were coded as 1 and -1, respectively, and the mean value as zero.

Table 5a.1 Factorial design 3^3 : factors and their levels

Factors	Low (-1)	Middle(0)	High(+1)
A:Chitosan glutamate concentration(%w/v)	1.5	2	2.5
B:% Sumatriptan succinate loading(%w/w)	20	35	50
C:Volume of glutaraldehyde(μl)	7.5	16.25	25

The different formulations of the factorial design consisted of all possible combinations of all factors at all levels and were conducted in a fully randomized order. The matrix of the experiments and the results of the responses for every experiment are listed in Table 5a.4. To determine the experimental error, the experiment at the center point was replicated five times at different days. The statistical evaluation of the results was carried out by analysis of variance (ANOVA) using a commercially available statistical software package (DESIGN EXPERT V 7.0.4, Minneapolis, USA). The quadratic model was selected for this analysis. Finally the desirability function was used for the optimization of the process. During the optimization of a multivariable process, the responses have to be combined in order to produce a product of desired characteristics. The application of the

desirability function combines all the responses in one measurement (Lewis et al., 1999), and gives the possibility to predict the optimum levels for the independent variables.

The combination of the responses in one desirability function requires the calculation of the individual desirability functions. In this particular study there were not special requirements for the particle size of the optimum formulation, so the range of the values of the produced formulations was selected. The optimum formulation of this study should have a particle size ranging between 10-100 μm, with maximum % drug entrapment, maximum mucoadhesive strength in terms of detachment stress and maximum effective permeability. The individual desirability for each response was calculated using the following methods (Lewis et al., 1999).

For the particle size the formulations that have value within the range 10-100μm have a desirability function of 1, while the formulations that have values out of this range have a desirability value of 0. These can be described by the following equations:

$d_1 = 0$ for $Yi < Ymin$

$d_1 = 1$ for $Ymin < Yi < Ymin$

$d_1 = 0$ for $Yi > Ymax$

where d_1 is the individual desirability of the particle size.

The % drug entrapment, mucoadhesive strength and effective permeability were maximized in the optimization procedure. The desirability functions of these parameters were calculated by using the following equation:

d_2, d_3 and d_4 = Yi-Ymin/Ymax-Ymin

where d_2 is the individual desirability of % drug entrapment, d_3 is the individual desirability of mucoadhesive strength and d_4 is the individual desirability of effective permeability.

The values of Ymax and Ymin for % entrapment are 88.7% and 47%, Ymax and Ymin for mucoadhesive strength are 5705.22293dyne/cm^2 and 1460.636943 dyne/cm^2 and Ymax and Ymin for effective permeability are 8.4×10^{-5} cms^{-1} and 5.51×10^{-5} cms^{-1}, Yi is the experimental result The overall desirability values were calculated from the individual values by using the following equation:

$D = (d_1 d_2 d_3 d_4)^{1/4}$

5a.4. RESULTS AND DISCUSSION

5a.4.1. Preparation of chitosan glutamate microspheres by emulsification crosslinking method

Preliminary studies revealed that in absence of span-85 in the external phase led to aggregation of chitosan glutamate microspheres and no discrete microspheres were formed. A thin jelly like material was obtained, which on drying was converted into hard unbreakable lumps. This indicated that there is an agglomeration of the chitosan glutamate microdrops in the internal phase of the emulsion. Hence 2% span 85 was added in the external phase to produce discrete microspheres

5a.4.1.1. Entrapment efficiency and particle size

For determining the entrapment efficiency, it was necessary to degrade the matrix of the microspheres so that the total drug associated with the microspheres could be determined. For degradation, microspheres were incubated with 0.1N hydrochloric acid for 24 hours. The effect of various formulation and process parameters on microsphere formation, drug entrapment and particles size during preliminary screening was as follows:

5a.4.1.1a.Effect of stirring speed

To study the effect of stirring speed on the entrapment efficiency and particles size, the microspheres were prepared using three different stirring speeds viz. 1500rpm, 2500rpm and 3500rpm. As shown in Table 5a.2, with an increase in the stirring speed from 1500rpm to 2500 rpm, there was a decrease in the particle size with more uniform distribution from 19.43 to 15.27. With further increase in the stirring speed to 3500 rpm, there was no significant decrease in the particles size. Thus, 2500 rpm was selected as an optimum stirring speed. The stirring speed does not have any influence on the entrapment efficiency of the microspheres.

Table 5a.2 Effect of stirring speed on the entrapment efficiency and particle size of sumatriptan succinate loaded chitosan glutamate microspheres

Stirring speed (rpm)	% Entrapment efficiency	Particle size(μm)
1500	66.3 ± 1.7	19.43 ± 1.32
2500	65.8 ± 2.2	15.27 ± 0.45
3500	63.7 ± 1 4	14.86 ± 0.51

Chitosan concentration: 2%

Drug loading: 35%

Volume of glutaraldehyde: 16.25 μl

5a.4.1.1.b. Effect of volume ratio of water: oil phase

Three different ratios of volume of water: oil phase, 1:5, 1:10 and 1:20. The microspheres were aggregated in the case of 1:5 ratio and were found to be discrete in case of other two volume ratios. However, there was no significant difference in particle size and entrapment efficiency of microspheres prepared using 1:10 and 1:20 water: oil phase ratio. Thus, 1:10 was selected as an optimum ratio of volume of the water: oil phase.

5a.4.1.1.c. Effect of composition of external phase

Four different compositions of external phase were used to study the effect of the parameter on the entrapment efficiency and particle size. Light liquid paraffin, heavy liquid paraffin, mixture of light and heavy liquid paraffin (1:1) and soyabean oil were used. Light and heavy liquid paraffin as an external phase, resulted in aggregates of microspheres and hence were not used further. Use of mixture of light and heavy liquid paraffin (1:1) and soyabean oil gave discrete microspheres. Particle size of the microspheres obtained using soyabean oil as external phase was lower and uniform as compared to other phase compositions. As shown in Table 5a.3, % encapsulation efficiency was not affected by change in the external phase. Thus soyabean oil was selected as an ideal external phase for the preparation of chitosan glutamate microspheres.

Table 5a.3 Effect of composition of external phase on the entrapment efficiency and particle size of sumatriptan succinate loaded chitosan glutamate microspheres

External phase	% Entrapment efficiency	Particle size(μm)
Light.heavy liquid	64.7 ± 1.9	17.84 ± 1.07
Soyabean oil	65.8 ± 2 2	15.27 ± 0.45

Chitosan concentration: 2%

Drug loading: 35%

Volume of glutaraldehyde: 16.25 μl

5a.4.1.1.d. Effect of emulsification time

Three different emulsification times were chosen to study the effect of emulsification time on the characteristics of the microspheres. There was no significant effect of emulsification time on the entrapment efficiency of the microspheres It was found that increase in emulsification time from 10 minutes to 15 minutes led to a decrease in particle size from 20 43μm to 15.27μm. Further increase in emulsification time from 15 to 20 minutes did not affected particle size significantly, thus 15 minutes was chosen as an optimum emulsification time.

5a.4.1.2. Effect of chitosan glutamate concentration, drug loading and volume of crosslinking agent

A 3^3 factorial design was used to investigate the combined effect of three different variables in the preparation of chitosan glutamate microspheres loaded with sumatriptan succinate. Chitosan glutamate concentration, drug loading and volume of crosslinking agent were selected as independent variables and its effect on dependent variable particle size, % drug entrapment; mucoadhesive strength and effective permeability were studied. Potential variables such as stirring speed, volume ratio of water: oil phase, composition of external phase, emulsification time and concentration of span 85 were kept constant in the experimental design based on the preliminary screening.

Factors/levels					Responses				Overall desirability
Formulation code	ES[a]	Polymer concentration (%w/v)	Drug loading (%w/w)	Crosslinking agent volume (µl)	Particle size µm	Drug entrapment %	Mucoadhesive strength dyne/cm^2	Effective permeability cm/sec	
1S	9	1 5	20	7 5	9 64	53 9	5705 22	6 47E-05	0 000
2S	23	1 5	20	16 25	7 52	47	5592 87	6 90E-05	0 000
3S	6	1 5	20	25	10 93	60	4381 91	7 56E-05	0 537
4S	11	1 5	35	7 5	10 91	58 6	5418 09	6 34E-05	0 400
5S	1	1 5	35	16 25	11 93	58 7	2771 46	6 26E-05	0 421
6S	4	1 5	35	25	12 37	70 4	1872 61	6 15E-05	0 331
7S	20	1 5	50	7 5	11 41	66 7	3071 08	6 12E-05	0 000
8S	30	1 5	50	16 25	12 38	60 9	1697 83	5 98E-05	0 237
9S	32	1 5	50	25	12 53	71 5	1460 64	5 79E-05	0 000
10S	15	2	20	7 5	14 05	58	4331 97	6 78E-05	0 536
11S	5	2	20	16 25	13 09	61 8	4319 49	6 28E-05	0 500
12S	29	2	20	25	12 68	55 2	4269 55	6 37E-05	0 389
13S	25	2	35	7 5	14 72	70 4	3121 02	6 42E-05	0 509
14S	24	2	35	16 25	15 27	65 8	3058 60	6 02E-05	0 396
15S	13	2	35	25	17 13	68 2	2821 40	5 92E-05	0 264
16S	26	2	50	7 5	16 64	73 2	1947 77	6 09E-05	0 289
17S	22	2	50	16 25	16 79	82 2	1835 16	5 51E-05	0 000
18S	14	2	50	25	17 93	75 5	1747 77	5 98E-05	0 360
19S	3	2 5	20	7 5	19 54	75 6	5592 87	8 40E-05	0 909
20S	19	2 5	20	16 25	18 77	72 6	5505 22	7 57E-05	0 809
21S	8	2 5	20	25	18 02	75 1	4494 27	7 09E-05	0 611
22S	16	2 5	35	7 5	20 07	88 2	5418 09	7 59E-05	0 913
23S	17	2 5	35	16 25	18 93	80 2	4381 91	6 97E-05	0 704
24S	2	2 5	35	25	19 95	87 3	3282 31	7 01E-05	0 733
25S	12	2 5	50	7 5	19 63	88 5	5617 83	7 77E-05	0 933
26S	28	2 5	50	16 25	20 36	82 5	3308 28	6 76E-05	0 570
27S	10	2 5	50	25	20 13	88 7	3071 08	5 62E-05	0 345

Table 5a.4 Factorial 3^3 matrix of experiments and results for the measured responses and the desirability

[a] ES, experimental sequence

Table 5a.5 ANOVA results (P values): effect of the variable on various responses

Factors	Particle size		Drug entrapment		Mucoadhesive strength		Effective permeability	
	Coefficient	P	Coefficient	P	Coefficient	P	Coefficient	P
A	4.21	< 0 0001*	10.61	< 0.0001*	483.34	0.0052*	4 01E-06	< 0.0001*
B	1.31	< 0.0001*	7.25	< 0.0001*	-1135 33	< 0 0001*	-4.34E-06	< 0.0001*
C	0.28	0.1859	1 04	0.3023	-712.36	0 0002*	-2.50E-06	0 0018*
AB	-0.37	0.1575	-0.15	0.9022	487.94	0.0173*	9 81E-08	0 9071
AC	-0.42	0.1124	-1.99	0.116	58 18	0.7569	-3.85E-06	0.0002*
BC	0.38	0.1509	0 37	0.7589	-156.07	0.4104	-1.65E-06	0.0624
A^2	-0.089	0.8049	3.66	0.0463*	985.45	0.0015*	6.45E-06	< 0 0001*
B^2	-0.59	0.1156	-2.59	0.1455	203 32	0.4476	9.36E-07	0.4349
C^2	0 45	0.215	3.42	0.0603	149.1	0.5761	1.65E-06	0.1762
Constant	15.45	-	67 26	-	2815.35	-	5.98E-05	-
r^2	0 97	-	0.92	-	0 87	-	0.90	-

Regression coefficient, * statistically significant (P<0.05)

5a.4.1.2.1 Particle size

From the results in Table 5a.5 it can be concluded that the effects of the polymer concentration and % drug loading on the particle size were found statistically significant (P<0 05). The results obtained from the Table 5a.4 show that the largest microspheres were obtained at the high level of the polymer concentration. Especially the batches with larger particle size were produced with increase in polymer concentration, followed by increased drug loading. However, the concentration of the crosslinking agent did not significantly affect the particle size. The increase in the particle size observed with an increase in the polymer and/or drug concentrations could be attributed to an increase in the relative viscosity of the medium, which may have caused an increase in the interfacial tension. This resulted in the formation of larger globules during emulsification. Similar results were obtained by studies conducted by various research groups (Denkbas et al, 2002).

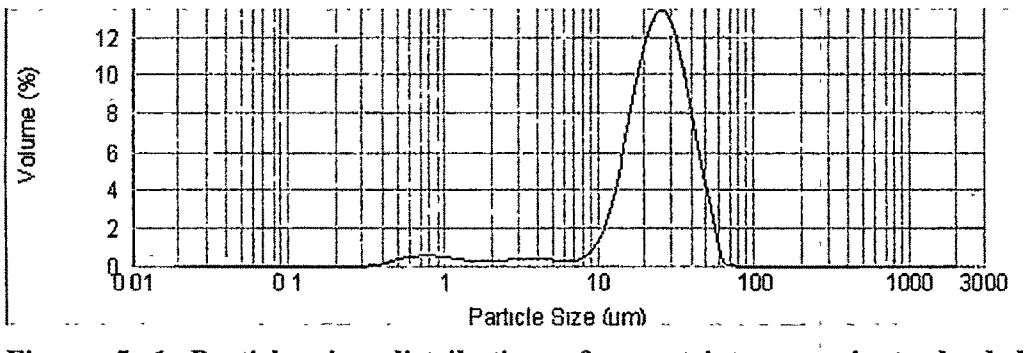

Figure 5a.1 Particle size distribution of sumatriptan succinate loaded chitosan glutamate microspheres

5a.4.1.2.2 Drug entrapment

As it could be seen from Table 5a.5, the drug entrapment was found to be significantly affected by polymer concentration and drug loading. The increase in drug entrapment with an increase in drug loading may be due to an increase in the drug concentration. Similar behavior with an increase in the polymer concentration may be attributed to increased viscosity, which results in the formation of larger microspheres, thus increasing the incorporation. Similar results were obtained by studies conducted by various research groups (Denkbas et al, 2002). The method adopted for the preparation of microspheres could be responsible for the observed higher incorporation efficiency. An aqueous solution of chitosan

glutamate containing drug was dispersed in an oil phase to form a w/o type of emulsion The addition of crosslinking agent will result in the formation of gel instantaneously and entrapping drug in the resultant matrix of the cross-linked chitosan glutamate.

5a.4.1.2. 3 Mucoadhesive strength

Mucoadhesion studies were carried out to ensure the adhesion of the formulation to the mucosa for a prolonged period of time at the site of absorption. The model used for mucoadhesive strength measurement was validated by studying effect of initial contact time of the tissue with formulation. 3 minutes was found to be optimum time to achieve maximum detachment stress. At lower contact time formulations did not have sufficient time to interact with mucosal membrane whereas increase in contact time greater than 3 min did not affected mucoadhesive strength further. The mucoadhesion studies indicated that the crosslinked chitosan glutamate microspheres showed very good mucoadhesion. As seen from Table 5a 5 mucoadhesive strength was found to be significantly affected by all the three factors, with polymer concentration exhibiting direct proportionality to mucoadhesive strength whereas drug loading and crosslinking agent volume exhibiting inverse relationship with mucoadhesive strength Increase in polymer concentration will result in the increased viscosity of the medium which will result in increased migration of polymer to the surface This would increase the probability of amino groups for binding with sialic acid residues at the mucosal membrane resulting in increased mucoadhesive strength. Increase in drug loading in the formulation will result in reduced availability and accessibility of amino groups of chitosan glutamate responsible for mucoadhesion at the site of interaction with mucosal membrane, thus increased drug loading will result in reduced mucoadhesive strength Aldehyde groups of glutaraldehyde forms covalent imine bonds with the amino groups of chitosan, due to the resonance established with adjacent double ethylenic bonds via a Schiff reaction. Although the highest amount of glutaraldehyde added was very less resulting in very low crosslinking density, addition of increasing volume of glutaraldehyde will result in decrease in number of free amino group. Thus keeping all other parameters constant number of free amino groups oriented towards mucosal membrane will reduce with increase in crosslinking agent volume and thus mucoadhesive strength will be decreased

5a.4.1.2. 4 Effective permeability

It has been proposed that chitosan salts open the tight junctions in a reversible way (Artursson et al , 1994; Kotze et al , 1997) Chitosan acts primarily by an interaction between the positively charged amino groups on the C-2 position with negatively charged sites on the cell membranes and tight junctions to allow for opening of the tight junctions Chitosan glutamate most probably allows for the paracellular transport of hydrophilic compounds by an indirect mechanism, whereby the integrity of the tight junctions is altered by changes in intracellular F-actin (Artursson et al., 1994). It is also known that pharmacological agents which interact with cytoskeletal F-actin simultaneously increase the paracellular permeability (Meza et al , 1982). This is in agreement with the hypothesis that F-actin is directly or indirectly associated with the proteins in the tight junctions (Madara, 1987). As seen from the results in Table 5a.5, all the three factors, i.e. polymer concentration, drug loading and crosslinking agent concentration were found to be significantly affecting effective permeability of sumatriptan succinate across sheep nasal mucosa Increase in drug loading in the formulation will result in reduced availability and accessibility of groups of chitosan glutamate responsible for tight junction opening at the site of interaction with mucosal membrane, thus effective permeability of the drug was inversely proportional to drug loading in the formulation. Polymer concentration exhibited linear relationship with effective permeability, this effect could be explained by the fact that the increased viscosity of the microsphere preparative mixture hinders drug migration towards the external surface during microsphere preparation resulting in comparatively less amount of surface associated drug and more amount of chitosan glutamate on the surface and hence more number of reactive groups are available and oriented towards mucosal membrane for opening of tight junction. Volume of crosslinking agent exhibited inverse relationship with effective permeability, with increase in volume of crosslinking agent. Although the highest amount of glutaraldehyde added was very less resulting in very low crosslinking density, addition of increasing volume of glutaraldehyde will result in decrease in number of free amino group as discussed in previous section Thus increase in crosslinking agent volume will result in reduced effective permeability.

5a.4.1.3. Interactions between the factors

An interaction is the failure of a factor to produce the same effect on the response at the different levels of the other factor (Montgomery, 1999). The ANOVA results (Table 5a.5) showed that the interaction AB had significant influence on the mucoadhesive strength of the microspheres. Especially this interaction was synergistic, as it led to an increase in the mucoadhesive strength The interaction AC was found to have significant influence on effective permeability. Also polynomial factor A^2 was found to have statistically significant influence on drug entrapment, mucoadhesive strength and effective permeability The analysis of the results of the Table 5a 4 by multiple regression analysis leads to equations that adequately describe the influence of the selected factors on the particle size, % drug entrapment, mucoadhesive strength and effective permeability of the formulations. In the Table 5a.5 regression coefficients of these equations are presented.

5a.4.1.4. Optimization of the process using the desirability function

Generally the aim of the optimization of pharmaceutical formulations is to find the optimum levels of the variables, which affect a process, where a product of good quality characteristics could be produced. During the optimization procedure, all the measured responses that may affect the quality of the product should be taken into consideration. Some of these responses have to be minimized and some have to be maximized, in order to produce a product of desired characteristics Using the desirability function, all the selected responses were combined in one overall response, the overall desirability. As it has been already discussed the overall desirability response was calculated from the individual desirability of each of the responses The results of each of these overall desirability responses are included in the optimization procedure. Desirability function was utilized to find out the best batch out of 27 batches. Batch 25S showed the highest overall desirability of 0.933. Therefore, this batch was considered to be the best batch and the values of independent variables of this batch were considered to be optimum values for the preparation of microspheres The final formulation of microsphere containing sumatriptan succinate is given in Table 5a.6.

Table 5a.6 Final formulation of microsphere containing sumatriptan succinate

Independent variable	Optimum values
Chitosan glutamate concentration(%w/v)	2 5
% Sumatriptan succinate loading(%w/w)	50
Volume of glutaraldehyde(µl)	7 5

Response surface plots were obtained for the measured response based on the model using Sigma Plot software. The relationship between the independent variables and the response can be further explained by using these plots. Figures 5a.2,5a.3 and 5a.4 show the response surface plot for overall desirability as a function of any two factors among A,B and C, while the other factor is kept constant The study of these plots showed that the highest values for the desirability could be obtained at higher values of chitosan glutamate concentration and lower values of the crosslinking agent volume. Addition of increased amount of drug resulted in higher overall desirability. In order to assess the reliability of the equations that describe the influence of the factors on the microspheres characteristics, five additional experiments were conducted by varying the three independent variables. For each of these test formulations the responses were estimated by using the equations and the experimental procedure. In Table 5a.7 the comparison between the experimental and predicted values of the responses for the additional experiments is presented. It can be seen that in all cases there was a reasonable agreement between the predicted and the experimental values, since low values of the bias were found. For this reason it can be concluded that the equations describe adequately the influence of the selected independent variables on the responses under study.

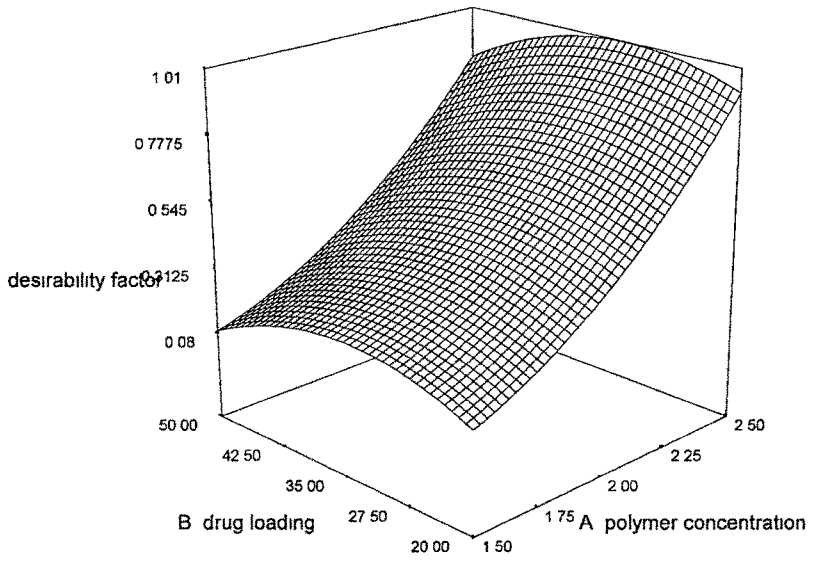

Figure 5a.2 Effect of polymer concentration and drug loading on overall desirability function (crosslinking agent volume=7.5µl)

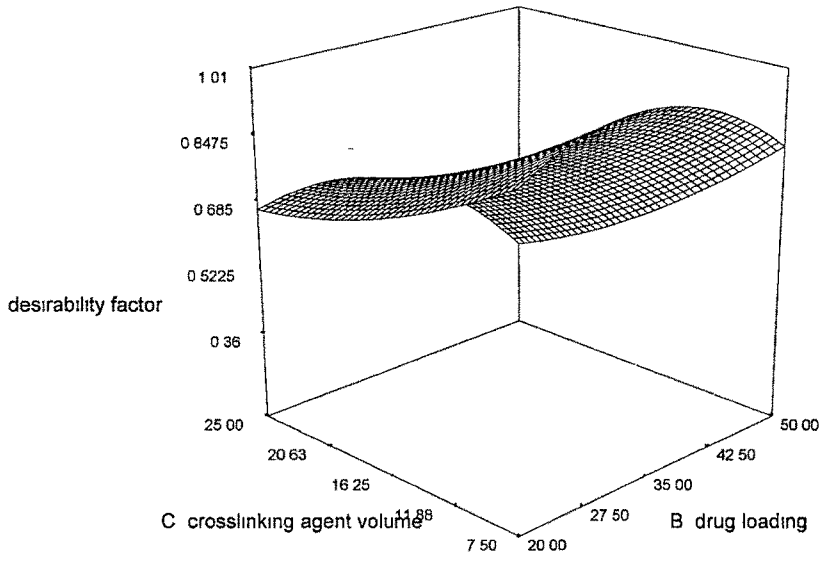

Figure 5a.3 Effect of drug loading and crosslinking agent volume on overall desirability function (polymer concentration=2.5%)

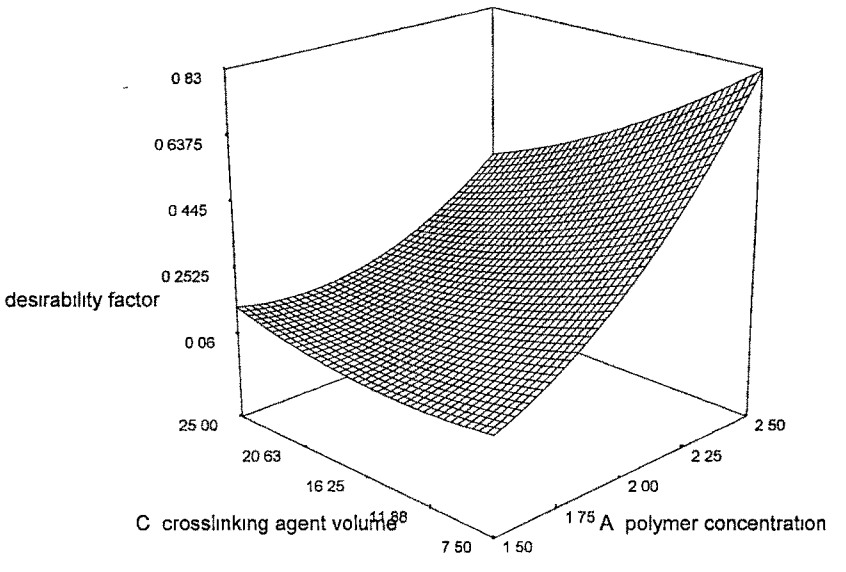

Figure 5a.4 Effect of polymer concentration and crosslinking agent volume on overall desirability function (drug loading=50)

Table 5a.7 Comparison between predicted and experimental values for the test formulations

Responses	Test	Factors/levels			Predicted values	Experimental values	Bias%*
		A	B	C			
Particle size µm	1	+1	0	-1	20.166	20.130	0.2
	2	+1	-1	+1	17.993	18.100	0.6
	3	+1	+1	0	19.929	20.221	1.5
	4	-0.6	0.2	0.6	13.706	13.650	0.4
	5	-0.4	0.6	-0.2	14.302	14.012	2.0
Drug entrapment %	1	+1	0	-1	85.895	87.200	1.5
	2	+1	-1	+1	73.932	74.320	0.5
	3	+1	+1	0	86.032	83.650	2.8
	4	-0.6	0.2	0.6	66.194	64.326	2.8
	5	-0.4	0.6	-0.2	66.775	68.294	2.3
Mucoadhesive strength dyne/cm^2	1	+1	0	-1	5087.43	5321.09	4.6
	2	+1	-1	+1	4785.85	4561.17	4.7
	3	+1	+1	0	3840.08	3356.36	12.6
	4	-0 6	0.2	0.6	2189.22	2129.65	2.7
	5	-0.4	0.6	-0.2	2226.40	2156.25	3.2
Effective permeability cm/sec	1	+1	0	-1	7.83E-05	7.62E-05	2.6
	2	+1	-1	+1	7.24E-05	7.11E-05	1.7
	3	+1	+1	0	6.69E-05	6.64E-05	0.8
	4	-0.6	0.2	0.6	5.92E-05	5.83E-05	1.5
	5	-0.4	0.6	-0.2	5.74E-05	5.65E-05	1.6

*, Bias was calculated using the equation :{(predicted value-experimental value)/predicted value} x 100

5a.4.2 .Histopathological evaluation of mucosa

Effect of the formulation on structure of nasal membrane was studied after *in vitro* permeation study of 25S. The microscopic observations indicate that the optimized formulation has no significant effect on the microscopic structure of mucosa. As seen in figure 5a.5 neither cell necrosis nor removal of the epithelium from the nasal mucosa was observed after permeation of 25S. The epithelium layer was intact and there were no alterations in basal membrane and superficial part of sub mucosa as compared to PBS treated mucosa. Thus chitosan glutamate microsphere formulation seems to be safe with respect to nasal administration.

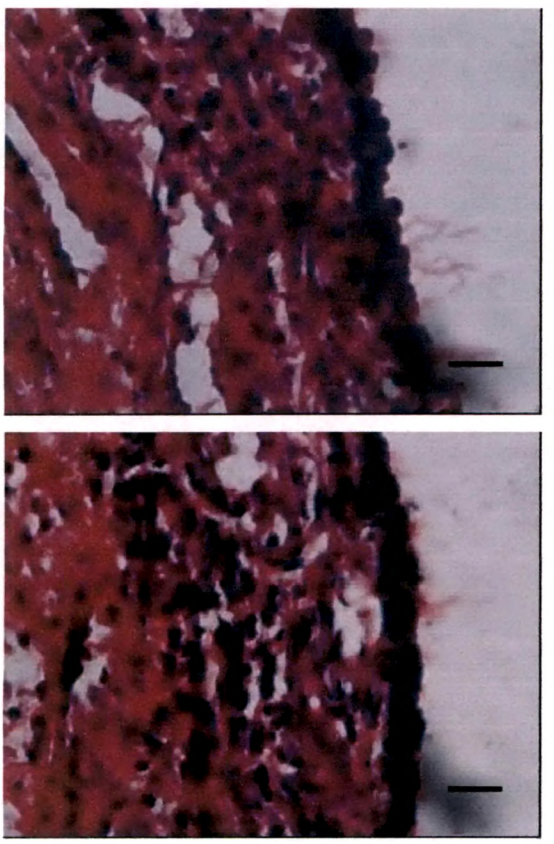

Figure 5a. 5 : Histopathological evaluations of sections of sheep nasal mucosal membrane (a) Mucosal layer after incubation with PBS (pH 6.4) in diffusion chamber (b) Mucosal layer after incubation in diffusion chamber with 25S formulation . (H x E), (line = 12.5μm).

5a.4. 3 Determination of residual glutaraldehyde

The residual glutaraldehyde in the microspheres was below quantification limit of the analytical method. Therefore glutaraldehyde, if present in the microspheres was less than 2.5 ppm (which is well below the permissible limit).

5a.4.4 Angle of repose

Angle of repose, representative of the flowability of the particles was38.66 for 25S. Angle of repose value for chitosan glutamate micrsopheres indicates its free flowing property.

5a.4.5 Morphological analysis

SEM micrographs of the 25S are given in Figure 5a.6. The microspheres exhibited spherical shape and smooth surface.

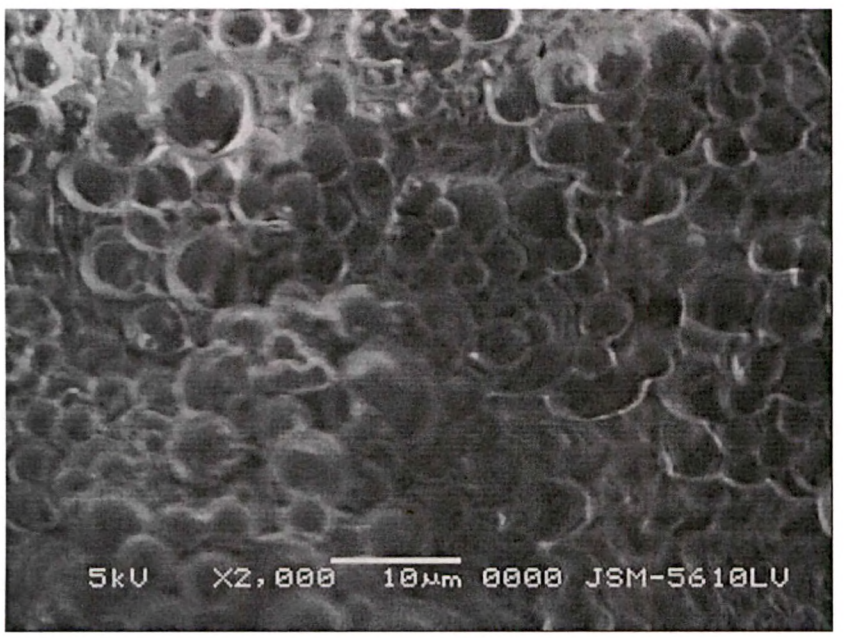

Figure 5a.6 Scanning electron micrograph of spray dried sumatriptan succinate loaded chitosan glutamate microspheres (25S).

5a.4.6 Infrared spectra

FT-IR spectra of chitosan glutamate is given in Figure5a.7 In the IR spectra a very broad band detected between 2856 and 3419 cm^{-1} , that can be attributed to the presence of OH groups stretching of carboxyl group and $-NH_2$ groups. Peak at 1313 cm^{-1} is due amide III band, characteristic of chitosan. Peak at 1076 cm^{-1} could be due to –C-O stretching vibration. Significant peak at 1660 cm^{-1} is due to –COO stretching vibration and amide vibration. The presence of band at 2856 cm^{-1} is due to the –C-H stretching vibrations. The FTIR spectra of sumatriptan succinate and sumatriptan succinate loaded chitosan glutamate a microsphere (25S) are given in figure5a.8 and 5a.9 respectively.

Broad band observed at 3272 cm^{-1} in the IR spectra of sumatriptan succinate correspond to –NH and –OH stretching vibrations. Sharp peak at 1647 cm^{-1} ,1299 cm^{-1} and 1120 $cm^{-1}$is due to –C=O stretching vibration, -CN stretching vibration and –S=O (sulphonamide) stretching vibration respectively. Sharp peak at 806 cm^{-1} is due to -C=C aromatic bending vibration. Prominent band between 2929 and 3419 cm^{-1}, corresponding to stretching vibration of –OH group and $-NH_2$ groups of chitosan glutamate still remained in the microspheres, band in this region is also found in sumatriptan succinate. Also peak 1319 cm^{-1} corresponding to chitosan glutamate was found in the microspheres. Other peaks observed in the microspheres were sharp peak at 1100 cm^{-1}, which could be attributed to sulphonamide stretching vibration (S=O), characteristic peak of sumatriptan succinate. Additionally, sharp peal at 1319 cm^{-1}, due to (C-N) stretching vibration of sumatriptan succinate was also observed Other characteristic peak found in the microspheres corresponding to drug was found at 1558 cm^{-1}(N-H deformation). IR spectra do no indicate any interaction between drug and polymer under preparation conditions Band at around 1700 cm^{-1} characteristic of aldehyde group was absent, indicating that there was no residual glutaraldehyde present in the microspheres.

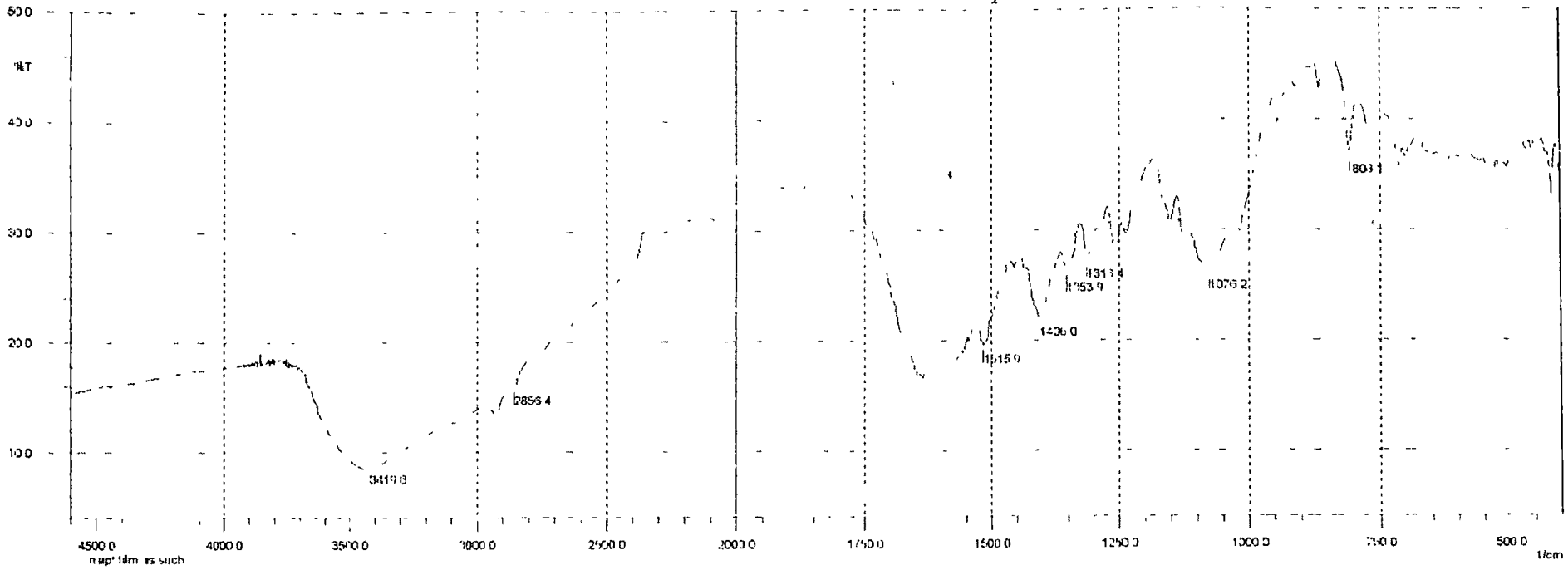

Figure 5a.7 Infrared spectra of chitosan glutamate

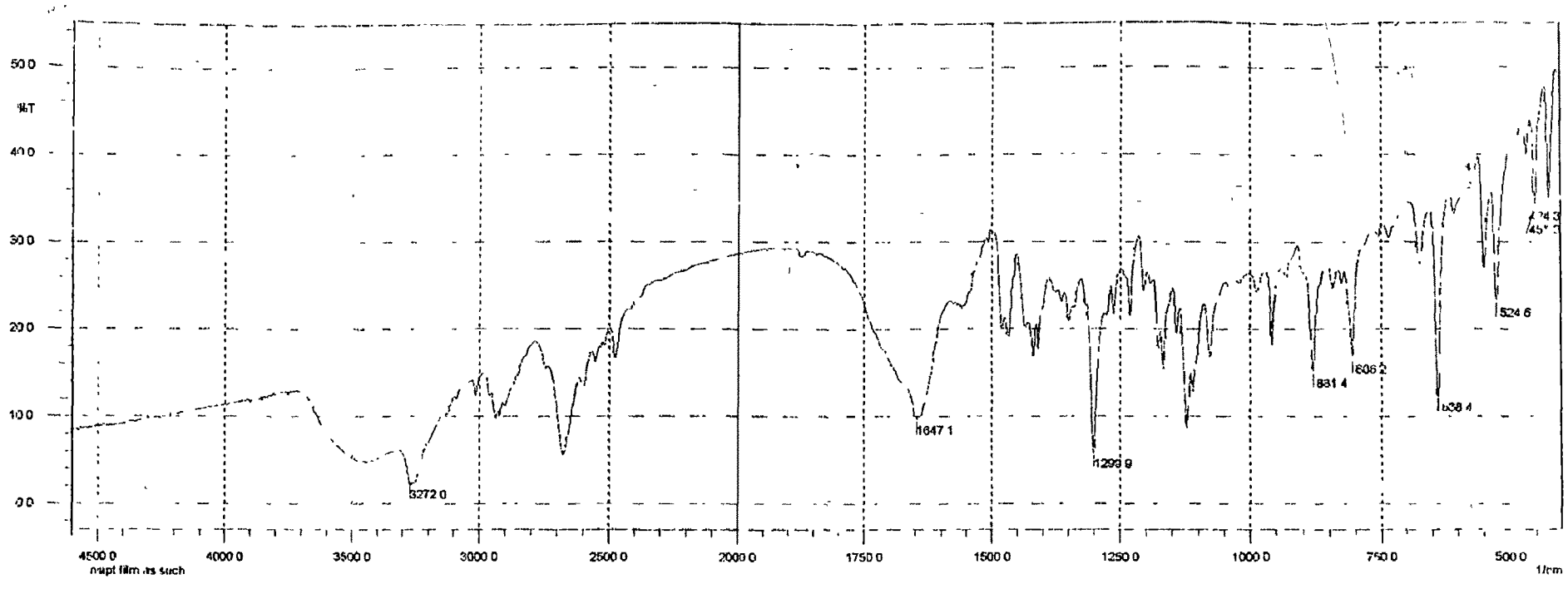

Figure 5a.8 Infrared spectra of Sumatriptan succinate

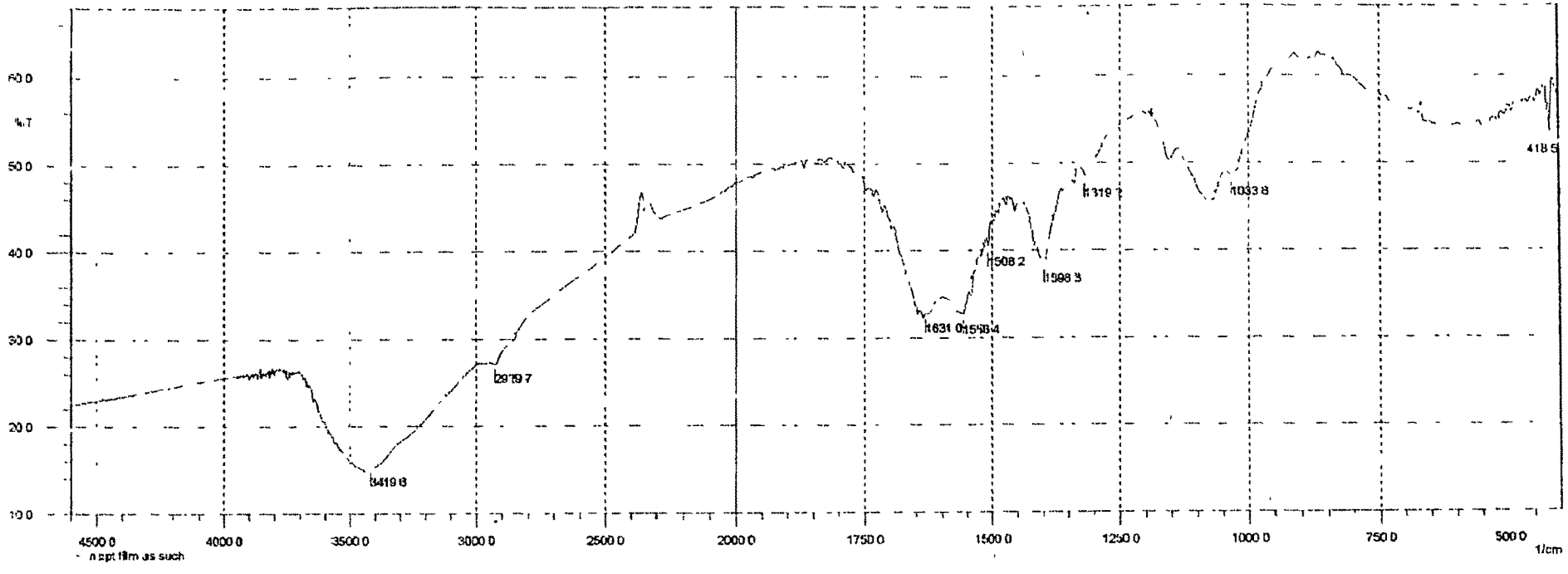

Figure 5a.9 Infrared spectra of Chitosan glutamate microspheres loaded with sumatriptan succinate (25S)

5a.4.7 Stability study

The results of the short term stability of the optimized formulation indicate that the prepared formulation is highly stable. The %entrapment efficiency for 25S changed from 88.5% (Initial) to 87.3 %(Room temperature for 6 months), also change in particle size was from 19.63μm (Initial) to 20.17μm (Room temperature for 6 months). Thus there was no significant change in drug entrapment or particle size of the microspheres on storage at room temperature for 6 months. Formulation remained in the form of free flowing powder which accounts for their excellent stability.

5a.5 CONCLUSION

The microspheres of sumatriptan succinate incorporated in chitosan glutamate were obtained by the emulsification crosslinking technique, it showed acceptable particle size range and satisfactory drug entrapment, mucoadhesive potential and increase in effective permeability. The prepared microspheres were smooth in surface without any interactions between drug and polymer. The multiple regression analysis of the results led to equations that describe adequately the influence of the selected variables on the responses under study. The desirability function led to the optimum values of the factors at which the produced microspheres showed most desirable characteristics. Chitosan glutamate microspheres also exhibited permeation enhancing effect across sheep nasal mucosal membrane. Thus, chitosan glutamate microspheres loaded with sumatriptan succinate could be effectively used in the treatment of migraine by intranasal route of administration, as it would retain the formulation for prolonged period of time at the site of absorption in the nasal cavity by reducing nasal clearance due to its mucoadhesive potential and also enhance absorption across nasal membrane with enhanced delivery to intracranially located target sites.

5b. CARBOPOL MICRSOPHERES

5b.1. OPTIMIZATION OF SPRAY DRYING PROCESS FOR CARBOPOL MICROSPHERES

5b.1.1. INTRODUCTION

Spray-drying is extensively used in the pharmaceutical industry to produce raw drug or excipients or drying of solutions, suspensions, or emulsions. Recently, the process received much attention for the preparation of various formulation including dried liposomes, microspheres, drying of preformed microcapsules, gastro retentive microspheres, and controlled-release systems (Palmieri et al, 2001). The growth in pharmaceutical spray drying is driven by a number of advantages over competing technologies including lower processing costs, ease and speed of scale-up, its ability to transform liquid feed into dry powder in a one step, continuous particle processing operation and its application to a wide variety of materials (Broadhead et al, 1992). The use of spray-drying has been regarded as a promising alternative to traditional techniques for the production of microspheres. Spray drying serves as a microencapsulation technique where an active material is dissolved or suspended in a melt or polymer solution.This solution/suspension is then fed into the spray-drying apparatus. Basically, the polymer/drug solution is mixed rapidly with air and forced through a small diameter orifice. Nebulization of the polymer/drug solution occurs at the nozzle and the resultant droplets are very quickly dried by evaporation (under high-pressure air) before collection. Significant advantages of using this technique include ability to handle labile materials because of the short contact time in the dryer, no residual surfactant on the surface of the microparticles; in addition, the operation is economical. Furthermore, it generally yields microparticles of narrow size distribution and characterized by high encapsulation efficiency (Giunchedi et al, 1998). Microsphere morphology, particle size and particle size distribution strongly depends on the production process. Parameters that can affect microparticle size and morphology include temperature of drying, air pressure, nozzle diameter and flow rate. Spray-drying technique has inconveniences related to processing variables that must be well controlled to minimize problems such as low yields, sticking, or high residual moisture content (Billon et al, 2000), which are particularly encountered with laboratory scale spray-dryers during process development and scale up. The driving

force for drying is controlled by the water content and the difference in the inlet and outlet temperatures of the drying air. Drying time of the droplets depends on the residence time of the droplets in the spray drier which, in turn, is determined by number of variables. It is important to note that all the parameters are closely interrelated and so, changing one process parameter would disturb other parameters set previously. Optimization of various process parameters is therefore very much important for obtaining maximum yield and overcoming problems like sticking, particularly with microparticles containing mucoadhesive polymers Hence prior to preparing drug loaded microspheres, spray drying process parameters were optimized for preparing placebo microspheres with maximum yield and particle size distribution.

5b.1.2 EXPERIMENTAL

5b.1.2.1.Carbopol Microsphere Preparation

Carbopol was hydrated in distilled water at concentrations of 0.005 g/mL. This solution were spray-dried by using JISL Mini Spray-Dryer, LSD48 model having 0.7 mm nozzle at 90/100°C inlet temperature, 80/100 cuft/min aspiration volume and 15/20 ml/min flow rate with air pressure 2.5kg/cm^2. These batches of spray-dried products were collected from cyclone reservoir. The collected powders were stored under vacuum in a desiccator.

5b.1.2.2. Experimental Conditions

Influence of spray drying process parameters on formulation of mucoadhesive carbopol microspheres was evaluated by employing 2^3 factorial design, whereby particle size and yield were considered as response variables Independent variables studied at lower and higher levels included the inlet temperatures, flow rate and aspiration volume. Two replicate runs for higher and lower value of each variable were conducted resulting in a total of 16 experimental runs. The experimental design matrix is shown in Table 5b.1. The results were analyzed statistically and graphs showing the magnitude of effects for each variable and interactions were generated by Stat-Ease software.

Table 5b.1. 2^3 Experimental Design

Sample № (replicate a and b)	A-Inlet Temperature°C	B-Aspiration volume cuft/min	C-Flow rate ml/min
1	90	80	15
2	100	80	15
3	90	100	15
4	100	100	15
5	90	80	20
6	100	80	20
7	90	100	20
8	100	100	20

5b.1.2.2a. Theory

The inlet temperature (A), aspiration volume (B) and Flow rate (C) were taken as independent variables to optimize spray drying process. The response parameters selected for analysis were %yield (Y1) and microsphere diameter (Y2). The magnitude of the main effect (ME) for each variable(X) on a particular response (Y) was calculated by the following formula

M.E.X =average (all high Y responses) - average (all low Y responses)

To calculate the effect of inlet temperature on the %yield, the average of all the yields at lower inlet temperature is subtracted from the average of all yields at higher inlet temperature. The same procedure can be applied to find the effect of flow rate and aspiration volume on both the responses i.e %yield and mean particle size.

The interactions between two variables on a specific response are found by the following procedure

Variable 1(A)	Variable 2 (B) Average Effect
At High A	$\frac{1}{2}[(B_{high} - B_{low})_1 + (B_{high} - B_{low})_2]$
At Low A	$\frac{1}{2}[(B_{high} - B_{low})_1 + (B_{high} - B_{low})_2]$

A × B Interaction = 1/2 (High Average Effect– Low Average Effect)

Interaction between inlet temperature and flow rate for the %yield can be calculated as given below

Flow rate	Inlet temperature Average Effect
At 20ml/min	½[(Y1№6 – Y1№5) + (Y1№8 – Y1№7)]
At15 ml/mın	½[(Y1№4 – Y1№3) +(Y1№2 – Y1№1)]

Interaction between inlet temperature and flow rate = 1/2 (20ml/min Effect– 15ml/min Effect)

where Y1 is the yield and the subscripts indicate the run numbers.

In addition, a three-factor interaction for %yield can be calculated. The interaction between inlet temperature and aspiration volume for the variable flow rate can be calculated as follows

A	B and C interaction
At High A	½[$(C_{high} - C_{low})_{B\,high} - (C_{high} - C_{low})_{B\,low}$]
At Low A	½[$(C_{high} - C_{low})_{B\,high} - (C_{high} - C_{low})_{B\,low}$]

A × B×C Interaction = 1/2 (High Average Effect– Low Average Effect)

Flow rate	Interaction between Inlet temperature and aspiration volume
At 20ml/min	½[(Y1№8 – Y1№7) - (Y1№6 – Y1№5)] = DH
At 15 ml/min	½[(Y1№4 – Y1№3) -(Y1№2 – Y1№1)] = DL

Interaction between inlet temperature, aspiration volume and flow rate = 1/2 (DH–DL)

Standard error was calculated based on a pooled variance(σ2) for 16 experiments which was in turn calculated by adding the sum of the squares of the differences between each replicate, for all eight conditions, and dividing by *N* {= 16}(Narayan et al, 2001).

5b.1.2.3. Characterization of Spray-Dried Samples

5b.1.2.3a. % Yield

The percentage of production yield (w/w) was calculated from the weight of dried microspheres (W_1) recovered from each of three batches and the initial dry weight of starting materials (W_2) as the following equation.

$$\Pr oductionYield = \left[W_1 / W_2 \right] \times 100$$

5b.1.2.3b. Particle Size Analysis

The particle size distributions of the prepared polymer microspheres were determined as described in section 5a.2 4.

5b.1.2.3c. Morphology Analysis

Morphological analysis of the microspheres was done as described in section 5a.2.9.

5b.1.3. RESULTS AND DISCUSSION

Spray drying is a solvent evaporation process. The solvent in the droplets is removed very quickly due to heat energy provided in the spray drier. The thermal efficiency of the spray drying is related to the heat energy input and the amount of heat used in the evaporation process. The optimum spray drying efficiency can be achieved from a balance of the two The yield of microspheres was expected to be greater at the high temperature due to rapid evaporation of solvent. At each run condition, the average yields of samples at inlet temperature of 100°C (samples 2, 4, 6, and 8) were higher than their respective counterparts (samples 1, 3, 5, and 7) where an inlet temperature was 90°C. Lower yield values at lower inlet temperature were possibly caused by adherence of the carbopol slurry onto the sides of the atomization chamber due to incomplete evaporation of the solvent. Particle size was also found to be marginally affected by inlet temperature, even though it is insignificant ($P<0$ 05). This increase in particle size at lower inlet temperature may be attributed to incomplete drying of the particles resulting in possible inter-droplet agglomeration. As evident from the data in Table 5b 2, aspiration volume was found to be contributing factor affecting yield however the effect on particle size was insignificant ($P<0.05$). The yield of microspheres for samples 3, 4, 7, 8 with higher aspiration volumes were lower as compared to their counterparts (samples 1, 2, 5, 6). This can be attributed to turbulence and recirculation patterns in the atomization flow as reported by Bodmeier (Bodmeier et al, 1988), at higher aspiration volume which forces droplets to collide prematurely with each other and with the sides of the chamber before drying. Samples 5, 6, 7, 8 with higher flow rate exhibited lower yield with larger particle size as compared to their counterparts (samples 1, 2, 3, 4) with lower flow rate Decrease in yield with increase in particle size at higher flow rate could be probably due to increased output of polymer slurry resulting in formation of larger droplet Increase in surface area of droplet

will result in inadequate drying, further resulting in collision and sticking to the walls of atomization chamber prior to drying.

Qualitative estimates of the influence of individual variables (inlet temperature, aspiration volume and flow rate) could be made by inspection of the data in Table 5b.2. However, it would be difficult to make predictions as to whether interactions actually existed between the variables, or which single variable had the most dominant effect. To achieve this, factorial design statistics were computed for all eight runs. The *ME* and interactions, calculated for the responses are listed in Table 5b. 3. The variables *A, B and C* represent inlet temperature, aspiration volume and flow rate, respectively. Interactions between two and among three variables are shown as *AB, BC, AC and ABC* The highest contributing variable for % yield as seen from the magnitude of effects is A and B, interactions among the variables except AB doesn't seem to have much effect on % yield. Similarly, flow rate(C) seems to be the only contributing factor, which influences particle size. Other variables and their mutual interaction do not seem to have prominent effect on particle size. Standard error was calculated for both the responses and its value for % yield was greater than particle size suggesting higher variability of data for % yield as compared to particle size.

Table 5b. 2. % Yield and Particle size results of experimental design

Sample.№	% Yield		Particle size(μm)	
	Mean	S.D.	Mean	S.D.
1	16 ±	1.27	5.46 ±	0.48
2	30 ±	1 98	5.3 ±	0.55
3	14 ±	1.56	5 92 ±	0.23
4	16.3 ±	0.85	5.73 ±	0.61
5	15.3 ±	0.85	7.03 ±	0.25
6	24 ±	2.4	6.98 ±	0.22
7	12.95 ±	1 06	7.21 ±	0.25
8	15.95 ±	1.56	7.17 ±	0.27

Table 5b. 3.Magnitude of effect of variables and interaction on response.

Variable	Effect for % Yield(Y1)	Effect for Particle size(Y2)
A	7*	-0.055
B	-6 53*	0.37
C	-2.03*	1.44*
A-B Interaction	-4.35*	0.05
B-C Interaction	1.33	-0.19
C-A Interaction	-1.15	0.013
A-B-C Interaction	1.5	-0 048
Standard Error	0.765	0.194

* Statistically significant ($p<0.05$)

In order to determine whether variables significantly effected response or were merely contributions from experimental error or noise, the effects on both %yield and particle size were plotted linearly on scaled normal plots generated by Stat-Ease software. Figure 5b.1 and 5b.2 shows the linearized normal probability plots of %yield and particles size respectively, against the magnitude of the variable effect. The points that lie directly on the straight line fall within the normal distribution of experimental noise, indicating a

negligible contribution to the response. Points that deviate considerably from the line are factors that cannot be explained simply as chance occurrences and are the variables actually affecting the responses.

Variable A, B, C and interaction AB are found to be contributing factors affecting % yield as they are deviating from the normal probability line in Figure 5b 1. Variable A seems to have maximum effect on % yield followed by variable B, interaction AB and variable C in descending order. Figure 5b.2 implies that the only contributing variable affecting particle size is C as no other point is found to be deviating from the normal probability line.

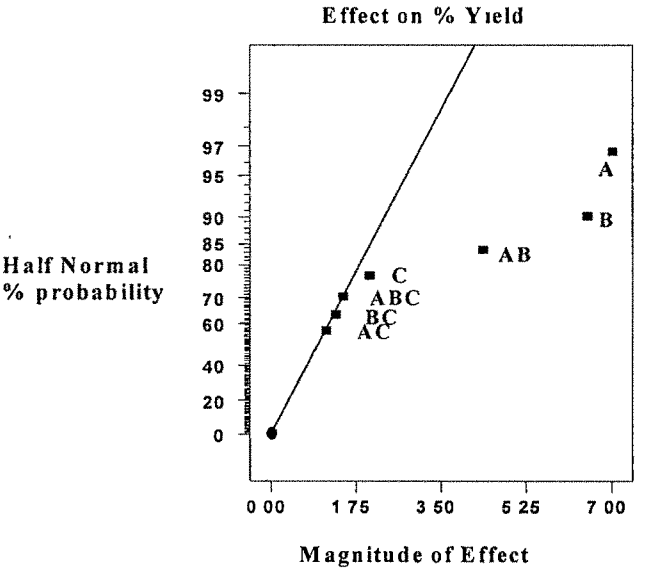

Figure 5b. 1. Linearized normal probability plot of the variable effects on % yield of spray dried microspheres. A = Inlet temperature, B= Aspiration volume, C= Flow rate.

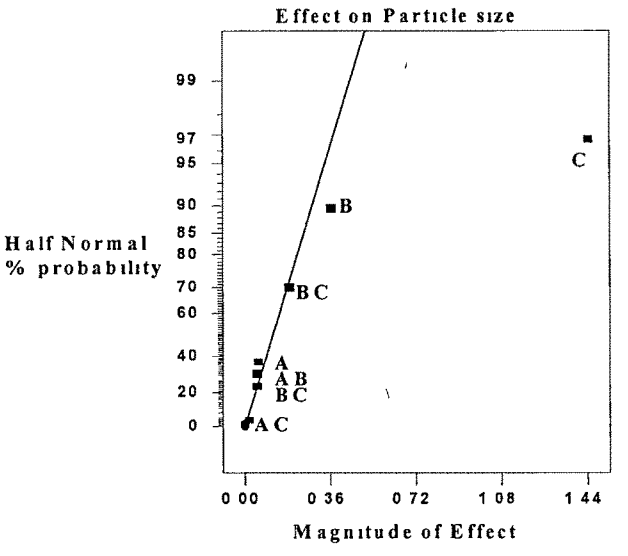

Figure 5b.2. Linearized normal probability plot of the variable effects on particle size of spray dried microspheres. A = Inlet temperature, B= Aspiration volume, C= Flow rate.

Cubical graph showing effects of variables on the responses were plotted using Stat-Ease software; this graph shows how three variables combine to affect the response. Variables are plotted as A (inlet temperature)-X axis, B (Aspiration volume)-Y-axis and C (Flow rate)-Z axis. Figure 5b.3 shows effect of variables on % Yield, cube clearly shows trend of increasing % yield with increasing inlet temperature, decreasing aspiration volume and flow rate. Figure 5b 4 shows trend of decreasing particle size with decreasing Flow rate. Maximum yield could be obtained at A+B-C- combination of variable, similarly lowest particle size could also be achieved using the same A+B-C- combination of variables.

From cubical graph analysis and analysis of run data, the best possible combination of spray drying conditions to achieve the highest yield with desired particle size for the particular system under study were inlet temperature of 100°C, aspiration volume of 80 cuft/min and Flow rate of 15ml/min and hence sample № 2 was found to be the most suitable Highest yield obtained with optimum spray drying process parameters is 30%, lower yield with spray drying process for small scale batch was expected as carbopol is highly hygroscopic material with adhesive properties, which has a natural tendency of adhering to the walls of cyclone separator and other glass chambers. Lower yield accounts for the loss of the particles that are adhered and difficult to collect from the chambers, moreover batch size used for optimization of the parameters was small which

resulted in % losses to be greater However, when we spray dried batch with 4 times higher volume than that used for factorial design study, at the optimum spray drying process parameters yield obtained was 53.7%. Thus although yield is lower for the small scale batch it increased with increase in batch size as % losses will reduced remarkably.

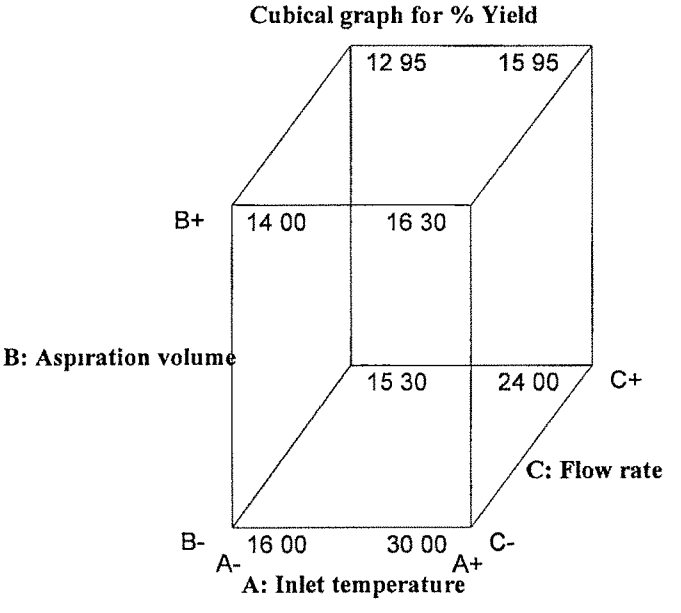

Figure 5b.3 Cubical graph showing effect of all the parameters on % Yield.

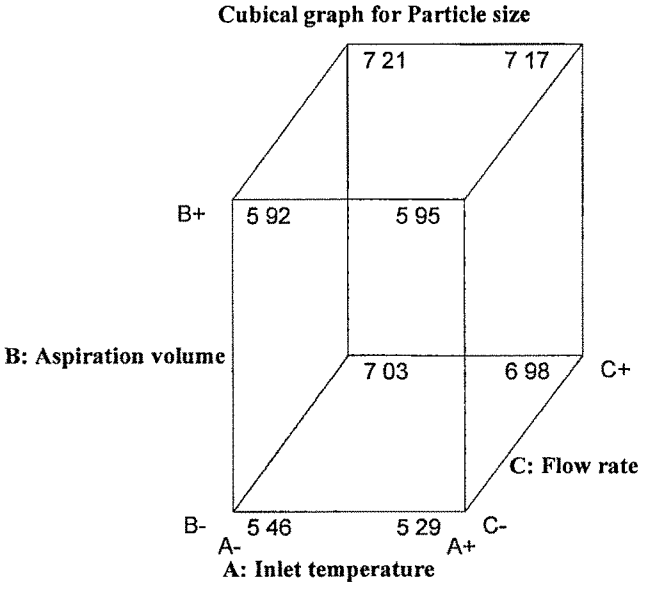

Figure5b.4. Cubical graph showing effect of all the parameters on Particle size.

5b.1.3.1. Morphological studies

Scanning electron micrograph studies of sample № 2 shows microspheres having a depressed surface morphology (slightly wrinkled surface).

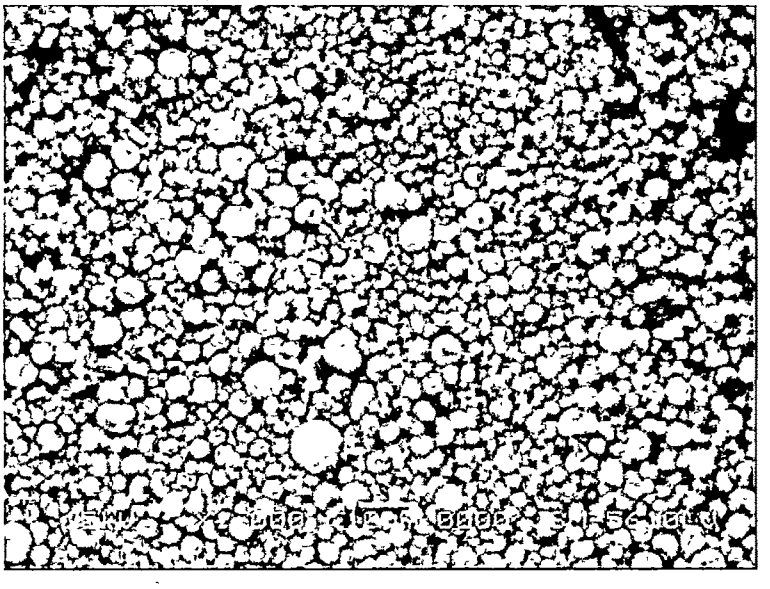

Figure5b.5. Scanning electron micrograph of spray dried microspheres, sample № 2.

5b.2.SUMATRIPTAN SUCCINATE LOADED CARBOPOL MICRSOPHERES

5b.2.1. INTRODUCTION

Carbopol microspheres containing sumatriptan succinate prepared by spray drying process could be one of the potential carriers for nasal drug delivery, because of their high mucoadhesive potential and permeation enhancing effect. These systems could be effectively used in the treatment of migraine particularly by intranasal route of administration, as it would retain the formulation for prolonged period of time at the site of absorption in the nasal cavity by reducing nasal clearance due to its mucoadhesive potential and also enhance absorption across nasal membrane with enhanced delivery to intracranially located target sites. This local transfer of sumatriptan to target sites will not only result in rapid onset of action but increased residence time of the formulation in the nasal cavity will result in highly effective antimigraine therapy. Particle properties being considerably important in such applications, the present part of the chapter reports a detailed study of effect of drug to polymer ratio on % yield, drug entrapment, particle size, mucoadhesive potential and permeation enhancing effect of the microspheres. Microspheres were also characterized for its surface morphology, angle of repose, infrared spectophotometry and toxicological effect on the nasal mucosal membrane

5b.2.2. EXPERIMENTAL

5b.2.2.1.Preparation of microspheres

Carbopol was hydrated in distilled water at concentrations of 0.005 g/mL sumatriptan succinate was dissolved at different percentages in the polymer solutions containing 5% iso propyl alcohol (Table 5b.4). This solution were spray-dried by using JISL Mini Spray-Dryer, LSD48 model having 0.7 mm nozzle at pre optimized spray drying process parameters of 100°C inlet temperature,100 cuft/min aspiration volume and 20 ml/min flow rate with air pressure 2.5kg/cm^2. These batches of spray-dried products were collected from cyclone reservoir. The collected powders were stored under vacuum in a desiccator.

5b.2.2.2.Characterization of the microspheres

A. Production Yield

Determined as discussed in section 5b.1.3.3

B. Drug loading

Drug content of sumatriptan succinate loaded microspheres was determined by drug determination following dissolution in water for 24 hours. Drug content was determined using UV spectrophotometer (Shimadzu UV, 1601) by measurement of absorbance at 283 nm by the method described in chapter 3. Encapsulation efficiency was calculated from the ratio of actual to theoretical drug content and expressed as a percentage. The analysis was performed three times for each batch of microspheres.

C. Particle size analysis

Particle size was determined as described in section 5a.2.4.

D. Evaluation of Mucoadhesive Strength

Mucoadhesive strength was determined as described in section 5a.2.5.

E. In Vitro Permeation Studies

In vitro permeation studies of the formulations were conducted as described in section 5a.2.6.

F. Statistical analysis

Data were expressed as mean with standard deviation (S.D). Statistical analysis of data was performed using ANOVA followed by Dunnett's Multiple Comparison test. A *p*-value of less than 0.001 was considered significant.

G. Histopathological evaluation of mucosa

Histopathological evaluation of tissue incubated in PBS (pH 6.4) after collection was compared with tissue incubated in the diffusion chamber with microsphere formulation (CS-3). Tissue was fixed in 10% buffered formalin (pH 7.2), routinely processed and embedded in paraffin. Paraffin sections (7 μm) were cut on glass slides and stained with hematoxylin and eosin (H & E). Sections were examined under a light microscope, to

detect any damage to the tissue during *in vitro* permeation by a pathologist blinded to the study.

H. Angle of repose

Angle of repose of the optimized formulation was determined as described in section 5a.2.8.

I. Morphology Analysis

Morphology analysis of the optimized formulation was done as discussed in section 5a 2.9

J. Infrared spectroscopy

FTIR spectral measurement of the optimized formulation was performed as described in section 5a.2.10.

K. Stability study

The optimized batch was subjected to stability studies. Formulation was stored in vials at room temperature for six months. The change in % entrapment efficiency and particle size was determined after 6 months.

5b.2.3.RESULTS AND DISCUSSION

Spray drying is a solvent evaporation process. The solvent in the droplets is removed very quickly due to heat energy provided in the spray drier. The thermal efficiency of the spray drying is related to the heat energy input (controlled by inlet temperature and blower) and the amount of heat used in the evaporation process. The optimum spray drying efficiency can be achieved from a balance of the amount of the energy input and the amount of the energy needed, which is related to the amount of the sample input. It is important to note that success of spray drying process depends on number of interrelated parameters. Changing a process parameter will therefore lead to a change in the others. Hence preliminary studies were done to optimize various process parameters like inlet temperature, feed rate and aspiration volume to achieve maximum yield and desired particle size. It was found that carbopol microspheres with desired particle size suitable for nasal administration with maximum yield can be prepared using optimized spray

drying conditions i.e. inlet temperature 100°C, Aspiration volume 80 cuft/min and flow rate 15ml/min.

5b.2.3.1.Characterization of microspheres

Sumatriptan succinate loaded microspheres were produced with high drug encapsulation efficiency (Table 5b.4.). Encapsulation efficiency ranged from 96.4% to 99.5%. Spray drying technique is generally characterized by high drug encapsulation efficiency (Conte et al, 1994). The encapsulation efficiency showed low standard deviation which implied good uniformity of drug distribution. The yield of production ranged from 54 to 61% (Table 5b 4) Similar percentages were obtained in some studies on spray drying technique as reported by Canan et al, Conte et al and Whitschi et al (Canan et al, 2003, Conte et al, 1999 and Whitschi et al 1999). This relatively low percentage depends on the technical characteristics of the spray dryer; much of the spray dried powder adhering to the cyclone walls is lost during spray drying. Considering the high encapsulation efficiency and sufficient production yield, it can be concluded that spray drying method is a simple and suitable technique for producing sumatriptan loaded carbopol microspheres.

A. Particle size

The particle size of microspheres ranged from 12.52-16.79 μm (Table 5b.4). Such particle size was considered to be suitable for nasal administration by insufflation. Figure 5b.6 shows particles size distribution of sumatriptan succinate loaded carbopol 934P microspheres. It was also noted that increasing the drug to polymer ratio slightly increased the size of microspheres upto 1:1 ratio, further increase in drug content reduced the particle size. It was also observed that addition of drug increased particle size drastically as compared to placebo microspheres. Placebo microspheres seemed to be hollow microspheres, which collapsed during evaporation of aqueous phase in spray drying process in absence of drug; however addition of drug resulted in better morphology with bigger particle size.

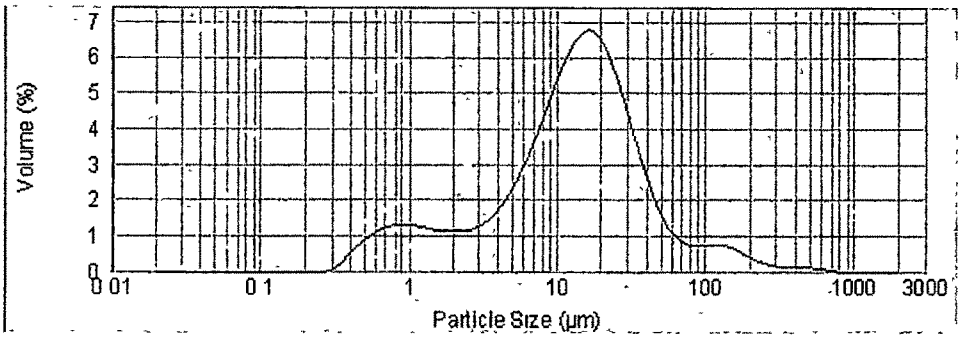

Figure 5b.6 Particle size distribution of sumatriptan succinate loaded carbopol 934P microspheres

Table 5b.4. Influence of Drug/polymer ratio of %Yield, encapsulation efficiency and particle size of spray dried carbopol microspheres

Formulation code	Drug/polymer ratio	Production Yield(% ± S D)	Theoretical Drug content (%w/w)	Actual Drug content (%w/w)(mean ±S D)	Encapsulation efficiency(%)	Particle size µm(mean ±S D)
CS1	1 03	55 ± 3 26	25	24 53 ± 2 01	98 1	14 05 ± 1 02
CS2	1 02	58 ± 2 98	33 3	33 10 ± 2 97	99 4	14 72 ± 1 37
CS3	1 01	61 ± 3 21	50	49 75 ± 3 66	99 5	16 79 ± 1 42
CS4	2 01	54 ± 2 22	66 7	64 30 ± 4 58	96 4	13 09 ± 1 06
CS5	3 01	56 ± 2 69	75	73 05 ± 4 32	97 4	12.52 ± 0 98

B. Mucoadhesive strength

The model used for mucoadhesive strength measurement was validated by studying effect of initial contact time of the tissue with formulation. 3 minutes was found to be optimum time to achieve maximum detachment stress. At lower contact time formulations did not have sufficient time to interact with mucosal membrane whereas increase in contact time greater than 3 min did not affected mucoadhesive strength further. Mucoadhesive strength in terms of detachment stress is given in Figure 5b.7. The results showed that the microspheres had good mucoadhesive properties and could adequately adhere on nasal mucosa. Earlier work with carbopol polymers has clearly indicated that it is the availability of hydrophilic functional group such as carboxyl groups that determines bioadhesion (Efentakis et al, 2000), carbopol has very high percentage (58% - 68%) of carboxylic groups that gradually undergoes hydrogen bonding with sugar residues in oligosaccharide chains in the mucus membrane resulting in formation of strengthened network between polymer and mucus membrane. Thus carbopol having high density of available hydrogen bonding groups would be able to interact more strongly with mucin

glycoproteins. It is speculated that the higher mucoadhesive strength of delivery system may lead to the prolonged retention and increased absorption across mucosal tissues (Kunisawa et al, 2000). The results showed that with increasing polymer ratio, the mucoadhesive strength increased. Mucoadhesive strength was significantly lower ($P<0.001$) for CS-4 and CS-5 compared to CS-1. It is also evident from the results that although mucoadhesive strength increased with increasing polymer concentration, increase in mucoadhesive strength for CS-3 and CS-2 was not significant ($P<0.001$) compared to CS-1 (increase in polymer concentration beyond 50%w/w). Similar results have been previously demonstrated by Vidgren et al (Vidgren et al, 1992). This could be probably due to limited availability of the space for the polymer chain of carbopol to extend within the mucus. Polymer structure is looser at concentration up to 50% w/w, and space available is sufficient for polymer chains to extend within the mucus. Hence, further increase in polymer concentration does not have any significant enhancement of mucoadhesive potential.

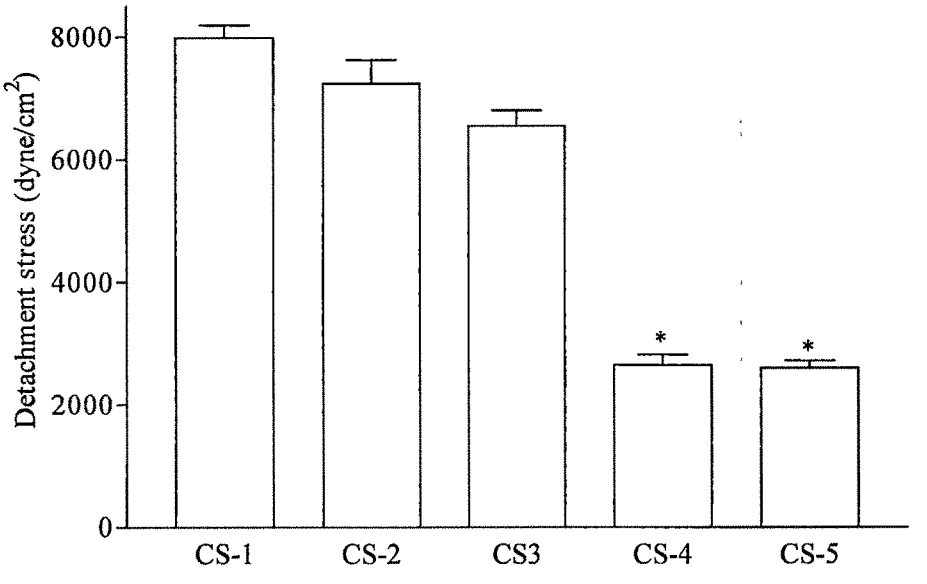

Figure 5b.7.Influence of Carbopol 934P concentration on the detachment stress measured *in vitro*, mean ± SD (n=3); $^{*}P < 0.001$ versus CS-1

C. In vitro permeation studies

Anionic polymers such as polycarbophil or carbopol are reported to demonstrate permeation enhancing properties. These polymers were shown to express a high Ca^{2+} binding ability. The depletion of Ca^{2+}ions from the extra cellular cell medium has been

shown to increase the permeation of sodium-fluorescein, bacitracin, a vasopressin analogue and insulin (Lueben et al, 1994 and Lueben et al, 1997).

Our previous experiments (described in chapter 4) studying effect of calcium chelator ethylene-glycol-bis-[β-aminoethylether]-N,N,N',N'-tetraacetic acid (EGTA) on permeability of sumatriptan across sheep nasal mucosal membrane exhibited increased permeability of sumatriptan in pure drug solution on exposure of membrane to EGTA, indicated by upward shift of time-permeation profile which was significant over the whole time period of 240 min. This study confirms importance of chelating calcium for enhancing permeability of sumatriptan. Permeation profiles and the effective permeability coefficient (peff) for pure drug solution and carbopol microspheres are given in Figure 5b 8 and Table 5b.5 respectively

Peff for CS-1, CS-2 and CS-3 was significantly increased ($P<0.001$) as compared to pure drug solution, while Peff was not significantly different for formulation CS-4 and CS-5. Presence of anionic polymer (50%w/w or above) dramatically increased permeation coefficient of the drug Presence of carbopol results in very rapid dissolution and release of highly soluble drug due to rapid swelling and dissolution of carbopol at pH 6.4. This could be attributed to increase in ionized carboxyl group with increasing concentration of carbopol. Increase in permeation of the drug from the formulation can be further explained on the basis that increase in carbopol concentration will result in increased number of carboxyl groups responsible for complexation of Ca^{2+}. Thus with increase in carbopol concentration number of Ca^{2+} binding affinity sites will increase and hence integrity of tight junctions will be altered to a greater extent further resulting in increased permeation of hydrophilic drug. However, it is also evident that increase in carbopol concentration greater than 50%w/w in the formulation (CS-1 and CS-2) further did not significantly increased permeation of drug compared to CS-3. As discussed earlier, space available for extending of the polymer chain is limited and 50% is sufficient concentration to extend polymer chain within the space available, further increase in concentration of the polymer did not significantly increased permeation as vicinity for Ca^{2+} binding sites did not increased further. Concerning, local tissue damage staining of sheep nasal mucosa with trypan blue after permeation studies with various formulations did not show any dead cells.

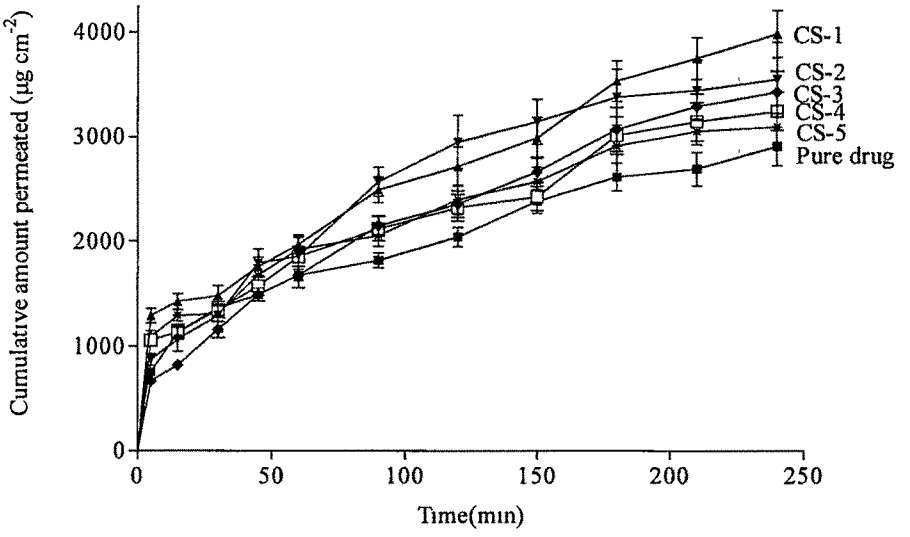

Figure5b.8. Cumulative amount of sumatriptan permeated across sheep nasal mucosal membrane from pure drug solution and from various carbopol microspheres formulations at 34°C in franz diffusion, data are expressed as mean ± S.D (n=3).

Table 5b.5.Effective permeability of various formulations across sheep nasal mucosa, data are expressed as mean ± SD (n=3);*$P < 0.001$ versus pure drug solution

Formulation code	Peff (x 10^{-5}) (cm s^{-1}) mean ± S.D
Pure solution	5.58 ± 0.365
CS1	7.98 ± 0.249*
CS2	7.95 ± 0.764*
CS3	7.91 ± 0.447*
CS4	6.42 ± 0.339
CS5	5.88 ± 0.369

Considering results obtained from above discussed characterization formulation CS-3 was considered to be the one exhibiting best possible combination of mucoadhesive strength and permeation enhancing effect. Increase in polymer concentration beyond 50 %w/w, apart from resulting in bulkier dosage form(which may not be ideal for nasal delivery system) had no significant advantage in terms of mucoadhesive potential or permeation enhancing effect. Hence, CS-3 with drug to polymer ratio (1:1) was selected for further studies.

D. Histopathological evaluation of mucosa

Effect of the formulation on structure of nasal membrane was studied after *in vitro* permeation study of CS-3. The microscopic observations indicate that the optimized formulation has no significant effect on the microscopic structure of mucosa. As seen in Figure 5b.9 neither cell necrosis nor was removal of the epithelium from the nasal mucosa observed after permeation of CS-3. The epithelium layer was intact and there were no alterations in basal membrane and superficial part of sub mucosa as compared to PBS treated mucosa. Thus carbopol microsphere formulation seems to be safe with respect to nasal administration.

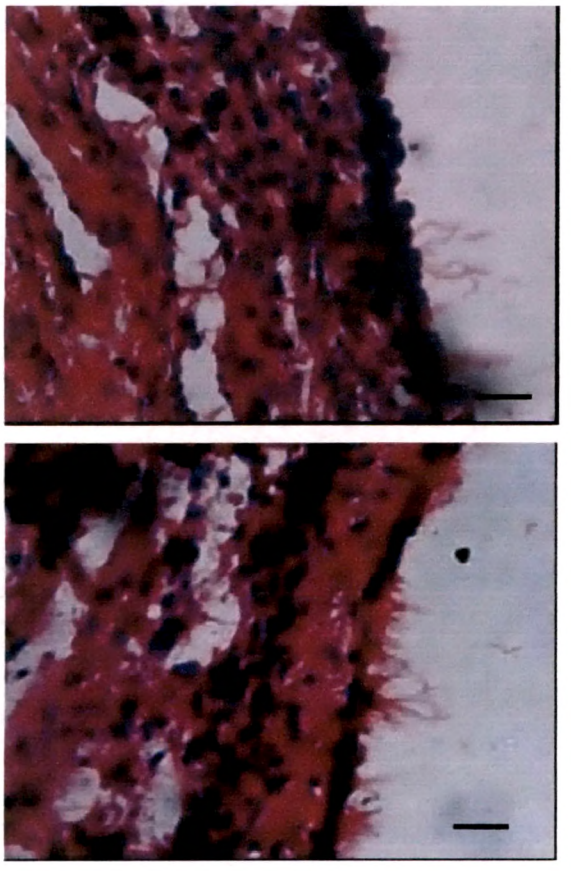

Figure 5b. 9 : Histopathological evaluations of sections of sheep nasal mucosal membrane (a) Mucosal layer after incubation with PBS (pH 6.4) in diffusion chamber (b) Mucosal layer after incubation in diffusion chamber with CS-3 formulation . (H x E), (line = 50μm).

E. Angle of repose

Angle of repose, representative of the flowability of the particles was 39.62 for CS-3. Angle of repose value for carbopol microspheres indicates its free flowing property.

F. Morphological analysis

SEM micrographs of the CS-3 are given in Figure5b.10. The microspheres exhibited spherical shape and smooth surface. Scanning electron micrographs of the preliminary

study revealed that empty microspheres (without drug loading) exhibited slightly crumpled surface resulting from the hollow microspheres. The results showed that the incorporation of drug affected the morphological characteristics of the spray dried microspheres extensively. With incorporation of drug, more spherical microspheres with smoother surface were obtained.

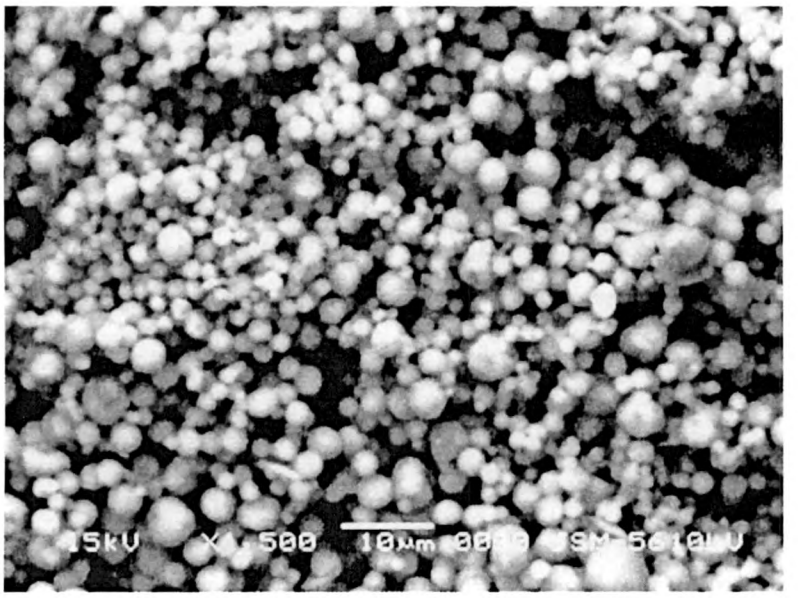

Figure 5b . 10. Scanning electron micrograph of spray dried sumatriptan succinate loaded carbopol microspheres (CS-3).

G. Infrared spectra

FT-IR spectra of Carbopol 934P is given in Figure5b.11 Carbopol 934P has the carbonyl stretching band of the carboxyl groups that are in mutual association at 1716 cm^{-1}.A very broad band detected between 2960 and 3463 cm^{-1}can be attributed to the presence of OH groups stretching of carboxyl group. FTIR spectra of sumatriptan succinate and sumatriptan succinate loaded carbopol a microsphere (CS-3) are given in figure5a.8(previous section) and 5b.12 respectively. Prominent peak at 1716cm cm^{-1}, corresponding to stretching vibration of carbonyl(C=O) bands still remained in the spray dried microspheres. Also broad band corresponding to hydroxyl stretching (O-H) corrsoponding to that of carbopol was found in the range of 2931-3367 cm^{-1}. Other peaks observed in the microspheres were sharp peak at 1159 cm^{-1}, which could be attributed to sulphonamide stretching vibration (S=O), characteristic peak of sumatriptan succinate. Additionally, sharp peal at 1309 cm^{-1}, due to (C-N) stretching vibration of sumatriptan

succinate was also observed. Other characteristic peak found in the microspheres corresponding to drug were peak found at 1558 cm^{-1}(N-H deformation) and at 806 cm^{-1}, which is due to (C=C) aromatic bending vibration of sumatriptan succinate. IR spectra do no indicate any interaction between drug and polymer under spray drying conditions

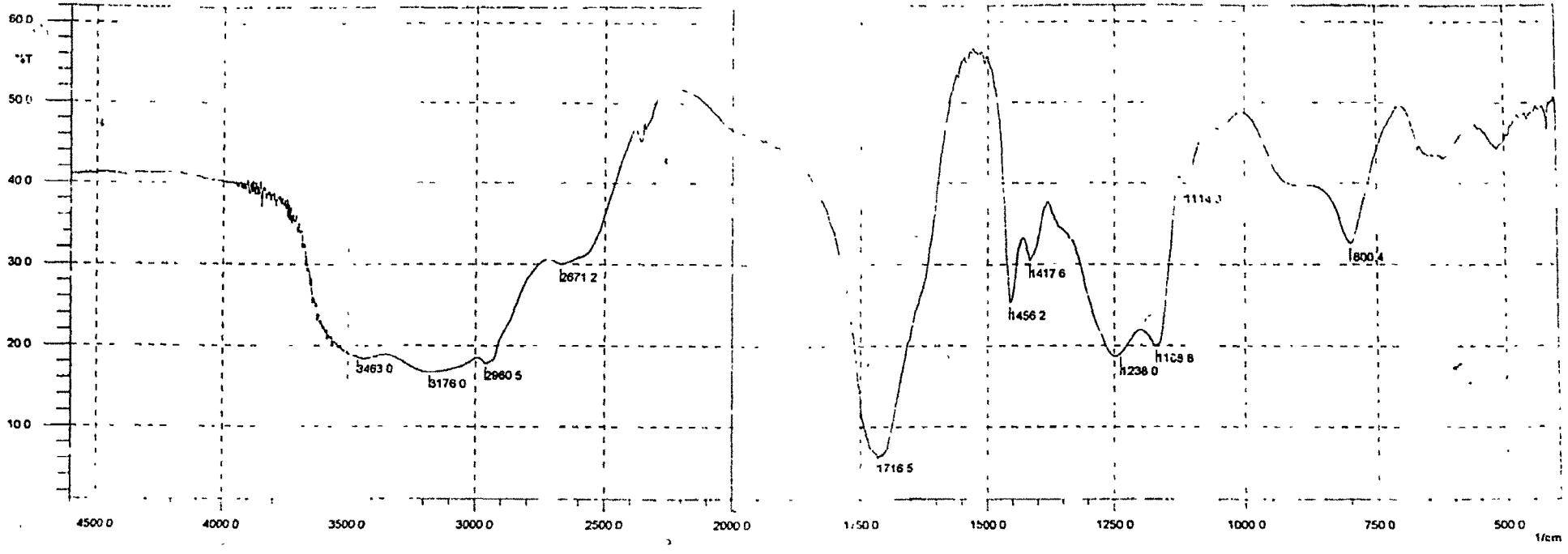

Figure 5b.11 Infrared spectra of carbopol 934P

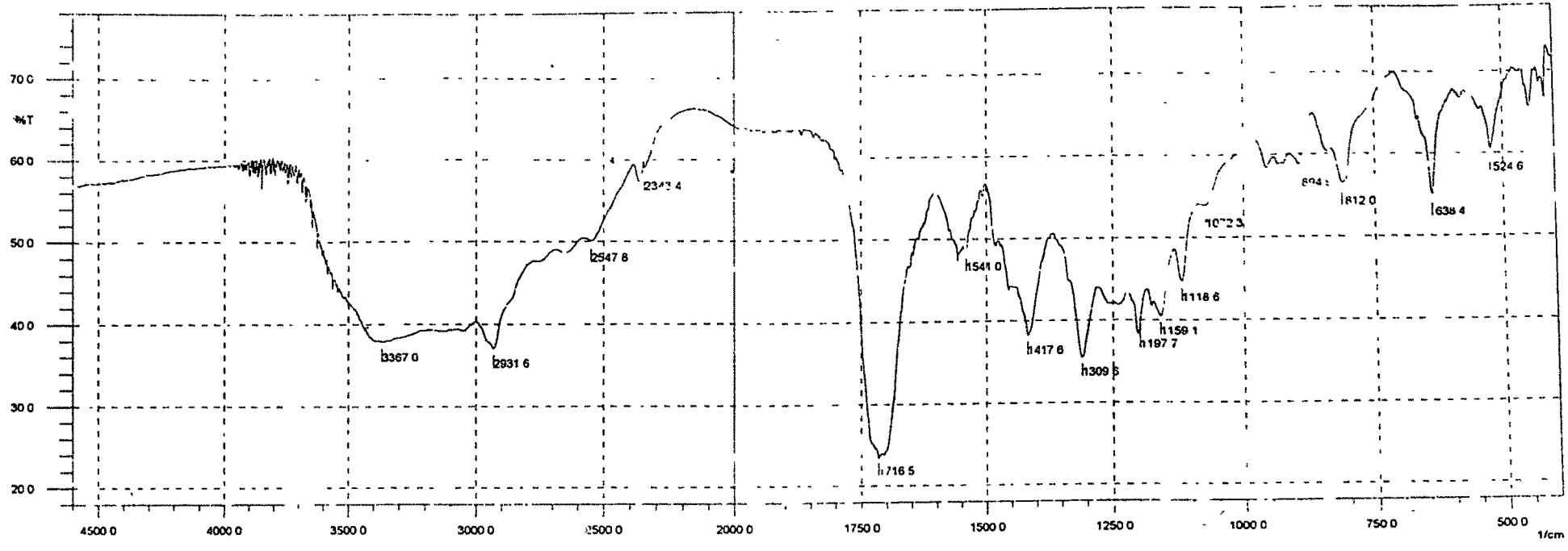

Figure 5b.12 Infrared spectra of carbopol 934P loaded sumatriptan succinate microspheres (CS-3)

H. Stability study

The results of the short term stability of the optimized formulation indicate that the prepared formulation is highly stable. The %entrapment efficiency for CS-3 changed from 99.5% (Initial) to 98.6 %(Room temperature for 6 months), also change in particle size was from 16.79 μm (Initial) to 17.21μm (Room temperature for 6 months). Thus there was no significant change in drug entrapment or particle size of the microspheres on storage at room temperature for 6 months. Formulation remained in the form of free flowing powder which accounts for their excellent stability.

5b.2.4. CONCLUSION

Factorial design proved to be a valuable technique for the production of spray-dried mucoadhesive microspheres. Use of factorial design minimized number of experiments for studying various parameters involved in the study, while still accomplishing a detailed evaluation of the dominant variable effects and interactions. Thus carbopol microspheres with desired particle size (5-20μm) suitable for nasal administration , maximum yield and required mucoadhesive strength can be prepared using optimum spray drying conditions i.e. inlet temperature 100°C, Aspiration volume 80 cuft/min and flow rate 15ml/min. Sumatriptan succinate loaded carbopol microparticles were prepared by varying drug to polymer ratio by optimized spray drying process. Microparticles obtained had desired particle size with good mucoadhesive strength that reduced with increase in drug content of the formulations. Carbopol microparticles also exhibited permeation enhancing effect across sheep nasal mucosal membrane. Thus, carbopol microspheres loaded with sumatriptan succinate microspheres could be effectively used in the treatment of migraine by intranasal route of administration, as it would retain the formulation for prolonged period of time at the site of absorption in the nasal cavity by reducing nasal clearance due to its mucoadhesive potential and also enhance absorption across nasal membrane with enhanced delivery to intracranially located target sites.

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