



## DEVELOPMENT OF MUCOADHESIVE MICROSPHERES FOR NASAL DELIVERY OF SUMATRIPTAN

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### ABSTRACT

In the present work mucoadhesive nasal microspheres containing sumatriptan were formulated and evaluated with the aim of increasing its bioavailability. Microspheres composed of Chitosan were obtained using emulsification cross linking method with different polymer: drug ratios. The prepared microspheres were evaluated for physical characteristics such as particle size, particle shape, surface morphology, drug entrapment efficiency, *in vitro* mucoadhesion, *ex-vivo* drug permeation studies and *in vivo* bioavailability. The particle size of the prepared microspheres was in the range of 46-68  $\mu\text{m}$ , which was suitable for nasal administration. SEM analysis of the microspheres revealed that all the prepared microspheres possess similar surface morphology. The *ex-vivo* permeation of sumatriptan succinate from chitosan microspheres exhibited a biphasic diffusion mechanism. Initially the microspheres showed immediate permeation of drug due the diffusion of non-encapsulated drug from the surface of microspheres followed by much slower diffusion of entrapped drug. *In-vivo* studies showed increased relative bioavailability with nasal route as compared to the oral route.

**Keywords:** Sumatriptan, Bioadhesion, Chitosan, Bioavailability.

### INTRODUCTION

Nasal drug delivery has frequently been proposed as the most feasible alternative to parenteral injections. This is due to the high permeability of the nasal epithelium, allowing a higher molecular mass cut-off at approximately 1000 Da, and rapid drug absorption rate with plasma drug profiles sometimes almost identical to those from intravenous injections<sup>1</sup>. Conventionally, the nasal route has been used for delivery of drugs for treatment of local diseases such as nasal allergy, nasal congestion and nasal infections. Recent years have shown that the nasal route can be exploited for the systemic delivery of polar drugs having low molecular weight peptides and proteins that are not easily administered via other routes than by injection. From the pharmacokinetic standpoint, absorption is rapid which provides a faster onset of action compared to oral and intramuscular administration. Hepatic first-pass metabolism is also avoided, allowing increased, reliable bioavailability<sup>2</sup>. Sumatriptan is an agonist for a vascular 5-hydroxytryptamine receptor subtype (probably a member of the 5-HT<sub>1D</sub> family)<sup>3</sup>. The vascular 5-HT<sub>1</sub>-receptor subtype that sumatriptan activates is present on the human basilar artery, and in the vasculature of human dura mater and mediates vasoconstriction, this action in humans correlates with the relief of migraine headache. Sumatriptan has previously been shown to have a low oral bioavailability in human volunteers (15%), the intranasal route may be a viable alternative for self-administration where this limitation could be overcome. The problem associated with nasal delivery of sumatriptan solution is lower retention of solution in nasal cavity (15min) resulting in lower bioavailability. To overcome the rapid clearance and to facilitate the absorption, in the present study

mucoadhesive polymer chitosan is used as a carrier to formulate microspheres. Chitosan being positively charged is able to interact strongly with the nasal epithelial cells and the overlying mucous thereby providing a longer contact time for drug transport across the nasal membrane, in addition it increases the Paracellular transport of polar drugs by transiently opening the tight junctions between the epithelial cells.

### MATERIALS AND METHODS

#### Chemicals

Sumatriptan succinate was kindly given by Natco Pharma Ltd, Hyderabad, Chitosan was provided by India sea foods, Cochin, heavy liquid paraffin, light liquid paraffin, hexane, glutaraldehyde, acetic acid, dioctyl sodium sulphosuccinate was provided by SD fine chemicals, Mumbai. All other solvents and chemicals used were of analytical grade.

#### Animals

New Zealand white rabbits weighing around 2-2.5 kg were used. They were maintained in a clean room. Animal ethical committee of K.L.E.S's College of Pharmacy Belgaum approved the protocol of this animal study.

#### Method

A series of batches of microspheres were prepared by simple emulsification cross linking method to optimize the parameters like stirring rate, surfactant concentration (dioctyl sodium sulphosuccinate) and volume of crosslinking agent, glutaraldehyde. After optimization of these parameters, six batches were prepared namely SM-1, SM-2, SM-3, SM-4, SM-5 and SM-6 using drug polymer



ratio of 1:1, 1:1.5, 1:2, 1:2.5, 1:3 and 1:3.5 respectively. The detailed procedure follows: Chitosan solution was prepared in 5% acetic acid in which drug was dispersed, this was then dispersed in 125 ml (1:1) heavy and light liquid paraffin containing dioctyl sodium sulphosuccinate (0.05%w/v) under constant overhead stirring at 2000 rpm. Initially 4ml of glutaraldehyde saturated toluene (GST) was added, further after 30mins 4ml of GST was added drop wise. 4ml of aqueous 25% glutaraldehyde was added after 1hr to it with continued stirring up to 5hrs. The microspheres were filtered, washed with n-hexane and ice cold water and then air-dried<sup>4</sup>.

#### Characterization of prepared microspheres

**Percentage yield:** Total amount of microspheres obtained were weighed and the percentage yield was calculated, taking into consideration the weight of drug and polymer.

**Particle size determination:** Particle size was determined by optical microscopy with the help of calibrated eyepiece micrometer. The size of around 100 microspheres was measured and their average particle size determined.

**Surface morphology:** Surface morphology of microspheres was studied using scanning electron microscopy (SEM) JEOL SEM, Model-6360 Japan. Microspheres were sprinkled on double side tape, sputter-coated with platinum and examined under the microscope at 15KV<sup>5</sup>.

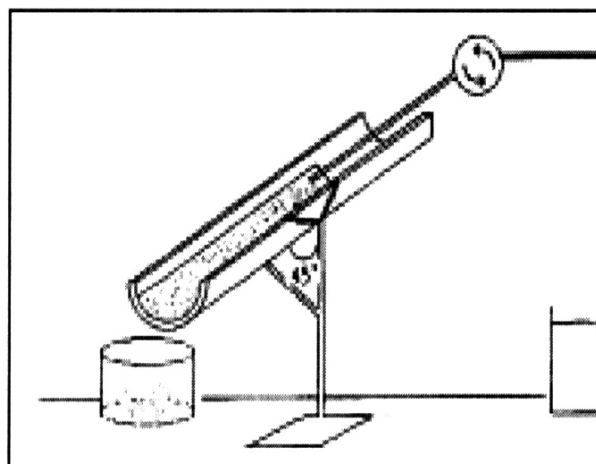
**Percentage drug encapsulation:** Encapsulation was determined by taking a known quantity of microspheres (approximately 25 mg) in a 25 ml volumetric flask, sufficient quantity of phosphate buffer pH 6.8 was added to make up the volume to 25 ml<sup>6</sup>. The suspension was shaken vigorously and then kept aside for 24 hrs at room temperature with intermittent shaking. Supernatant was collected by centrifugation and drug content in supernatant was determined by using UV spectrophotometer at 265nm.

**Equilibrium swelling degree:** The equilibrium swelling degree (ESD) of microspheres was determined by swelling 5gms of dried microspheres in 5 ml of phosphate buffer pH 6.8 overnight in a measuring cylinder<sup>7</sup>.

**Mucoadhesion studies (Falling film technique):** 100mg of microsphere sample was placed over a nasal mucosal segment mounted on a tilted slide at an angle of 45° as shown<sup>7</sup> (Figure 1). The effluent was run over the segment. The effluent was collected on a whatmann filter paper and weight of detached particles were determined. Percentage of mucoadhesion was determined using following formula.

$$\% \text{ Mucoadhesion} = \frac{\text{Wt. of sample} - \text{Wt. of detached sample} \times 100}{\text{Wt. of Sample}}$$

**Figure 1:** Falling Film Technique to Measure Microsphere Mucoadhesion

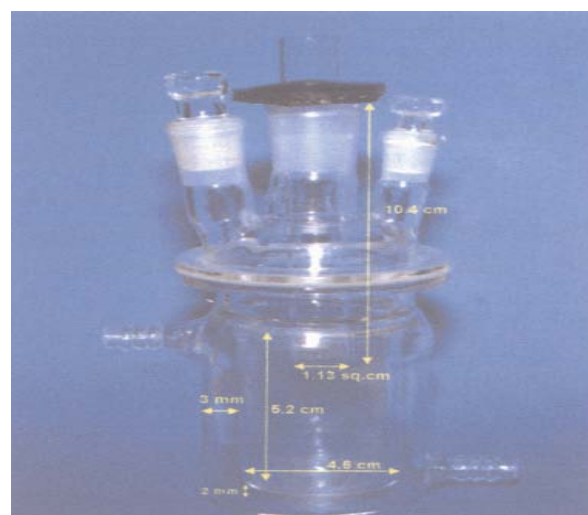


#### Ex-vivo drug permeation study

Ex-vivo drug permeation study was performed using a glass fabricated nasal diffusion cell [Figure 2]. The water-jacketed recipient chamber has a total capacity of 60 ml and flanged top of about 3mm, the lid has three opening, each for sampling, thermometer, and a donor tube chamber<sup>8</sup>. The 10 cm long donor chamber tube has an internal diameter of 1.13 cm. The nasal mucosa of sheep was separated from sub layer bony tissue and was stored in distilled water containing few drops of gentamycin sulphate injection. After the complete removal of blood from mucosal surface, it was attached to donor chamber tube. The donor chamber tube was placed in such a way that nasal mucosa just touches the diffusion medium (phosphate buffer of pH 6.8) in recipient chamber

Permeation study of Sumatriptan from microspheres was studied at  $37 \pm 2^{\circ}\text{C}$ . 1 gm of microspheres were placed on the mucosal surface at zero time. Samples (1ml) were withdrawn at different time intervals for up to 8 hours which were further diluted up to 10 ml and measured spectrophotometrically at 265nm to evaluate the amount of drug permeated.

**Figure 2:** Nasal Diffusion Cell



### **In vivo bioavailability studies**

*In vivo* bioavailability studies were conducted in healthy male rabbits weighing 2-2.5 kg. Twelve rabbits were divided into two groups of six each and were fasted for 24 hours<sup>9-11</sup>. One group was fed with Oral tablet preparation, the nasal formulation (microspheres) was administered to the second group of unanesthetized rabbits equally between both nostrils with polyethylene tubes. The formulation was released from the tube using a syringe containing compressed air. 2 ml of blood sample was collected from the marginal ear vein of the rabbits into heparinized centrifuge tubes at 1, 1.5, 2, 2.5, 3, 4, 5 and 6 hours after the drug administration during the study. Blood samples were centrifuged at 1500 rpm and plasma was separated. A five-point calibration standard, ranging from 20 to 100 ng/ml was prepared by spiking the drug free rabbit plasma containing EDTA with an appropriate amount of sumatriptan and internal standard respectively.

### **Extraction procedure for plasma samples**

1ml aliquot of rabbit plasma sample was placed in a screw cap glass tube, 40µl of internal standard working solution (150 µg/ml ofloxacin), 1 ml of 2M sodium hydroxide solution was added and the mixture was vortexed for 30s<sup>12</sup>. The plasma samples containing sumatriptan were then extracted with 4 ml of ethyl acetate. The mixture was shaken for 5 min and centrifuged for 15 min at 10°C. Extraction procedure with 4 ml ethyl acetate was repeated twice for each sample; both the extracts were mixed together in a culture tube, and then evaporated to dryness. The extracted residue was reconstituted in 0.3 ml of the mobile phase and injected into the HPLC system.

### **Data analysis**

The area under the curve (AUC) was obtained using the trapezoidal rule, the data from time zero to last experimental data point of 6h were used in calculating the bioavailability of both nasal and the oral formulation. The C<sub>max</sub> and T<sub>max</sub> were also obtained from plasma-time profile curve. The bioavailability was calculated using the following equation:

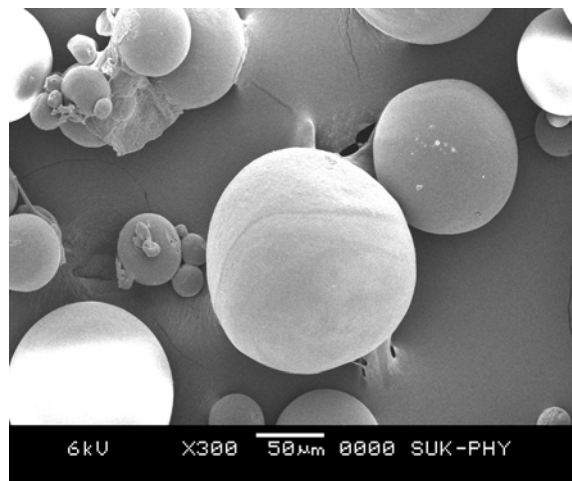
$$F = \frac{[AUC]_{IN} [Dose (mg)]_{Oral}}{[AUC]_{Oral} [Dose (mg)]_{IN}}$$

## **RESULTS AND DISCUSSION**

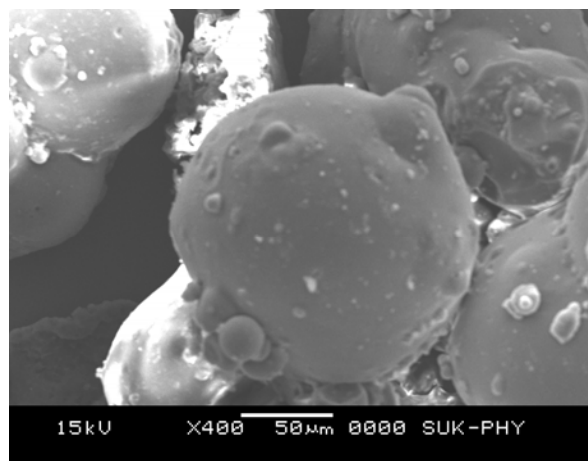
The percentage yield obtained in all the batches was in the range of 57-84%. The loss of material during preparation of microspheres was due to process parameters as well as during filtration of microspheres. Spherical particles were obtained for all the batches. All the microspheres obtained were in the size range of 46-68µm, which was suitable for intranasal administration. It was observed that with the increase in the concentration

of polymer (Chitosan) there was an increase in the particle size of the obtained microspheres. This was due to increase in viscosity and hence difficulty in dispersion and subdivision of droplets. The scanning electron microscopy shows that microspheres obtained were fairly smooth and spherical in shape. The Sumatriptan Succinate loaded microspheres revealed drug crystals in microspheres confirming the dispersion of drug throughout the matrix. The SEM micrographs of drug free and drug-loaded microspheres are shown in the fig. No: 3 and 4.

**Figure 3:** SEM Micrograph of Sumatriptan-Free Chitosan Microspheres



**Figure 4:** SEM Micrograph of Sumatriptan-loaded Chitosan Microspheres.



With an increase in the concentration of the polymer, the encapsulation efficiency also increased. It was observed that as the concentration of polymer increased in microspheres swelling degree also increased marginally. It was also observed that with increase in polymer concentration percent mucoadhesion was also increased. Furthermore, the swelling behavior and the mucoadhesive effect of these formulations increase the residential time of the drug in the nasal cavity, protecting the drug from the mucociliary clearance due to rapid

turnover of the mucous. The physicochemical characteristics of SM1-SM6 batches is shown in table no 1

The *ex vivo* permeation of sumatriptan succinate from chitosan microspheres exhibited a biphasic pattern, initially showing a rapid permeation due to diffusion of nonencapsulated drug from the surface of microspheres

followed by much slower diffusion of entrapped drug. Increase in polymer concentration attributed to higher density of polymer matrix with resultant increase in diffusion path length that the drug molecule has to travel, thereby significantly decreased the rate and extent of drug permeation [Figure 5].

**Table 1:** Evaluation of microspheres

Batch Code	Polymer: Drug	% Yield # ±SD	Particle Size # in µm ±SD	% Drug Encapsulation# ±SD	Swelling Index# in mg/ml±SD	% # Mucoadhesion ±SD
SM1	1:1	60.70±2.41	49.76 ± 3.15	54.8±4.32	3.73±1.46	82.24±0.23
SM2	1:1.5	75.73±3.05	55.20 ± 4.28	66.8±5.67	4.08±0.79	88.92±0.48
SM3	1:2	62.84±4.86	56.04 ± 6.08	69.3±3.43	4.12±2.86	92.12±0.43
SM4	1:2.5	66.66±4.21	57.80 ± 3.46	73.3±6.84	4.36±0.35	93.67±0.56
SM5	1:3	84.37±5.15	60.28 ± 5.82	76.5±5.72	4.48±0.92	95.54±0.34
SM6	1:3.5	72.16±2.89	62.40 ± 7.04	79.6±5.89	5.00±1.28	96.18±0.64

SM1 to SM6 represents the various nasal formulations of sumatriptan succinate,

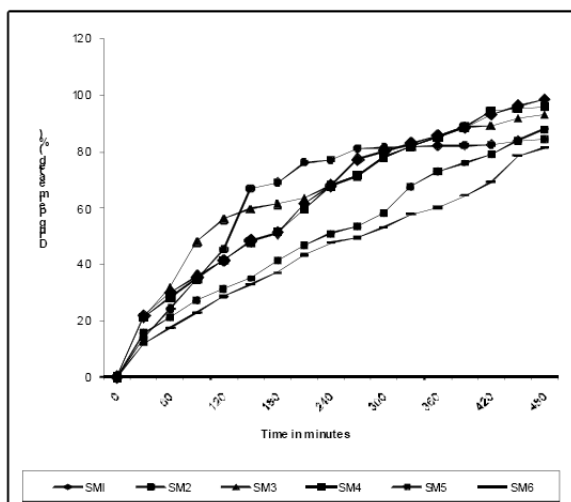
# Each value represents a mean of 3 determinations (n=3)

**Table 2:** Pharmacokinetic parameters of tablet and nasal microspheres of sumatriptan succinate.

Dosage form	Tmax (hrs)	Cmax ng/ml*	Kel	AUC* (ng.hr/ml)	Elimination t1/2
Oral (tablet)	2	35 ±0.6	-0.147	126.25±5.8	4.71
Intranasal (microspheres)	2	40 ±0.4	-0.115	270±2.4	6.02

\* Mean±SD (n=3)

**Figure 5:** Percentage of drug permeated for formulations SM-1 to SM-6



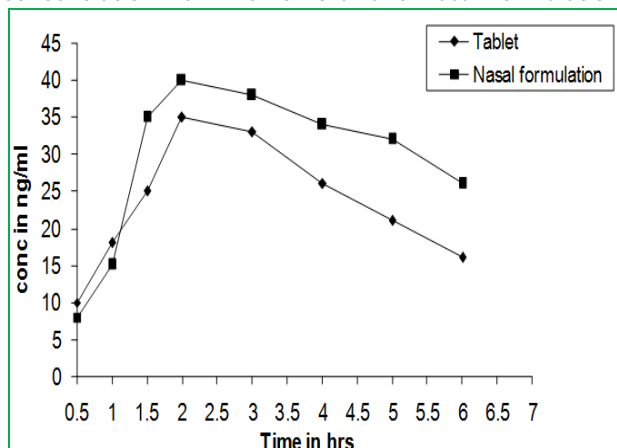
Chitosan being insoluble, the release of drug has been controlled by swelling control release mechanism. The results showed that concentration of Chitosan can vary the release profile of drug from the microspheres. The batches SM-1, SM-2, SM-3 showed a release of 98.6%, 96%, and 93.21% within 8 hrs where as SM-4, SM-5, SM-6 showed a release of 88.1, 84.4, and 81.5% respectively within 8 hr.

The HPLC analysis of the plasma showed a peak plasma concentration ( $C_{max}$ ) of 35ng/ml after oral dosing and the

time to attain the peak plasma concentration i.e.,  $T_{max}$  was around 2 hrs while in the case of the nasal microspheres the  $C_{max}$  was 40ng/ml and the  $T_{max}$  was 2 hours. The mean pharmacokinetic parameters of the oral and the nasal formulation were estimated and are given in table No 2

Assessment of the  $AUC_{0-t}$  from the plasma concentration-time curve showed that the relative bioavailability was increased to about 2.68 times with the nasal formulation as compared to the tablet formulation fig 6.

**Figure 6:** Plot for plasma concentration of Sumatriptan concentration Vs Time for oral and nasal formulation.



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