



## PREPARATION AND *IN VITRO* CHARACTERISATION OF MUCOADHESIVE MICROSPHERES OF MIDAZOLAM: NOSE TO BRAIN ADMINISTRATION

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### ABSTRACT

HPMC microspheres of midazolam for nose to brain delivery were developed with the aim to avoid first pass metabolism, to improve the patient compliance, to use an alternative therapy to conventional dosage form, to improve the therapeutic efficacy of midazolam. The purpose of this research was also to formulate and systematically evaluate the performances of mucoadhesive microspheres of midazolam. The emulsion cross linking technique was used to prepare Midazolam microspheres containing HPMC. Glutaraldehyde was used as a cross-linking agent. Results of preliminary trials indicate that volume of cross-linking agent, time for cross-linking, polymer-to-drug ratio and speed of rotation affected characteristics of microspheres. Microspheres were discrete, spherical and free flowing. The microspheres exhibited good mucoadhesive and also showed high percentage drug entrapment efficiency. Spherical microspheres were obtained in all batches in range of 7-18  $\mu\text{m}$ . The best batch exhibited a high drug entrapment efficiency of 97% and a swelling index of 1.29; *in vitro* bioadhesion was 94%. The drug release was also sustained for 12 hours. The polymer- to-drug ratio had a more significant effect.

**Key words:** Midazolam, Nose Brain, Emulsion cross-linking, HPMC, Microspheres

### INTRODUCTION

Intranasal (IN) delivery has been shown to non invasively deliver drugs from the nose to the brain in minutes along the olfactory and trigeminal nerve pathways, bypassing the blood brain barrier (BBB). The unique relationship between nasal cavity and cranial cavity tissues makes IN delivery to the brain feasible. An IN delivery provides some drugs with short channels to by pass the BBB especially for those with fairly low brain concentrations after a routine delivery, thus greatly enhancing the therapeutic effect on brain diseases. The BBB is a system of layers of cells at the cerebral capillary endothelium and the arachnoid membranes, which are connected by tight junctions (zonulae occludens) and which together separate the brain and cerebrospinal fluid (CSF) from the blood.

The nasal mucosa is nearby the brain, CSF and the drug concentrations can exceed plasma concentrations. IN delivery provides a noninvasive method of bypassing the BBB to rapidly deliver therapeutic agents to the brain, spinal cord, lymphatics and to the vessel walls of the cerebrovasculature for treating central nervous system (CNS) disorders. IN delivery also offers the advantage of simple administration, cost effectiveness and convenient.

This novel delivery method allows drugs, therapeutic proteins, polynucleotides and viral vectors that do not normally cross the BBB to be delivered to the central nervous system. Additionally, IN targeting of drugs to the CNS avoids first pass elimination by the liver allowing a lower therapeutic drug dose and fewer systemic side effects. Delivery from the nose to the CNS occurs within minutes along both the olfactory and trigeminal nerves. Delivery occurs by an extracellular route and does not require that the drugs bind to any receptor or undergo axonal transport.<sup>1-6</sup>

Midazolam is chemically 8-chloro- 6-(2-fluorophenyl)- 1-methyl-4H-imidazo[1,5-a] [1,4]benzodiazepine . It is used to produce sleepiness or drowsiness and to relieve anxiety before surgery. Midazolam is also given to produce amnesia so that the patient will not remember any discomfort or undesirable effects that may occur after a surgery or procedure. Midazolam undergoes first pass metabolism and it is generally given by oral or parenteral routes. An alternative route of drug delivery is needed since oral and intravenous routes for delivering drugs are sometimes impractical and/or inconvenient.<sup>7</sup> Direct transport of drugs to the brain circumventing the brain-barriers following intranasal administration provides a unique feature and better option to target

drugs to brain. The plasma half life of midazolam is 4 hrs and that is the reason it was used for the nose to brain drug delivery and the use of bioadhesive microspheres gives more residence time to facilitate absorption. HPMC was used to prepare the midazolam microspheres for the nose to brain drug delivery so as to increase the residence time and by pass the first pass metabolism by liver.<sup>8,9</sup>

### MATERIALS AND METHODS

#### Materials

Midazolam was obtained as a gift sample from Sun Pharmaceuticals Ltd., Vadodara, Gujarat. HPMC was obtained from Color con Asia, Mumbai. Glutaraldehyde, heavy paraffin, light paraffin, Tween 80 was procured from Loba chem Mumbai. All the reagents used were of AR grade.

#### Preparation of microspheres

Microspheres were prepared using HPMC by emulsion cross linking method. The aqueous phase was prepared by using 4% HPMC in phosphate buffer (pH 5.5). The drug was dissolved in it and the solution was extruded through a glass jacketed syringe in 50 ml of liquid paraffin (heavy and light 1:1 mixture) containing 0.5% Tween 80, with continuous stirring on Remi stirrer at 2000 rpm. After 3 hrs, 1ml of glutaraldehyde (25% solution, as crosslinking agent) was added and stirring was continued for 2 hrs. Microspheres obtained were filtered and washed several times with petroleum ether to remove oil and finally washed with water to remove excess of glutaraldehyde. Microspheres were then air dried.<sup>10-16</sup>

#### Particle size analysis

Particle size of the microspheres was determined by optical microscopy. Average of 100 microspheres were measured randomly and the average particle size was determined by using the Edmondson's equation  $D_{\text{mean}} = \frac{\sum n_d}{\sum n}$ , where n = number of microspheres observed and d = mean size range.<sup>17</sup>

#### Scanning electron microscopy (SEM) of microspheres

Shape and surface morphology of microspheres was studied using scanning electron microscope (Jeol, JSM 5610 LV, Japan)<sup>17,18</sup>

#### Swelling Index

Swelling Index was determined by measuring the extent of swelling of microspheres in phosphate buffer pH 6.6. To ensure the complete equilibrium, exactly weighed 100 mg of microspheres were allowed

to swell in buffer for 24hrs. The degree of swelling was calculated using following formula,  $\alpha = (W_s - W_o) / W_o$ , where  $\alpha$  is degree of swelling,  $W_o$  is the weight of microspheres before swelling and  $W_s$  is the weight of microspheres after swelling.<sup>19</sup>

#### Drug entrapment efficiency

Weighed quantity of microspheres were crushed and suspended in methanol to extract the drug from microspheres. After 24 hrs, the filtrate was assayed spectrophotometrically at 216 nm for drug content against methanol as blank. Corresponding drug concentrations in the samples were calculated. The drug entrapment efficiency was calculated using the following formula: (Practical drug content/ Theoretical drug content) X 100. The drug entrapment efficiency for batches SP1 to SP5 is reported in Table 1.<sup>20</sup>

#### In vitro mucoadhesive strength determination

The *in vitro* mucoadhesion of microspheres was carried out by modifying the method described by Ranga Rao and Buri<sup>[20]</sup> using sheep nasal mucosa. The dispersion (0.2 ml) of microspheres in phosphate buffer saline was placed on sheep nasal mucosa after fixing to the polyethylene support. The mucosa was then placed in the dessicator to maintain at >80% relative humidity and room temperature for 30 min. The mucosa was then observed under microscope and the number of particles attached to the particular area was counted.

After 30 min, the polyethylene support was introduced into a plastic tube cut in circular manner and held in an inclined position at an angle of 45°. Mucosa was washed for 5 min with phosphate buffer saline pH 7.4 at the rate of 22 ml/min using a peristaltic pump; tube carrying solution was placed 2-3 mm above the tissue so that the liquid flowed evenly over the mucosa. Tissue was again observed under microscope to see the number of microspheres remaining in the same field area. The adhesion number was found by the following equation:  $N_a = N/N_0 \times 100$ , where  $N_a$  is adhesion number,  $N_0$  is total number of particles in a particular area, and  $N$  is number of particles attached to the mucosa after washing.<sup>[11]</sup>

#### In vitro diffusion studies

Diffusion study was performed using the dialysis bag. For this study microspheres equivalent to 5 mg of midazolam were weighed and 5 ml of phosphate buffer saline pH 5.5 was added in it and the stirring was done at 60 rpm at 37°C. At specific time intervals, samples (5 ml) were withdrawn and filtered. Same volume (5 ml) of the phosphate buffer saline pH 5.5 was replaced after each sampling. The drug content in the sample was determined in the filtrate spectrophotometrically at 216 nm.

### RESULTS AND DISCUSSION

The microspheres of midazolam were prepared by the emulsification cross-linking method using glutaraldehyde as cross-linking agent. The microspheres obtained under these conditions were found to be spherical and without aggregation, and median size ranged from 7-18  $\mu\text{m}$  and are therefore suitable for nose to brain administration. Mean particle size, percent drug entrapment and % *in vitro* mucoadhesion of different batches of microspheres prepared are tabulated in Table 1.

Optimizations of various formulation parameters in preparation of midazolam microspheres were carried out. The heavy and light liquid paraffin (1:1) as external phase, Tween 80 (0.5% v/v) as stabilizing agent, and the stirring rate of 2000 rpm were found to be optimum to yield midazolam microspheres.

Glutaraldehyde 25% aqueous solution was selected as crosslinking agent due to its high rate of crosslinking and easy removal of the unreacted free glutaraldehyde.

With increase in polymer concentration in the microspheres from batch SP1 to SP5, the particle size of microspheres increased, which may be due to the fact that increase in the concentration of polymer increases the crosslinking, and hence the matrix density of the microspheres increased, and that may result in the increase in the

particle size of the microspheres. SEM of the microspheres is presented in Figure 1.

*In vitro* mucoadhesion of microspheres was the most important aspect of present investigation. It was found that, for batches SP1 to SP5, as the amount of polymer was increased, the % *in vitro* mucoadhesion also increased. This may be due to the fact that, as the amount of polymer increases, the binding with the sialic acid residues in mucus layer also increases, and that results in the increase in the *in vitro* mucoadhesion of microspheres.

The release pattern of formulation appears to be slow release. The *in vitro* percentage release of drug is indicated in Fig.2. Based on highest regression values R the best fit model follows Zero order release profile and the results of linear correlation coefficient ( $r^2$ ) values are tabulated in Table 2.

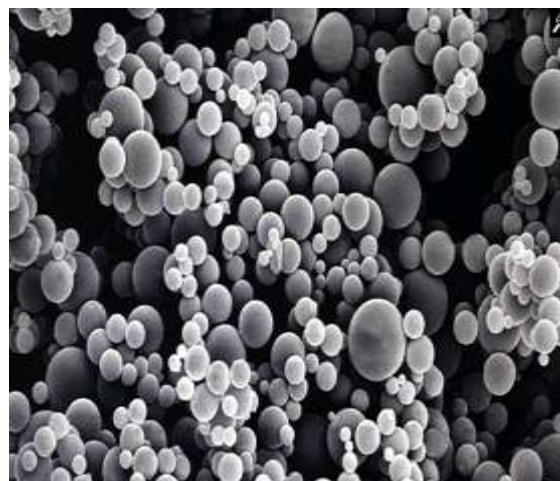


Figure -1: Scanning electron micrograph of Midazolam loaded microspheres

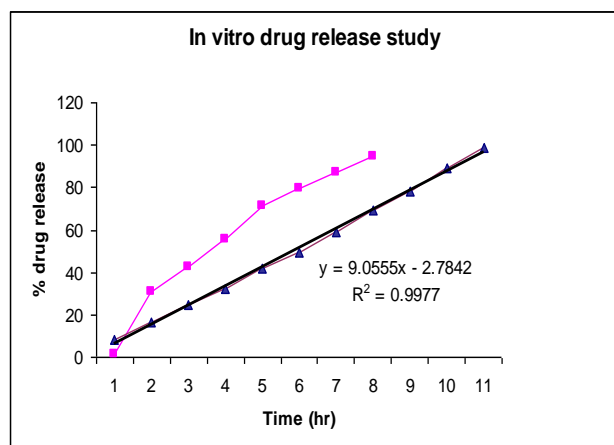


Figure -2: *In vitro* drug release study of drug solution and microsphere dispersion

### CONCLUSION

These results indicate that the HPMC microspheres have potential to deliver midazolam following nose to brain administration. Its possibility to avoid first pass metabolism of midazolam may ultimately show improvement of bioavailability than oral dosage, probably as a consequence of prolonged residence at the absorption site.

The emulsion crosslinking technique for the entrapment of midazolam in HPMC produced a high yield of discrete microspheres with minimal agglomeration, reproducible drug loading efficiency and release profiles from batch to batch. The release rate and mucoadhesion of HPMC could be modified by varying the process

**Table -1: Physical characteristics of prepared Midazolam loaded microspheres**

Formulation Code	Average Particle Size ( $\mu\text{m} \pm \text{SD}$ )*	Total entrapment efficiency (% $\pm$ SD)#	Degree of Swelling ( $\pm$ SD)#	Average bioadhesion ( $\alpha$ ) (% $\pm$ SD)#
SP1	8.9 $\pm$ 1.21	79 $\pm$ 1.19	0.79 $\pm$ 0.11	80.11 $\pm$ 0.15
SP2	9.3 $\pm$ 1.41	83 $\pm$ 1.14	0.84 $\pm$ 0.27	87.43 $\pm$ 0.24
SP3	9.7 $\pm$ 1.54	87 $\pm$ 1.24	0.93 $\pm$ 0.17	89.27 $\pm$ 0.64
SP4	10.1 $\pm$ 1.44	94 $\pm$ 1.20	1.20 $\pm$ 0.42	91.21 $\pm$ 0.29
SP5	10.8 $\pm$ 1.29	97 $\pm$ 1.01	1.29 $\pm$ 0.57	94.15 $\pm$ 0.18

n\* = 100, n# = 3

**Table -2: Linear correlation coefficient (r<sup>2</sup>) values**

Zero order	First order	Higuchi	Hixon-Crowell
0.9977	0.9176	0.9416	0.8304

parameters. Therefore we concluded that the water-in-oil emulsion crosslinking technique produced microspheres of a suitable size for nose to brain administration. The *in-vitro* mucoadhesive study demonstrated that HPMC adhered to mucus to a greater extent.

Midazolam microspheres were prepared by using different concentration of HPMC with intention to increase the mucoadhesion. HPMC solution of strength 1 to 8% was tried. It was found that formulation of microspheres with more than 5% HPMC is not possible due to drastic increase in the viscosity.

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