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Mucoadhesive Microspheres of Midazolam: Nose to Brain Delivery

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ABSTRACT

The purpose of this research was to formulate and systematically evaluate *in vitro* performances of mucoadhesive Midazolam microspheres for the nose to brain delivery and with the aim to avoid first pass metabolism, to improve the patient compliance, to use an alternative therapy to conventional dosage form and to improve the therapeutic efficacy of Midazolam. Midazolam microspheres were prepared by emulsion cross linking technique using Carbopol 934P and Hydroxy propyl methyl cellulose (HPMC) K4M as mucoadhesive polymers. Glutaraldehyde was used as a cross-linking agent. The prepared microspheres were evaluated with respect to the particle size, encapsulation efficiency, shape and surface properties, mucoadhesive property, *in vitro* drug release, Thermal Analysis & X-Ray Diffraction Studies. Microspheres both of Carbopol and HPMC were discrete, spherical and free flowing. The best batch exhibited a high drug entrapment efficiency of 93 ± 1.68 % for SD4 and 97± 1.01 for SP5. The drug release was also sustained up to 12 h. The preliminary results show that the drug loaded HPMC microspheres are much more suitable for the delivery of Midazolam to the brain. The polymer- to-drug ratio had a more significant effect. DSC study confirmed that drug is present in the molecular dispersion.

Key words: Midazolam, Nose Brain, Emulsion cross-linking, HPMC K4M, Carbopol 934P, Microspheres

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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 bypass 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. Nasal mucosa consists of a rich vasculature and a highly permeable structure for systemic absorption. Drug administration through the nasal cavity is easy and convenient. Subsequent to a drug's passage through the mucus, there are several mechanisms for absorption through the mucosa. These include transcellular or simple diffusion across the membrane (for lipophilic drugs), paracellular transport via movement between cell (for water soluble drugs) and transcytosis by vesicle carriers.

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 [1-6].

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 parentral routes. An alternative route of drug delivery is needed since oral and intravenous routes for delivering drugs are sometimes impractical and/or inconvenient [7].

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. Carbopol 934P & HPMC K4M were 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 [8, 9].

MATERIALS AND METHODS

Materials

Midazolam was obtained as a gift sample from Sun Pharmaceuticals Ltd., Vadodara, Gujarat. Carbopol 934P was obtained as a gift sample from Noveon Mumbai, India and HPMC K4M 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.

Optimized Method for Preparation of Microspheres

The Midazolam loaded mucoadhesive microspheres were prepared by emulsion cross linking method employing two different mucoadhesive polymers, viz. Carbopol 934P and HPMC K4 M.

a) Preparation of Carbopol 934P NF Microspheres:

Microspheres were prepared using carbopol by emulsion cross linking method. The aqueous phase was prepared by using different concentrations (0.5 to 4%) of carbopol 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 surfactant, with continuous stirring on Remi stirrer at 2000 rpm. After 3 h, 1 mL of glutaraldehyde (25% solution, as cross linking 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

b) Preparation of HPMC K4 M Microspheres:

The procedure employed for the preparation of HPMC K4M microspheres was same and the various batch from 1-5% of Polymer concentration was prepared as above [10-16].

Particle size analysis

Particle sizes of different batches of microspheres were determined by optical microscopy. Average of 100 microspheres were measured randomly and the average particle size was determined by using the Edmondson's equation $D_{mean} = \Sigma_{nd} / \Sigma_{n}$, where n = number of microspheres observed and d = mean size range [17].

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Scanning electron microscopy (SEM) of microspheres

Shape and surface morphology of microspheres was studied using scanning electron microscope (Jeol, JSM 5610 LV, Japan) [17, 18].

Swelling Index

Swelling Index was determined by measuring the extent of swelling of microspheres in phosphate buffer pH 5.5. To ensure the complete equilibrium, exactly weighed 100 mg of microspheres were allowed to swell in buffer for 24 hrs. The degree of swelling was calculated using following formula, α= (Ws-Wo)/ Wo, where α is degree of swelling, Wo is the weight of microspheres before swelling and Ws is the weight of microspheres after swelling [19].

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 and SD1 to SD5 is reported in Table 1 & Table 2 [10].

Table1. Physical characteristics of prepared Midazolam loaded Carbopol microspheres.

Formulation Code	Average Particle Size (µm ± SD)*	Total entrapment efficiency (%± SD)#	Degree of Swelling (± SD)#	Average bioadhesion (α) (%± SD)#
SD1	8.7 ± 1.71	78 ± 1.39	0.82 ± 0.21	84.21 ± 0.25
SD2	9.1 ± 1.21	80 ±1.24	0.86 ± 0.48	86.54 ± 0.64
SD3	9.8 ± 1.34	84 ± 1.84	0.91 ± 0.67	88.35 ± 0.84
SD4	10.3 ± 1.64	**93 ± 1.68**	1.11 ± 0.38	89.91 ± 0.19
SD5	11.5 ± 1.29	91 ± 1.11	1.25 ± 0.68	90.75 ± 0.48

n*= 100, n# = 3

Table 2. Physical characteristics of prepared Midazolam loaded HPMC microspheres.

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

n*= 100, n# = 3

In vitro mucoadhesive strength determination

The *in vitro* mucoadhesion of microspheres was carried out by modifying the method described by Ranga Rao and Buri [20] 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 desiccator 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: $Na = N/N_0 \times 100$, where Na 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 [11].

In vitro diffusion studies

Diffusion study was performed with modified diffusion apparatus. In order to localize the Microspheres, dialysis bag was used as diffusion membrane. For this study microspheres equivalent to 2 mg of Midazolam were weighed and 50 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.

Thermal Analysis

Differential scanning calorimetry (DSC) was performed on pure drug, drug loaded and blank microspheres of carbopol and HPMC. DSC measurement was done on a mettler Toledo DSC 822c.

X-Ray Diffraction (XRD) Studies

The crystallinities of Midazolam and Midazolam loaded HPMC & Carbopol microspheres were determined using an x-ray diffractometer (Brrucker Axs, 08 Advance).

RESULTS AND DISCUSSION

The microspheres of Midazolam were prepared using HPMC and Carbopol by the emulsification cross-linking method and glutaraldehyde as a cross-linking agent. The microspheres obtained under these conditions were found to be spherical and without aggregation, and median size ranged from 7-18 µm (for HPMC) and 8- 20 µm (for Carbopol)

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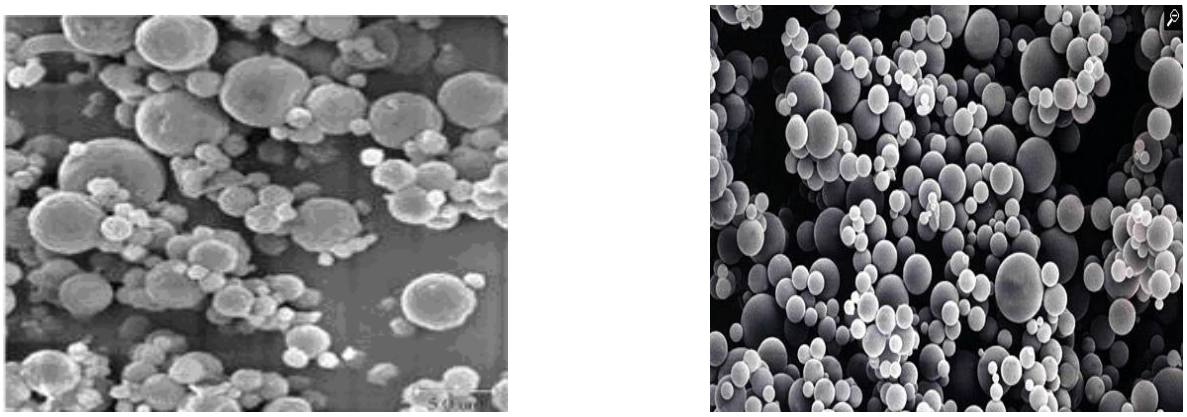
therefore these are suitable for nose to brain administration. Mean particle size, percent drug entrapment and % *in vitro* mucoadhesion of different batches of microspheres prepared are tabulated in Table1 & 2 for Carbopol and HPMC respectively. Optimizations of various formulation parameters in preparation 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 and for SD1 to SD5 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 Carbopol and HPMC microspheres is presented in Figure.1.



(A) **(B)**

Figure 1: Scanning electron micrograph of A) Midazolam loaded Carbopol Microspheres, B) Midazolam loaded HPMC Microspheres

In vitro mucoadhesion of microspheres was the most important aspect of present investigation. It was found that, for batches SP1 to SP5 & for SD1 to SD5, 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 Figure.2 and Figure.3.

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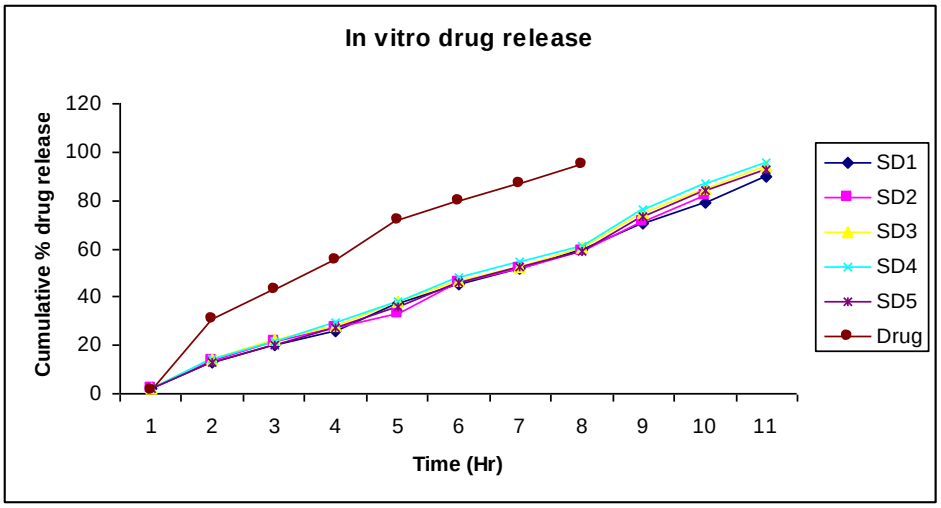

Figure 2: *In vitro* drug release study of drug solution and Carbopol Micro sphere dispersion

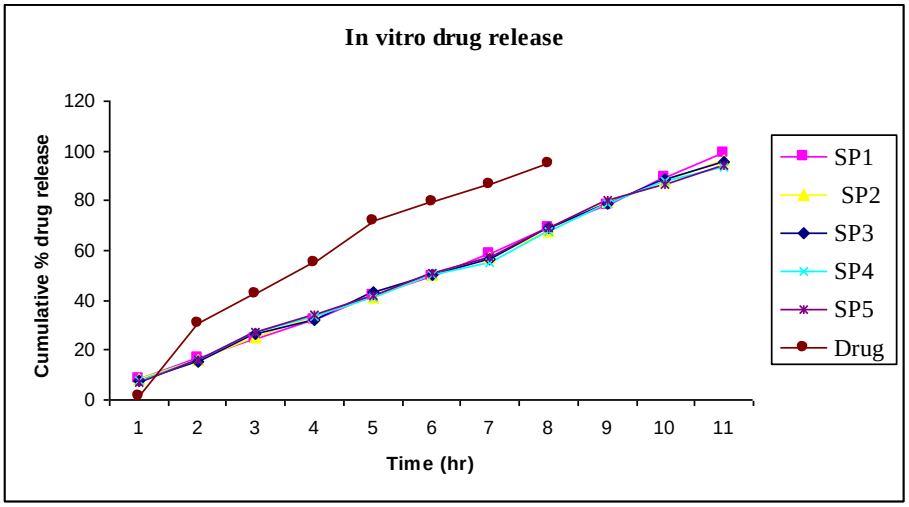

Figure 3: *In vitro* drug release study of drug solution and HPMC Micro sphere dispersion

DSC is very useful in the investigation of the thermal properties of microspheres, providing both qualitative and quantitative information about the physicochemical state of drug inside the Microspheres is indicated in Figure 4. Drug loaded microspheres doesn't show any endotherm may be due to the drug was present in the molecular dispersion or solid solution state in the polymeric microspheres loaded with drug. XRD spectra of pure Midazolam, drug loaded HPMC Microspheres and drug loaded Carbopol Microspheres is indicated in Figure 5. The well resolved sharp diffraction peaks of the characteristic moiety indicate crystallinity of Midazolam.

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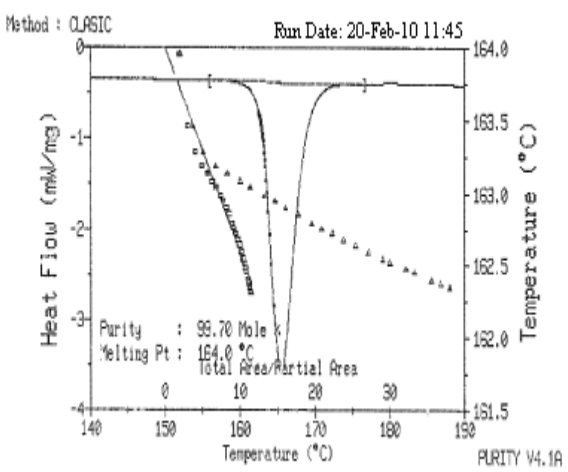
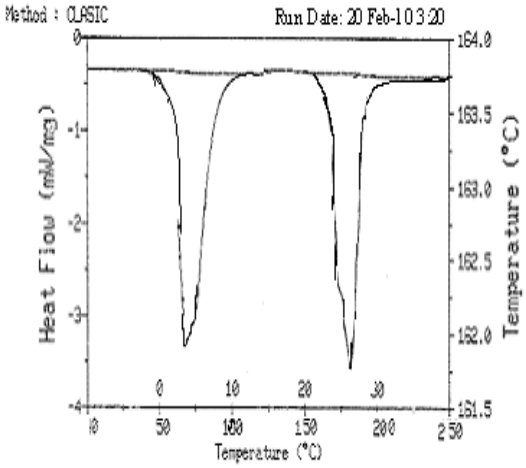
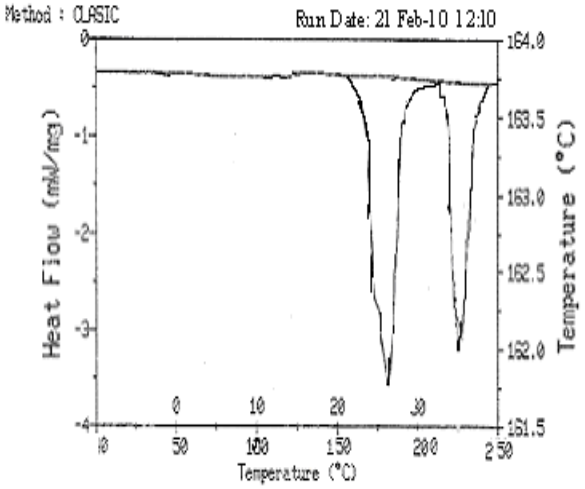


(A)



(B)



(C)

Figure 4: DSC thermogram of (A) Midazolam (Pure Drug), (B) Drug loaded HPMC Microspheres, (C) Drug loaded Carbopol Microspheres

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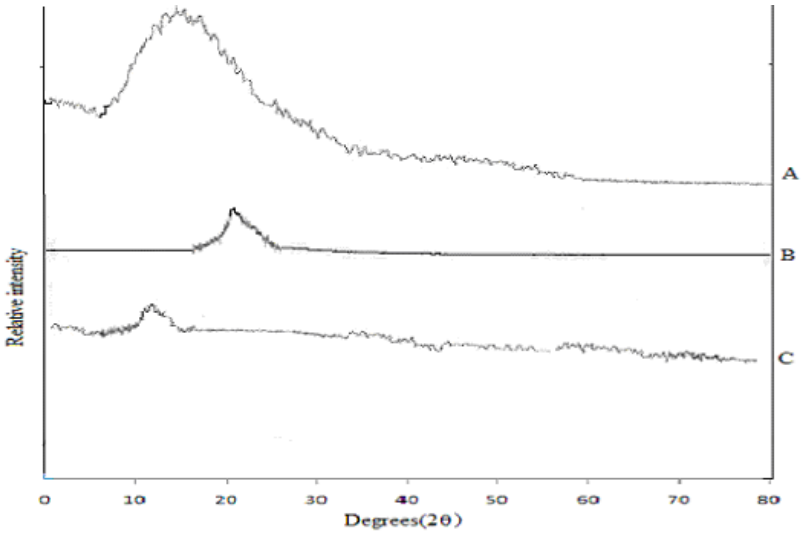

Figure 5: XRD spectra of A) Pure Midazolam B) Drug loaded HPMC Microspheres C) Drug loaded Carbopol Microspheres.

CONCLUSION

These results indicate that the Carbopol & 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 Carbopol & 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 Carbopol & HPMC could be modified by varying the process 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 microspheres adhered to mucus to a greater extent than Carbopol microspheres.

Midazolam microspheres were prepared by using different concentration of HPMC (1 to 8%) and Carbopol (0.5 to 4%) were tried with intention to increase the mucoadhesion. It was found that HPMC loaded microsphere formulation is much more suitable for the delivery of Midazolam to the brain.

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