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Formulation of Atenolol Mucoadhesive Microspheres for Nasal Delivery by Spray Drying Technique: *In vitro/ Ex vivo* Evaluation

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ABSTRACT

The aim of the study is to develope a formulation of atenolol for the nasal drug delivery system with consideration to parameters require for nasal administration, to get maximum utilization and efficacy of the drug also delivery of drug in case of emergency situation. The use of atenolol in conventional dosage form possesses several disadvantages like the absorption in GI tract is low, hence low bioavailability, require large amount of dose and has to pass first pass metabolism. This encourages us to formulate the dosage form with atenolol as a novel drug delivery which will show maximum potential utilization of the atenolol. The five formulations of microspheres were formulated with hydroxypropylmethyl cellulose K4M by spray drying technique. The in vitro and ex vivo studies of microspheres were performed. The Scattering Electron Microscopy (SEM), X-Ray Diffraction (XRD), and Histopathological study was also performed. The SEM demonstrated spherical particles with rough surface. The XRD studies show amorphous nature of drug entrapped in microspheres. The Histopathological study revealed intact nature of sheep nasal mucosal structure on treatment with microspheres. Particle size, swelling ability and mucoadhesion of microspheres was increased with the increase in drug: polymer ratio. The results indicate that atenolol spray dried microspheres formulated with HPMC K4M is a promising nasal delivery system.

Keywords: Microspheres, Atenolol, HPMC K4, Mucoadhesive.

INTRODUCTION

The drug atenolol is recognized and extensively prescribed as first choice of drugs in the treatment of majority of the hypertension population. The conventional formulation of these drugs is rapidly dissolved in upper gastric intestine and produces peak plasma concentration

within 1 to 4 hours and then declines quickly. Consequently, divided doses are recommended for maintaining the effective plasma concentration. However, conventional formulations exhibited drawbacks since they produce peaks and valley time drug plasma concentration on multiple dosing. The multiple dosing results in high and rapid plasma concentrations after each dose, which may be associated with undesirable beta-2 mediated effects like fatigue and bronchospasm. The conventional formulations fail to provide adequate protection against myocardial ischematic episode, which shows circadian pattern. Arterial blood pressure also exhibits circadian rhythm that leads to serious cardiac problems that occurs during early morning hours. The short half-life of the conventional formulations necessitates the multiple dosing which may lead to blood pressure variation over 24-hours and hence may increase target organ damage. Atenolol is subjected to first-pass metabolism, which increases the dose size of the drug. All the drawbacks necessitate the development of an even and effective drug delivery system which could utilizes all the potential of efficacy of the drug atenolol and should maintain the plasma concentration throughout 24 hours in order to achieve maximal cardio protection, improved patient compliance, maximal drug utilization and enhanced bioavailability[2]. Therefore, the development of mucoadhesive microspheres of atenolol could protect drug from first hepatic pass degradation and maintain a constant drug plasma level for extended period of time. This could maximize the drug utilization, improve bioavailability of drug, exhibit better patient compliance and finally applicable in emergency situation.

MATERIALS AND METHODS

Materials

Atenolol was supplied as a gift sample from IPCA Laboratories Ltd, Aurangabad. HPMC K4M was supplied as a gift sample from Colorcon Limited (Goa, India). All other chemicals and reagents were of analytical grade. Deionized water was used for all of the experiments. A freshly cut piece, 5 cm long of sheep nasal mucosa was obtained from a local abattoir house.

Table 1: Formulation composition

Polymer	Ratio (drug: polymer)	Formulation code	Drug (mg)	Polymer (mg)	Methanol (ml)	Dichloromethane (ml)
HPMC K4M	1:1	F1	200	200	30	60
	1:2	F2	200	400	50	100
	1:3	F3	200	600	70	140
	1:4	F4	200	800	90	180
	1:5	F5	200	1000	100	200

Method of Microspheres preparation

HPMC K4M microspheres were prepared by spray-drying technique with formulation composition as given in table 1. Methanol and dichloromethane used in the ratio of (1: 2) as a solvent to prepare different drug/polymer ratio (from 1: 1 to 1:5) microspheres. Feed solution was prepared by dissolving the drug and polymer in the solvent. Atenolol containing microspheres were obtained by spraying the feed solution with a spray dryer (JISL, India) using a standard 0.7 mm nozzle. The process parameters of the spray drying technique were: Inlet

temperature 60°C –750°C, outlet temperature 40°C –55°C, aspirator speed 40–50% and feed pump speed 9–10 ml/min[3].

Evaluation of microspheres

IR spectroscopy

The infrared absorption spectra of the Atenolol, Atenolol loaded microspheres and blank microspheres of complex with HPMC were obtained using a FT-IR spectrophotometer FTIR (Thermo Electron co. IR 200®)[4].

Particle size analysis

The mean particle size of the microspheres was measured using optical microscope (Olympus CX31). The microscope was equipped with the software Magnus pro 3.0 and Olympus master through a camera [5].

Morphology

The morphology of the microspheres was examined by scanning electron microscopy (JSM 6390 India). The sample was mounted on to an aluminum stub and sputter-coated for 120 s with platinum particles in an argon atmosphere [6].

In vitro Release of atenolol from the microspheres

The drug release test was carried out using a nasal diffusion cell microspheres loaded with Atenolol was placed in the reservoir tube, 100 ml of a release medium is kept and stirred at 100 rpm at 37° C the release media was of pH 6.8 phosphate buffer solution. An aliquot of the release medium was withdrawn at predetermined time intervals and an equivalent amount of fresh medium was added to the release medium. The samples were analyzed by UV spectrophotometer (UV- 1700 Shimadzu, Japan) at 258 nm [11].

X-Ray Diffraction study (XRD)

The crystallinities of Atenolol, Atenolol loaded microspheres and blank microspheres were evaluated by XRD measurement using an x-ray diffractometer (Brucker Axs, 08 Advance). All samples were measured in the 2ø angle range between 3–80° and 0.010 step sizes [8].

Measurement of adhesive force

By falling liquid film technique mucoadhesive microspheres were tested for adhesive force. A freshly cut piece of sheep nasal mucosa (10 cm long) was used. The mucosa was cleaned by washing with isotonic saline solution. A weighed amount of microspheres was placed on mucosal surface, attached over aluminum plate that was fixed in an angle of 45° relative to the horizontal plane. The phosphate buffer pH 6.6 was warmed at 37°C was peristaltically pumped at a rate of 5ml/min. After one hour perfusate was analysed for drug content. The adhered microspheres amount was estimated from the difference between the applied microspheres and the flowed microspheres amount. The ratio of the adhered microparticles was computed as percentage mucoadhesion. [9]

Histopathological study

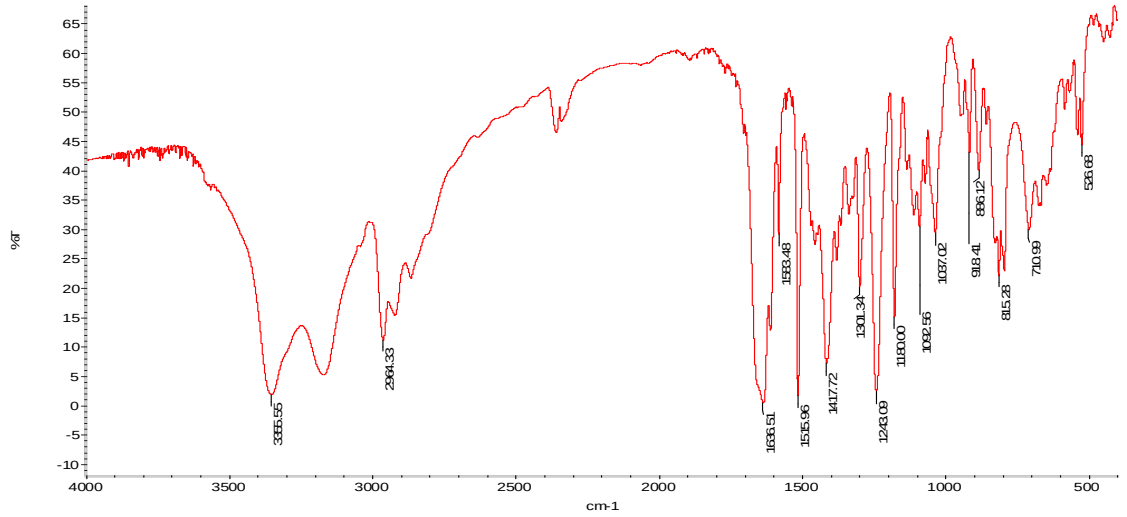
The histopathological evaluation of tissue incubated in phosphate buffer (pH 6.8) for 6 h after collection was compared with tissue incubated in diffusion chamber with formulation. Tissue

was fixed in 10% buffered formalin, routinely processed and embedded in paraffin. Sections were cut on glass slides and stained with hematoxylin and eosin. Sections were examined under a light microscope to detect and damage to the tissue [10].

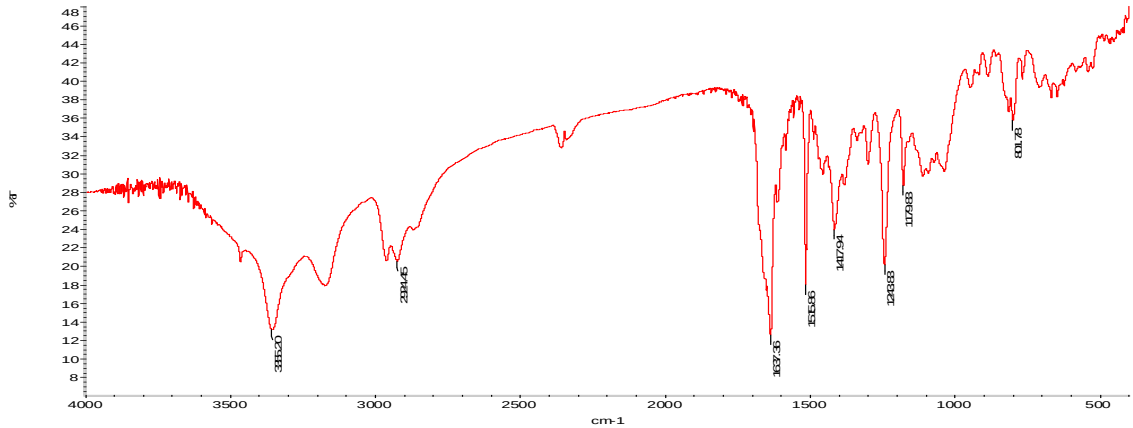
Ex vivo drug release study

The optimized formulation F1 studied for ex vivo release, by using sheep nasal mucosa within 1 hr as that obtained from the local slaughter house. The small section of nasal mucosa is attached to the glass tube of the nasal diffusion cell at the down side and kept the tube in position to just touching the buffer media in the cell. Further procedure of the study is similar to that of in vitro method [11].

Atenolol



Atenolol loaded microspheres



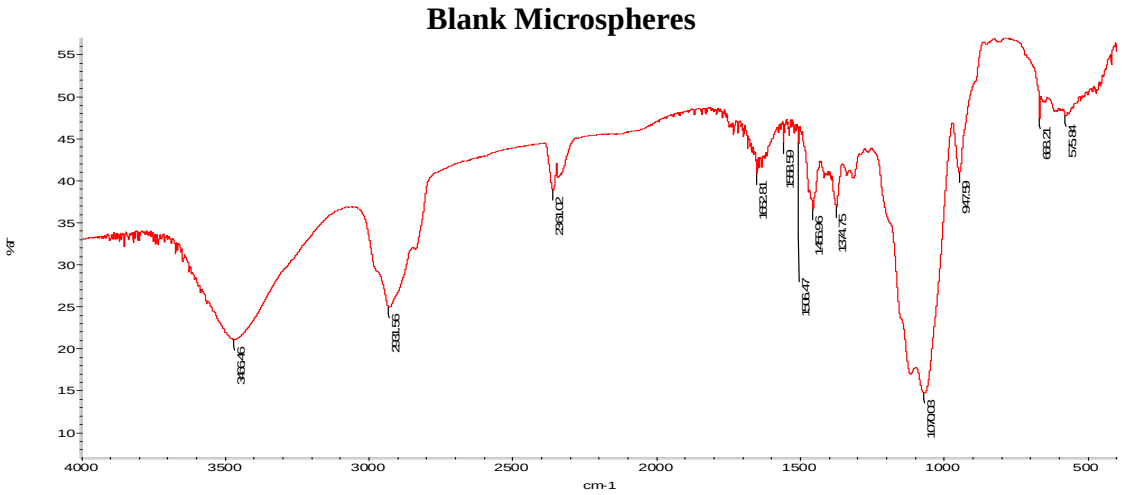


Figure 1: IR spectra of, atenolol, drug loaded microspheres and blank microspheres.

RESULTS AND DISCUSSION

IR spectroscopy

IR analysis confirms the drug and by analysis of the formulation reveals the presence of drug in the polymer complex. The graph of drug loaded microspheres reveals the characteristic peaks of the drug and the polymer which finds there is no any sort of interaction between the drug and polymer (Fig, 1).

Particle size analysis

A microscopic image analysis technique for determination of particle size is used, the average particle size of microspheres ranged from 25–50 mm, and such particles are considered to be suitable for nasal administration. It was also noted that with the increase in the drug:polymer ratio there was a slight increase in the size of microspheres (Table 2). The images of the scanned field are analyzed by the software. In all measurements at least 100 particles were examined (Fig.2).

Figure 2: optical microscopic image of microspheres of optimized formulation F1

Morphology

The morphology of the microspheres of optimized formulation F1 was examined by scanning electron microscopy. The SEM revealed a spherical shape with a rough surface morphology (Fig. 3). The inside of the microspheres was completely filled, indicating that the complexation had occurred.

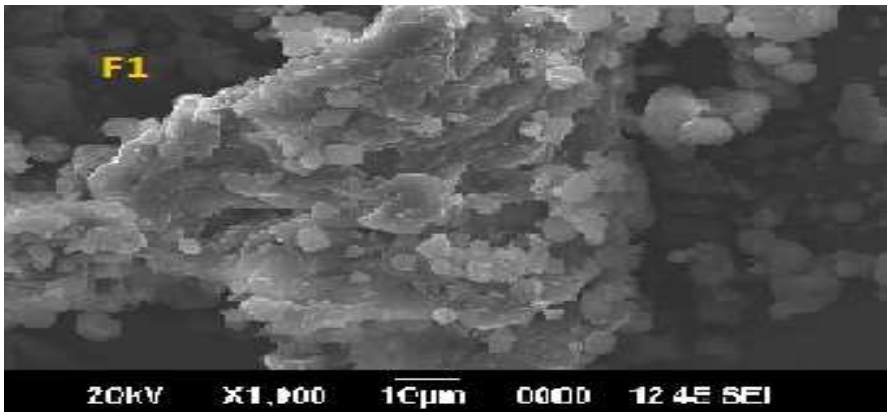


Figure 3: Morphology by SEM of optimized formulation F1

In vitro drug release

The microspheres bearing HPMC K4M and atenolol were spherical in shape and in the range of desired particle size. The microspheres swelled when in contact with moisture and released the drug, Fig. 4. Shows the release rates of the HPMC loaded microspheres in phosphate buffer 6.8. After 6 hr study it is found that the release of atenolol from the HPMC microspheres was in the range of 80-90% Korsmeyer peppa's model**.**

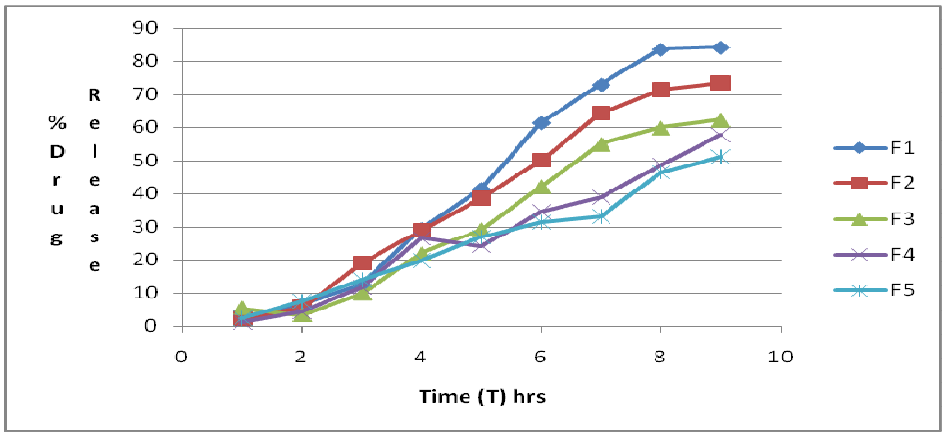
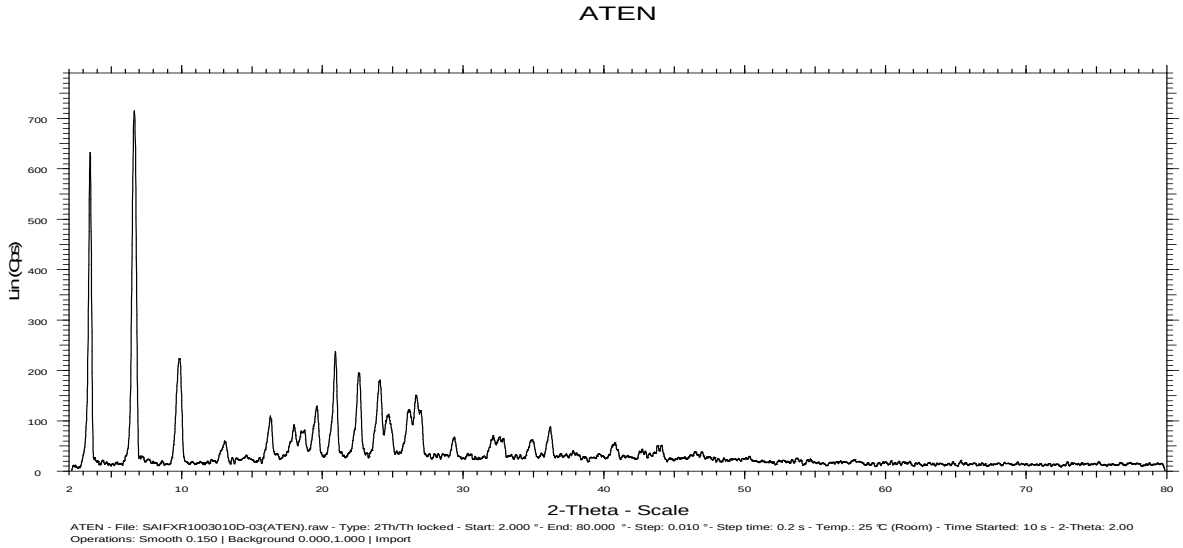


Figure 4: In vitro % drug release study

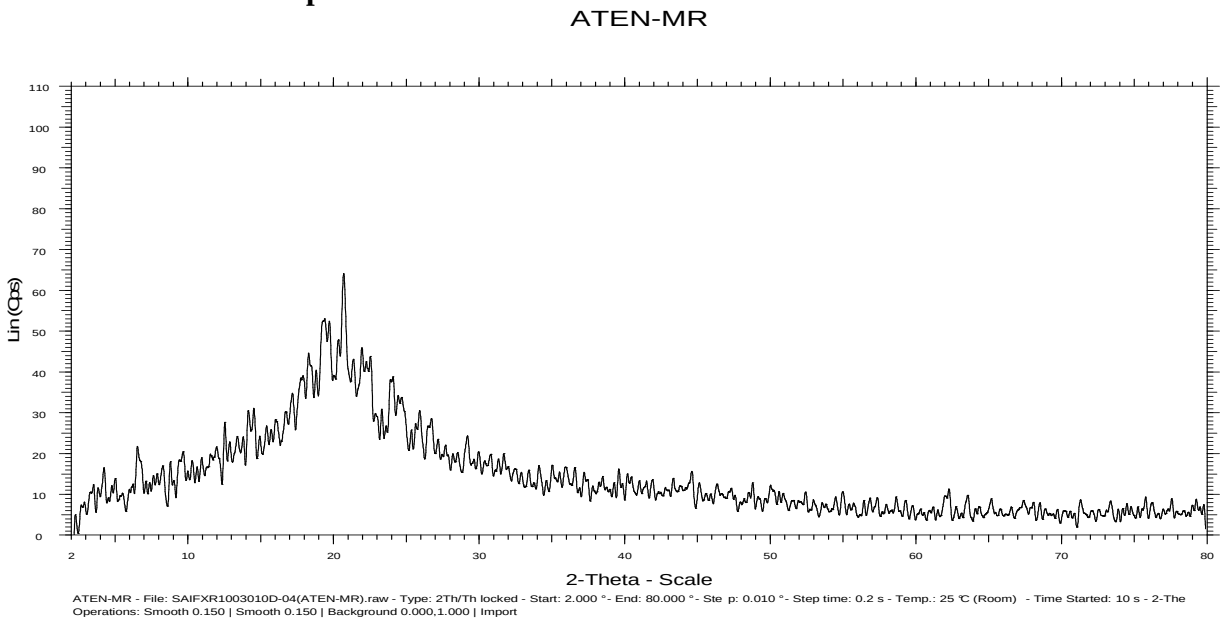
X-ray Diffraction study (XRD)

The X-ray diffraction spectra's were recorded for atenolol blank microspheres and drug loaded microspheres for investigating the crystallanity of the drug in the polymeric microspheres (Figure 5). The X-ray diffractogram of atenolol showed sharp peaks at diffraction angle 8.95◦ depicting a typical crystalline pattern. Blank microspheres showed less intense peaks, however atenolol loaded microspheres showed peaks, but of low intensity, indicating that some amount of drug was converted to amorphous form.

Atenolol



Atenolol loaded microspheres



Blank Microspheres

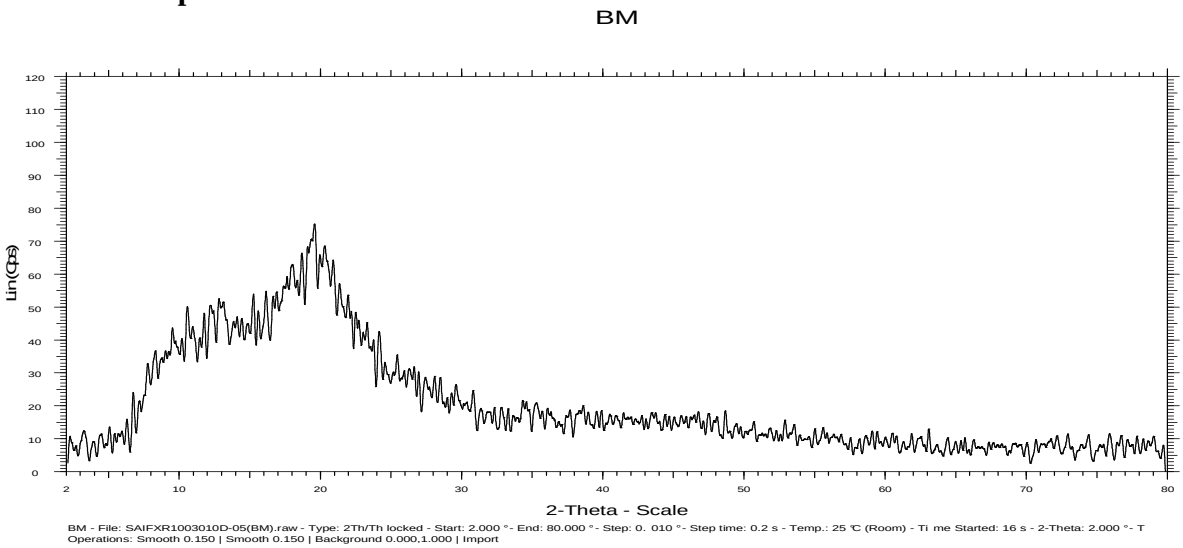


Figure 5: X-ray diffractogram of blank microspheres, drug loaded microspheres and atenolol

Measurement of adhesive force

Mucoadhesion studies were carried out to confirm the adhesion of formulation to the nasal mucosa for a prolonged period of time at the site of absorption. Results showed that the microspheres adequately adhere on nasal mucosa. The ratio of the adhered microspheres was expressed as percentage mucoadhesion. For all batches, percentage of mucoadhesion ranged from 80–90% (Table 2).

Table 2: Characterization of microspheres

Formulation code	Particle Size (in micron)	% Mucoadhesion
F1	25.27±0.97	80.12±1.25
F2	31.54±1.44	86.35 ±0.72
F3	38.64±1.82	88.98±1.35
F4	42.55±2.16	89.68±2.11
F5	47.88±1.54	91.24 ±0.21
F6	49.10±1.79	92.13 ±0.36

Histopathological study

With histopathological evaluation of the optimized formulation it was concluded that the formulation does not harms the nasal mucosa. The cell linings and the tissues were not destructed by the formulations (Fig.6).

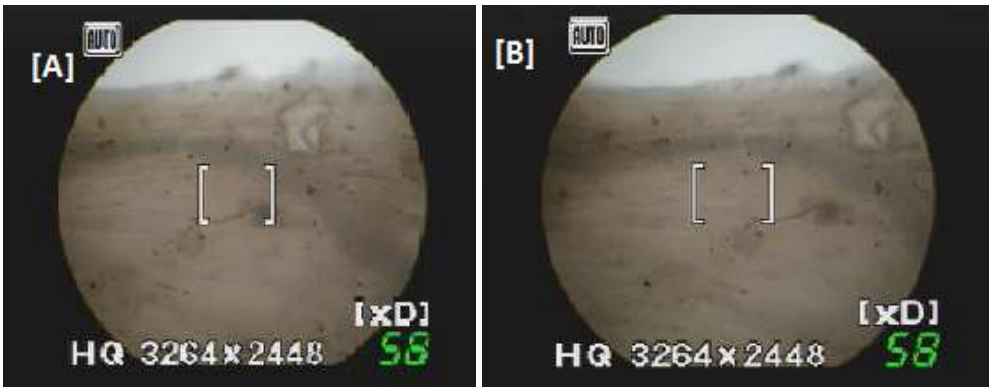


Figure 6: A) is untreated nasal mucosa, B) is treated nasal mucosa with f1 formulation

Ex vivo drug release study

The sample obtained were analysed by UV spectrophotometer at 258nm. The Fig.7 shows the release pattern of optimized formulation F1. The studies revealed a similar pattern of release of formulation performed with sheep nasal mucosa and that with dialysis membrane.

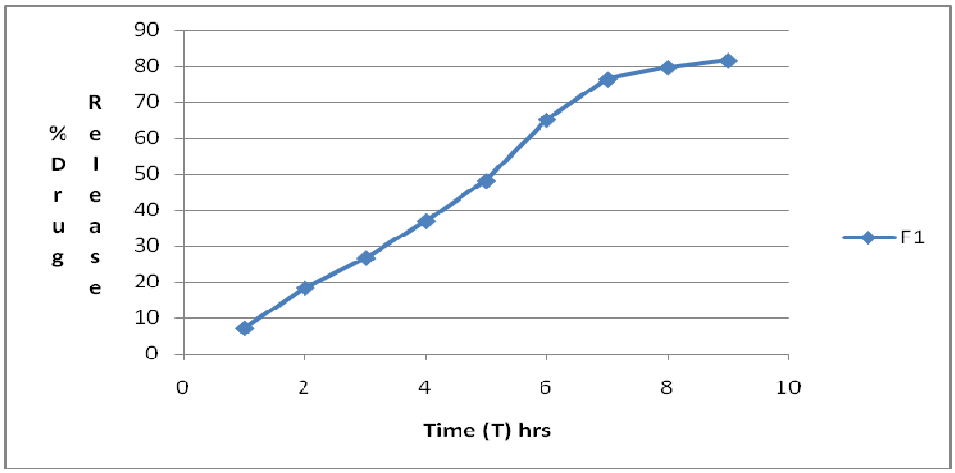


Figure 7: Ex vivo drug release study

CONCLUSION

A mucoadhesive microsphere was prepared by a spray drying technique. The release rate of the complex microspheres was significantly decreased with increase in the drug to polymer ratio. The results of the study indicate that HPMC K4M polymer is suitable for the development of mucoadhesive microspheres as a nasal drug delivery system for antihypertensive action. The designed drug and polymer system also holds promise to further study i.e. in vivo studies leading to IVIVC for commercialization.

REFERENCES

[1] Goodman and Gilman's "Manual of Pharmacology and therapeutics" X ed.**2001** published by Mc graw Hill pp. 256,

[2] Remington, The Science and Practice of Pharmacy, 21th edition, Vol. II, **2005**, pp. 1401.
[3] Snehal A. Jain a; Dheeraj S. Chauk a; Hitendra S. Mahajan a; Avinash R. Tekade; Surendra G. Gattani, *Journal of Microencapsulation*, **2009**, 1–11.
[4] Patil P.B. *International Journal of PharmTech Research*, Vol.1, No.3, pp 639-643, July-Sept **2009**.
[5] Hitendra Shaligram Mahajan and Surendra Ganeshlal Gattani Gellan, *Chem. Pharm. Bull.* **57**(4) 388—392 (**2009**): Vol. 57, No. 4
[6] Singh, U.V., Udupa, N., **1997**. *Pharm. Acta Helvetiae* 72, 165–173.
[7] Pisal S, Shelke V, Mahadik K, Kadam S. *AAPS Pharm Sci Tech* **2004**;5:63
[8] Hitendra S. Mahajan a; Surendra G. Gattani, *Pharmaceutical Development and Technology*, **2009**; 14(2): 226–232.
[9] Saraparn H, Vimolmas L, Narueporn S, Garnpimol CR. *AAPS PharmSciTech*. **2006**; 7:E1–10.
[10] Rita J. M., Pradip K. G., Manish L. U., Rayasa S. R., *AAPS Pharm- SciTech*, **7**, E1—E7 (**2006**).
[11] Dandagi, PM, Mastiholimath, VS, Gadad, AP, Iliger, SR. (**2007**) *Indian Journal of Pharmaceutical sciences*, 69, 402- 7.
[12] A. Martinaca et al, *International Journal of Pharmaceutics* 291 (**2005**) 69–77.
[13] Jin Yang Yu, Xiao Ling Hu, Ren Yuan Song, Shan Xi, *Advanced Materials Research* (Volumes 148 - 149), October:**2010**, 1192-1198
[14] Erik Bjork and Peter Edman, *International Journal of Pharmaceutics*, Volume 62, Issues 2-3, 31 July **1990**, Pages 187-192.
[15] A. V. Yadav and H.H. More, *Indian journal of pharmaceutical sciences* Volume: 70, **2008** Mar-Apr
[16] Zhao Quan, Deng Shu-hai et al, *Chinese Journal of Hospital Pharmacy*, ***2003****-05*
[17] Juan JT, Alfredo GA, Santiago TS, Luis G. *Bio. Pharma. Bull*. **2001**;24:1411–1416
[18] S. Dimova et al, *Toxicology in Vitro* 19 (**2005**) 107–122.