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Research Article

PLGA Based Mucoadhesive Microspheres for Nasal Delivery: *In Vitro / Ex Vivo* Studies

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ABSTRACT

In this study, a novel mucoadhesive microsphere for nasal administration was developed and investigated. The carvedilol loaded PLGA microspheres were prepared by a spray-drying technique that can be proposed as an alternative to the conventional methods for preparation of microspheres. The microspheres were evaluated with respect to the production yield, particle size, incorporation efficiency, swelling property, *in vitro* mucoadhesion, *in vitro* drug diffusion, *ex-vivo* permeation study, histopathological examination and stability study. Microspheres were characterized by differential scanning calorimetry, scanning electron microscopy and X-ray diffraction study to gain insights into the nature of interaction between the drug and polymer. It was found that the particle size, swelling ability and incorporation efficiency of microspheres increases with increasing drug to polymer ratio. Microspheres show adequate mucoadhesion and do not have any destructive effect on nasal mucosa. The results of morphological examination indicated that the PLGA 50:50 microspheres possessed a smooth and spherical appearance with average particle size of 10-25 µm. Release profiles of carvedilol from the microspheres displayed an anomalous transport mechanism. The investigations of these microspheres as vehicle for nasal delivery showed that PLGA microspheres may be considered as a promising nasal delivery system.

INTRODUCTION

PLGA polymers are widely used in the preparation of microspheres since, they are non-toxic, well-tolerated by the human body, biodegradable, biocompatible and drug release rate can be controlled by selection of their molecular weight and copolymer composition [1].

Poly (lactide-co-glycolic acid) (PLGA), a copolymer of poly (lactic acid) and poly (glycolic acid), has been studied extensively as a polymeric carrier for biodegradable microspheres. Thus, mainly due to their long history of safe human use in the form of surgical sutures and their commercial availability in various monomer ratios and molecular weights, a wide variety of drugs ranging from small molecular weight therapeutic agents to peptide hormones, antibiotics and chemotherapeutic drugs have been studied using these biodegradable polymers.

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PLGA microspheres have proved to be successful drug delivery systems for incorporating different classes of drugs, such as NSAIDs, peptides like LHRH agonists and steroid hormones[2]. By selection of the appropriate polymer composition with a known rate of degradation, the polymers can be exploited to produce a drug delivery system which releases an active agent at a predetermined rate.

Carvedilol (CV) ((±)-1-carbazol-4-yloxy)-3-[[2-(*o*-methoxyphenoxy) ethyl] amino]-2-propanol) is a β/α_1-adrenoceptor blocker used to treat moderate hypertension, angina pectoris and congestive heart failure[3]. It is well absorbed from the gastrointestinal tract but is subject to considerable first-pass metabolism in the liver; its absolute bioavailability is 25%.Several methods were used to prepare PLGA microspheres such as emulsion solvent evaporation, phase separation and spray drying method. Among all of this spray drying method is widely used to prepare microspheres. It is more advantageous as a one step process having high drug loading capacity and reproducibility.

Moreover, the process is more feasible for scaling up as compared to other microsphere fabrication methods[4].

The major objective of present study is to develop mucoadhesive microspheres by spray-drying method. The prepared mucoadhesive microspheres were evaluated in terms of production yield, particle size, drug loading, incorporation efficiency, swelling property, *in vitro* mucoadhesion, *in vitro* drug release, *ex vivo* permeation study, histopathological study and stability study. Prepared microspheres were characterized by differential scanning calorimetry, scanning electron microscopy and X-ray diffraction study.

MATERIALS AND METHODS

Carvedilol is kind gift from (Zydus Health Care Pvt. Ltd., Ahmadabad, India). Poly(d, l-lactide-co-glycolide acid) (PGLA 50:50) gift sample from Resomer RG-502H, inherent viscosity = 0.21 dl/g (Purac biomaterial, Gorinchem, Nederland). Methylene chloride were procured from Loba Chemie (Mumbai, India) and used as received. All other solvents and chemicals used were of analytical grade.

Preparation of Carvedilol loaded PLGA microspheres

Carvedilol loaded microspheres were prepared using PLGA 50:50 in five different drug to polymer ratios. Compositions were reported in Table 1. PLGA 50:50 was dissolved in 2% methylene chloride solution, carvedilol was added to above polymer solution. Microspheres were obtained by spraying the feed with spray drier (LU-222 Advanced, Labultima, India) using a standard 0.7 mm nozzle. When the liquid was fed to the nozzle with a peristaltic pump, atomization occurred by the force of the compressed air, disrupting the liquid into small droplets. The droplets together with hot air were blown into a chamber where the solvent in the droplets was evaporated and discharged out through an exhaust tube. The dry product was then collected in a collection pot. The spray drying conditions, inlet temperature, spray flow, compressed spray air flow (represented as the volume of the air input) and spray pressure were set at 60-70°C, 5-6 mL/min, 10 ml/min, 2 Kg /cm^2, respectively.

Table 1: Formulation composition of mucoadhesive microspheres

Polymer	Formulation Code	Drug: polymer ratio	Formulation composition	
			Drug (%W/W)	Polymer (%W/W)
PLGA 50:50	F1	1:1	50	50
	F2	1:1.5	39.99	59.85
	F3	1:2	33.30	66.70
	F4	1:3	25.00	75.00
	F5	1:4	20.00	80.00

PLGA, Polylactide co-glycolic acid

Characterization of mucoadhesive microspheres

Production Yield

The production yield of microspheres of various formulations were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microspheres and percent production yields were calculated as per the formula mentioned below[5].

$$\text{Production yield} = \frac{\text{Practical mass of microspheres}}{\text{Theoretical mass of drug and polymers}} \times 100 \quad \ldots (1)$$

Particle size analysis

The microspheres were evaluated for the particle size. An optical microscope (DMWB2-223, Motic, China) was used for this purpose. The microscope was equipped with the software, image manager through a camera. Analysis was carried out on the spray-dried microspheres dispersed in immersion oil. This slide was observed under the microscope. An image was clicked and used for the particle size analysis. The average particle size of the microspheres was expressed as the volume surface diameter; dvs (μm) [6] and standard deviation σ was calculated for each batch of microspheres.

Drug loading and incorporation efficiency

The weighed amount of microspheres were dissolved in distilled water and kept overnight. The drug content was measured

spectrophotometrically at 241 nm for CV. The drug loading and incorporation efficiency (%) were calculated by using equations (2) and (3), respectively.

Drug loading (%) =

$$\frac{M_{actual}}{\text{Weighted quantity of microsphers}} \text{ X } 100 \quad \text{.... (2)}$$

Incorporation efficiency (%) =

$$\frac{M_{actual}}{M_{therotical}} \text{ X } 100 \quad \text{.... (3)}$$

Where M_{actual} is the actual CV content in weighed quantity of powder of microspheres and $M_{theoretical\ is}$ the theoretical amount of CV in microspheres calculated from the quantity added in the spray-drying process[7].

Swelling property

The swelling ability of the microspheres in physiological media was determined by allowing them to swell to their equilibrium. Accurately weighed amount of microspheres (10 mg) were placed on Millipore filter (NY 11 0.22 µm) using a Franz diffusion cell (12.5 mL) with phosphate buffer (pH 6.6) and kept for 10 minute[8].

The following formula was used for calculation of degree of swelling.

$$\alpha = (Ws - Wo) / Ws \text{ ..... (4)}$$

Where, α = Degree of swelling, Wo=Initial weight of microspheres
and Ws = Weight of microspheres after swelling.

***In vitro* mucoadhesion study**

The mucoadhesive property was determined by falling liquid film technique. A freshly cut piece, 5 cm long of sheep nasal mucosa obtained from a local abattoir within 2 hour of killing the animal was cleaned by washing with isotonic saline solution. Accurately weighed quantity of microspheres was attached over a polyethylene plate that fixed in an angle of 45^0 relative to the horizontal plane, and pH 6.6 phosphate buffer warmed at 37^0C was peristaltically pumped at a rate of 5 mL/min over the tissue.[8] After 1 hour of administration of microspheres the concentration of the drug in the collected perfusate was measured spectrophotometrically. The microspheres amount corresponding to the drug amount in perfusate was calculated. The adhered microspheres amount was estimated from the difference between the applied microspheres and the flowed microspheres amount. The ratio of the adhered microspheres was computed as percent mucoadhesion[9] using following equation-(5)

% Mucoadhesion =

$$\frac{\text{Amount of drug in washout liquid}}{\text{Actual amount of drug in applied microparticles}} \text{ X } 100 \quad \text{.... (5)}$$

***In vitro* drug diffusion studies**

An in vitro drug release test of the microspheres was performed using Franz diffusion cell with dialysis membrane (cut-off Mol. Weight. 12000). The membrane was equilibrated before carefully dispersing the sample equivalent to 3.125 mg of drug onto the donor compartment. The donor compartment contained microspheres and receiver compartment was filled with phosphate buffer solution (pH 6.6) and maintained at 37°C ± 0.5°C. Samples were periodically withdrawn from the receptor compartment, replaced with the same amount of fresh buffer solution, and assayed by a spectrophotometer at 241 nm (UV-1700, Shimadzu®, Japan)

Drug release kinetics

To study the release kinetics of optimized formulation (F1), data obtained from *in vitro* drug release studies were plotted in various kinetic models: zero order (Equation 6) as cumulative amount of drug released Vs time, first order (Equation 7) as log cumulative percentage of drug remaining Vs time, and Higuchi's model (Equation 8) as cumulative percentage of drug released Vs square root of time.

Zero order

$$C = K_0 t \text{ ..... (6)}$$

Where K_0 is the zero-order rate constant expressed in units of concentration/time and t is the time in minutes. A graph of concentration Vs time would yield a straight line with a slope equal to K_0 andintercept the origin of the axes.

First order

$$\text{Log } C = \text{Log } C_0 - Kt/2.303 \text{ ..... (7)}$$

Higuchi Model

$$Q_t = Kt^{½} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (8)$$

Where Q_t is the amount of drug release in time t, K is the kinetic constant and t is the time in minutes.

Mechanism of drug release

To evaluate the mechanism of drug release from the CV loaded microspheres, data for the drug release was plotted in Korsmeyer- Peppas equation as log cumulative percentage of drug released Vs log time (Equation 9). The release exponent n and K value was calculated through the slope of the straight line.

$$M_t / M_\infty = Kt^n \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (9)$$

Where M_t represents amount of the released drug at time t, M_∞ is the total amount of drug released after an infinite time, K is the diffusion characteristic of drug/polymer system constant and n is an exponent that characterizes the mechanism of drug release. The value of n indicates the drug release mechanism from the delivery system. If the exponent n=0.5 then the drug release mechanism is Fickian diffusion, if n<0.5 the mechanism is quasi Fickian diffusion, if n is 0.5<n<1.0 then it is non-Fickian or anomalous diffusion, if n=1.0 mechanism is non Fickian case II diffusion and if n>1.0 mechanism is non Fickian super case II[11].

Scanning electron microscopy (SEM)

The morphology of the optimized formulations (F1) was studied using a scanning electron microscope (JSM 6390, JEOI, USA) operated at an accelerating voltage of 10 kV.

X-ray diffraction (XRD) study

X-ray diffractogram of the plane drug, blank microsphere and drug-loaded microsphere were recorded by a diffractogram (Brucker AXS 08, Advance®) using Cu-Ka line as a source of radiation which was operated at the voltage 35 kV and the current 25 mA. All samples were measured in the 2θ angle range between 3^0 and 80^0 and 0.010 step size[4].

Differential scanning calorimetry (DSC)

The thermal behavior of pure drug, drug-loaded microspheres and blank microspheres were studied using a differential scanning calorimeter (DSC 60, Shimadzu, Japan) at a heating rate of 10^0C min^{-1}. The measurements were performed at a heating range of 30-400^{0}C under nitrogen atmospheres.

***Ex vivo* permeation studies**

Fresh nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse.Tissue samples were inserted in Franz diffusion cells displaying a permeation area of 3.14 cm^2. Phosphate buffer (pH 6.6) was added to the acceptor chamber. The temperature was maintained at 37^{0}C. After a pre-incubation time of 20 minutes, formulation equivalent to 3.125 mg of carvedilol was placed in the donor chamber. At predetermined time points, 1-mL samples were withdrawn from the acceptor compartment, replacing the sampled volume with PBS pH 6.6 after each sampling, for a period of six hours[12].

The samples withdrawn were filtered and used for analysis. Blank samples (without Carvedilol) were run simultaneously throughout the experiment to check for any interference. The amount of permeated drug was determined using a UV-visible spectrophotometer at 241 nm. Permeability coefficient (P) was calculated by following formula

$$P = (dQ/dt) / (C_o \times A) \quad \ldots\ldots\ldots \quad (10)$$

Where, dQ/dt: Flux or Permeability rate (mg/ hr),

C_o: Initial concentration in donor compartment,

A: Effective surface area of nasal mucosa.

Histopathological examination of nasal mucosa

The histopathological evaluation of tissue incubated in phosphate buffer (pH 6.6) 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.

Stability Studies

The microsphere batches of optimized formulation were stored in a stability chamber (Remi CHM-10S®) at 40°C and 75% RH for 3 months and observed for the drug content at 1 month intervals. The drug content was determined after the study using the method of determination of drug loading.

RESULTS

The spray-drying method described here appeared to be a suitable and simple technique to prepare PLGA microspheres loaded with carvedilol. It is a one step process, easy and rapid, as it combines drying of the feed and embedding of the drug into a one step operation.

Characterization of mucoadhesive microspheres

Production Yields

Production yield was found in the range between 35 to 50 % (Table 2). These relatively low values may be because of the low quantity of feed used for the preparation of each batch and by the structure of the spray dryer apparatus that lacked a trap to capture the smallest and lightest particles[13].

Table 2: Production yield and particle size of microspheres

Formulation Code	Production Yield (%)	Average Particle Size (µM ± SD)#
F1	35.19	15.10 ± 1.29
F2	47.43	18.61 ± 2.25
F3	50.66	19.73 ± 1.23
F4	43.21	22.20 ± 1.35
F5	49.81	23.40 ± 1.52

#denotes average of 100 particles ± SD

Particle size analysis

Average particle size of microspheres ranged from 10 to 25 µm**,** such particles are considered to be suitable for nasal administration[14]. It was also noted that increasing drug to polymer ratio, slightly increased the size of microspheres (Table 2).

Drug loading and incorporation efficiency

Incorporation efficiency was high since it always exceeded 90%. An increasing the ratio of drug to polymer, the drug loading of microspheres was increased (Table 3).

Swelling Studies

Swelling capacity of the microspheres was mostly determined by polymer content in the preparation, since mucoadhesive polymers were the only component in the spray dried system with swelling abilities. The maximum degree of swelling was observed 0.83 and 0.89 for formulation F4 and F5 respectively (Table 3). Microspheres with lower polymer content (F1& F2) were characterized by lower swelling ability than the microspheres with higher polymer content (F4 & F5). This shows that increase in polymer ratio significantly increase the swelling ability of microspheres.

In-vitro mucoadhesion study

Mucoadhesion studies were carried out to ensure the adhesion of formulation to the 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 percent mucoadhesion. For all batches, the percentage of the originally applied mass of microspheres attached to the nasal mucosa range from about 80-97 % (Table 3).

In vitro drug release study

The release profile of CV from various batches of PLGA (50:50) microspheres at pH 6.6 phosphate buffer showed in Figure 1. The release pattern of all the formulations appears to be slow release with negligible or no burst effect. Cumulative percent drug release from microspheres using PLGA (50:50) polymer after 6 hour is shown in Figure 3. For nasal delivery too sustained release of drug is undesirable hence formulation (F1) is considered as suitable formulation and hence selected for further studies.

Drug release kinetics

To investigate the drug release mechanism, the release data were fitted to models representing zero order, first-order, and Higuchi's square root of time. The examination of coefficient of determination values indicated that the drug release from microspheres follow initial zero order followed by first order release kinetics. Optimized formulation (F1) follow anomalous transport mechanism for drug release (n= 0.695, k=1.819).

Scanning Electron Microscopy (SEM)

The optimized formulation blank and loaded microspheres were analyzed by SEM for studying particle shape and surface morphology (Figure 2).Microspheres are spherical in shape and possessed a smooth surface.

Table 3: Characterization of carvedilol loaded PLGA microspheres

Formulation Code	Drug loading* (% ± SD)	Incorporation Efficiency* (% ± SD)	Swelling index* (% ± SD)	Mucoadhesion* (% ± SD)
F1	47.63 ± 0.94	95.68 ± 1.09	0.60 ± 0.62	85.17± 1.03
F2	38.51 ± 1.22	96.85 ± 0.95	0.69 ± 0.86	87.82± 1.27
F3	32.26 ± 0.88	97.40 ± 0.72	0.78 ± 1.42	90.52 ± 1.14
F4	24.64 ± 1.28	98.43 ± 0.96	0.83 ± 1.79	93.56 ± 1.24
F5	20.13 ± 0.35	100.79 ± 1.46	0.89 ± 2.33	96.08 ± 0.94

*Value expressed as Mean ± SD, n=3

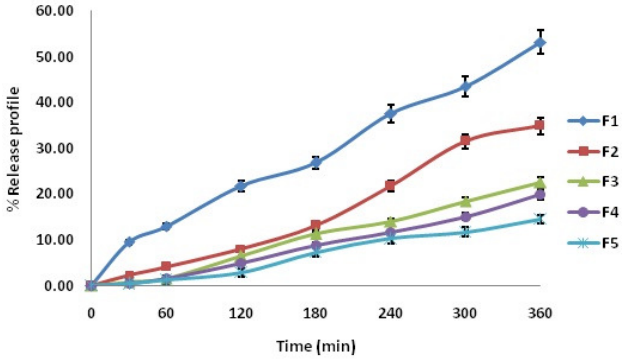

Figure 1: Drug release profile of CV microsphere.

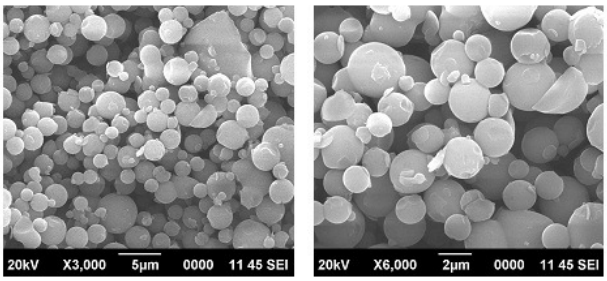

Figure 2: SEM image of CV loaded microspheres formulation.

X-ray diffraction study (XRD)

The X-ray diffraction spectra were recorded for pure CV, blank microsphere and drug loaded microsphere for investigating the crystallanity of the drug in the polymeric microspheres (Figure 3). The X-ray diffractogram of CV has sharp peaks at diffraction angle 8.107^0, 11.206^0, 12.347^0, 17.024^0, 18.157^0, 20.241^0, 21.687^0 and 26.235^0 which shows a typical crystalline pattern. Blank microspheres showed less intense peaks however, CV loaded microspheres shown peaks with low intensity indicating that partial conversion to amorphous form of drug as revealed by XRD studies.

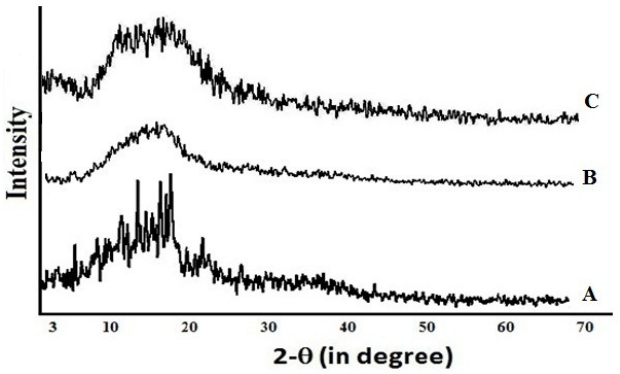

Figure 3: X-ray diffractograms of: (a) pure carvedilol; (b) carvedilol loaded microspheres; (c) blank microspheres.

Differential scanning calorimetry (DSC)

The DSC thermogram of drug (CV), drug loaded microspheres and blank microspheres are shown in Figure 4. The thermogram of CV exhibited sharp endothermic peak at 116.64-120.24^{0}C indicated melting point which was reported in literature. Characteristic peak of CV was well recognized in the drug loaded microspheres. Thus there was no interaction between CV and PLGA (50:50). Further, the decrease in intensity of CV endothermic peak in drug loaded microspheres may be due partial conversion of CV from crystalline to amorphous form. DSC studies revealed that carvedilol were molecularly dispersed inside of the microspheres.

Ex vivo permeation study

The optimized formulation (F1) of CV loaded microspheres was subjected to *ex vivo* permeation studies using sheep nasal mucosa. The percent drug permeated after 6 hours was found to be 45.31%. The permeability coefficient (P) was also calculated and found to be 0.00238 mg/hr for F1.

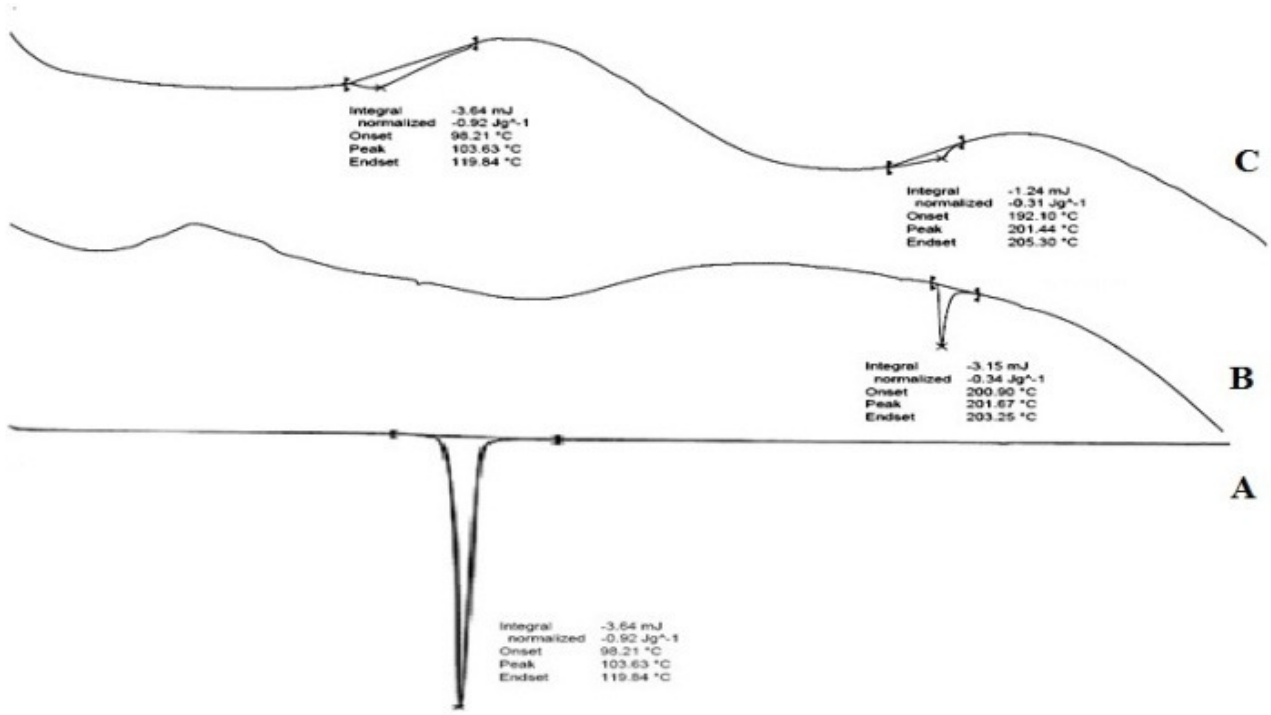

Figure 4: DSC of Carvedilol (A), blank microspheres (B) and drug loaded microspheres(C).

Histopathological study

The microphotographs were taken of nasal mucosa following incubation with microsphere formulations for more than 6 h (Figure 5). Examination of tissue showed ciliated respiratory epithelium and normal goblet cell appearance. None of the severe signs such as appearance of epithelial necrosis, sloughing of epithelial cells was detected. No change in mucosal structure was seen when treated with formulation F1 as compared to the control.

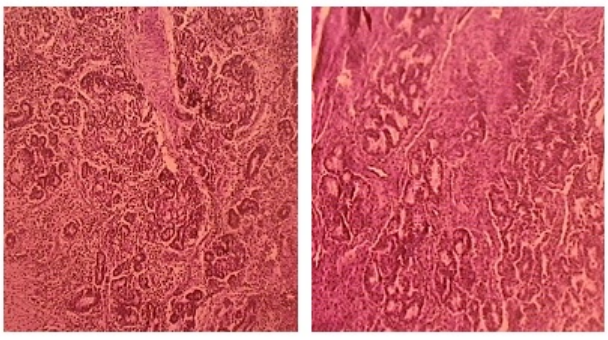

Figure 5: Light photomicrograph of the normal nasal mucosa (a) and microspheres treated mucosa (b).

Stability Studies

Formulations showing optimum mucoadhesive strength and drug content were selected for stability studies. According to ICH guidelines, a selected formulation (F1) was stored at 40°C temperature and 75% relative humidity (RH) for a period of 3 months. Formulations were evaluated at periodical intervals of 1 month for drug content, drug release and percentage mucoadhesion. Drug loss was minor, as observed after a month study. There was partial change in mucoadhesive strength.

DISCUSSION

These microparticles had no hole or rupture on the surface, such morphology would result in slow clearance and good deposition pattern in nasal cavity. The swelling study was an important at- tribute of studying clearance of drug from nasal cavity. It was suggested that administration of microparticles lowers clearance of the microparticles systems which may be probably due to the fact that the microparticles undergo a process of taking up water and swelling, which results in polymer/mucus mixture leading to reduced mucocilliary clearance. 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 high permeation rate and short lag time were thought to be due to the nasal cavity property in which it has a relatively high surface area and rich vasculature. From the stability studies of the optimized batches it was found that the microspheres remained stable even after

exposing to stress conditions of temperature and moisture.

In this study, mucoadhesive microspheres of carvedilol using PLGA (50:50) were developed using spray drying technique. At the body temperature these formulations could transform to hydrogels .The resultant gel formation decreases ciliary clearance rate and as a consequence the residence time of the formulation at the nasal mucosa is prolonged. The mucoadhesive properties of microspheres were attributed to spontaneous gel formation on nasal mucosa. PLGA is a biocompatible polymer, it does not cause any deleterious effect or toxic response in the nasal mucosal cavity even if used for prolonged periods was evaluated by histopathological studies. These results demonstrated that PLGA microspheres were potential to be used as a vehicle for the nasal delivery of carvedilol. However extensive pharmacokinetics and pharmocodynamic studies are required to establish a correlation, if any, before establishing nasal delivery as an alternative.

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