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Research Article

Spray Dried Nasal Mucoadhesive Microspheres of Carbamazepine: In Vitro / Ex Vivo Evaluation

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Abstract

The aim of this study was to investigate the role of biodegradable mucoadhesive microspheres for nasal delivery of carbamazepine. Microspheres were prepared by spray drying technique using hydoxypropyl methylcellulose. Microspheres were evaluated for particle size, drug content, swelling ability, and production yield. The drug excipients compatibility was checked with infrared spectroscopy (IR) and differential scanning calorimetry (DSC). The spray dried Microspheres were also studied for polymorphism and shape by X ray diffraction (XRD) and scanning electron microscopy (SEM). The release of drug from microspheres followed Non fickian diffusion kinetics. Ex vivo studies was performed with sheep nasal mucosa for mucoadhesion, histopathological and drug release studies. The production yield of optimized formulation was 41.75±1.56% with mean particle size of 22.12±1.53 µm. SEM demonstrated spherical particles with smooth surface. Particle size, swelling ability and encapsulation efficiency of microspheres was increased with the increase in the drug:polymer ratio. Drug interaction studies using DSC confirmed no interaction between carbamazepine and HPMC K4M. XRD studies revealed amorphous nature of drug entrapped in microspheres. The histopathological study indicated intact nature of sheep nasal mucosal structure on treatment with microsphere. Microspheres adhered well on nasal mucosa with percentage mucoadhesion for all batches ranging from 80% to 90%. The microspheres were found to be stable when stored at 40°C temperature and 75% relative humidity (RH) for a period of 3 months, as per ICH guidelines. Thus, carbamazepine spray dried microspheres formulated with HPMC K4M is a promising nasal delivery system

Keywords: *Carbamazepine, mucoadhesive microspheres, HPMC, spray drying, nasal drug delivery.*

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Introduction

The nasal drug delivery finds the alternative way of targeting the drug to central nervous system (CNS) with the advantage of its high vascularisation [1], and faster onset of action. Carbamazepine is an antiepileptic drug which is used effectively in epileptic seizures; carbamazepine is having very low water solubility and classified under the class II of BCS classification (Poorly soluble / highly permeable). One of the most persistent problems faced by these drugs with poor aqueous solubility is that their oral delivery is frequently associated with implications of low bioavailability and lack of dose proportionality. Efforts are going on to enhance the oral bioavailability of such lipophilic drugs in order to increase their clinical efficacy [2]. The drawback of carbamazepine is slow and variable absorption because of its poor aqueous solubility [3]. Microspheres swell in contact with nasal mucosa and form a gel which controls the rate of clearance from the nasal cavity. In the presence of microspheres, the nasal mucosa is dehydrated due to moisture uptake by the microspheres. The result is reversible shrinkage of the cells, providing a temporary physical separation of the tight (intercellular) junction, which increase the absorption of the drug [4]. Considering the usefulness of mucoadhesive microspheres in enhancing the solubility and bioavailability with added advantage of avoidance of the first pass hepatic metabolism, mucoadhesive microspheres can be explored as drug delivery carrier for carbamazepine via nasal route. Recently, microsphere formulation approach has been used in designing formulations for nasal drug delivery. The primary intention behind selection of microspheres is to serve a better chance for the drug to be absorbed by allowing a large surface area and prolonged contact between the drug and the mucosal membrane. Being BCS class II drug, carbamazepine presents challenge to formulation development scientists to formulate it into a suitable drug delivery carrier. The aim of this study was to investigate the role of biodegradable mucoadhesive microspheres for nasal delivery of carbamazepine

Materials and Methods

Materials

Carbamazepine was supplied as a gift from CTX life science (Surat India). HPMC K4M was supplied as a gift sample from Colorcon Limited (Goa, India). Al other chemicals 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.

Method of Microspheres Preparation

HPMC K4M microspheres were prepared by spray-drying technique with formulation composition as tabulated 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 1:6) microspheres. Feed solution was prepared by dissolving the drug and polymer in the solvent by using magnetic stirrer (DX Remi). Carbamazepine loaded microspheres were obtained by spraying the feed solution with a spray dryer (JISL, India) using a standard 0.7 mm nozzle. The spray dried microspheres were harvested from the apparatus collector and kept under vacuum for 48 hours. The process parameters of the spray drying technique were: Inlet temperature 70°C –80°C, outlet temperature 50°C –60°C, aspirator speed 40–50% and feed pump speed 9–10 ml/min [5].

Evaluation

Evaluation of Microspheres

Production yield: The production yields were calculated as practical mass upon theoretical mass multiplied by 100

*Particle size analysis***:** The microspheres were evaluated for the particle size. An optical Microscope (Olympus CX31) was used. The microscope was equipped with the software, Magnus pro 3.0 and Olympus master through a camera. Analysis was carried out on the spray-dried microspheres dispersed in immersion oil [6]. The average particle size of the microspheres was expressed as the volume surface diameter (mm).

Scanning electron microscopy (SEM): The morphology of the optimized formulation F1 was studied using a scanning electron microscope (JSM 6390 India) operated at an accelerating voltage of 20 kV.

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 285 nm for carbamazepine [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 mm) using a Franz type diffusion cell (12.5 ml) with phosphate buffer (pH 6.6) and kept for 5 min. The degree of swelling is calculated by initial weight of microspheres minus final weight of microspheres after swelling upon final weight of microspheres after swelling [8].

In vitro drug release studies*:*

The in vitro drug diffusion study of the microspheres was performed using Nasal diffusion cell with dialysis membrane (Mw cut-off 13500–14 000) (Figure 2). The water jacketed recipient chamber had the total capacity of 60 ml and a flanged top of 3 mm, the lid has three openings, one each for sampling, thermometer and a donor tube chamber. The donor chamber was a 10 cm

long tube with internal diameter of 1.13 cm. The donor chamber tube was placed in such a way that it just touched the diffusion medium in the receptor chamber [10]. The receptor compartment contained phosphate buffer solution pH 6.6 maintained at 37°C±1°C. The membrane was equilibrated before carefully dispersing the microspheres or solution equivalent to 10 mg of drug onto the donor side. Samples were periodically withdrawn from the receptor compartment, replaced with the same amount of fresh buffer solution of same temperature and assayed spectrophotometerically (UV-1700 Shimadzu, Japan) at 285 nm [11].

Drug release kinetics: The release data were fitted to the PCP disso version 2.08 software. The statistical treatment of data was done by Design Expert software version 7.1.6 .

Mechanism of drug release: To evaluate the mechanism of drug release from the carbamazepine loaded microspheres, data for the drug release was plotted in Korsmeyer-Peppas equation as log cumulative percentage of drug released Vs log -time (Equation 1). The release exponent n and K value are calculated through the slope of the straight line.

$$\frac{M_t}{M_\infty} = Kt^n \quad \text{...........................} (1)$$

Where Mt 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 = 0.5–1.0 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 diffusion [12].

Ex-vivo evaluation

Mucoadhesion studies: The mucoadhesive property was determined by falling liquid film technique. A freshly cut piece of sheep nasal mucosa (5 cm long) was cleaned with isotonic saline solution. An accurate weighed amount of microspheres were placed on mucosal surface, which was attached over a aluminum plate that fixed in an angle of 45° relative to the horizontal plane and pH 6.6 phosphate buffer warmed at 37°C was peristaltically pumped at a rate of 5ml/min over the tissue. After one hour of applying of microspheres, the collected perfusate was diluted and the concentration of the drug was spectrophotometrically determined. The applied 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 microparticles was computed as percentage mucoadhesion [9].

Histopathological study: Sheep nasal mucosa obtained from a local abattoir within 2 hour of killing the animal was cleaned with isotonic saline solution. The nasal mucosa was fixed in 10% neutral carbonate buffered formalin solution, routinely processed and embedded in paraffin. Paraffin sections (7 µm) were cut on glass slides and stained with hematoxylin and eosin. After 6 hours of applying the drug loaded microspheres, sections were examined under a light microscope to detect any damage to the tissue during in vitro permeation by a pathologist blinded to the study.

Ex-vivo drug release study: The release study was performed using sheep's nasal mucosa. The procedure and parameters similar to in vitro drug release study.

Differential scanning calorimetry (DSC): The thermal behavior of pure drug, drug-loaded microspheres and blank microspheres were studied using a differential scanning calorimeter Shimadzu Japan (DSC 60) at a heating rate of 20°C /min. The measurements were performed at a heating range of 50°–225°C under nitrogen atmospheres.

X-ray diffraction study (XRD): X-ray diffractogram of the drug, blank microsphere and drug-loaded microsphere were recorded by a diffractogram (Brucker AXS D8 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 2ø angle range between 3–80° and 0.010 step sizes [13].

Infrared spectroscopic study (IR): Fourier transformed infrared (FTIR) spectra of carbamazepine, blank microspheres and carbamazepine loaded microspheres were obtained on a FTIR (Thermo Electron co. IR 200®) using the KBr disk method (2 mg sample in 200 mg KBr). The scanning range was 450– 4000cm¯1 and the resolution was 1 cm^{-1}.

Stability of the microspheres: The microspheres batches of optimized formulation were stored in stability chamber (HMG INDIA) at 40°C and 75 % RH for 3 month and observed for the particle size, % mucoadhesion, % drug release at 1 month interval [14].

Analysis of data by design expert software (ANOVA Study): The data obtained for the selected polymer i.e. HPMC K4 is treated using Stat Ease Design Expert 7.1.4 software and analyzed statistically using analysis of variance (ANOVA).

Results

Six different formulations of HPMC K4M microspheres containing carbamazepine were prepared using spray-drying method, Table1.

Table 1: Formulation composition of mucoadhesive microspheres

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
	1:6	F6	200	1200	120	240

Evaluation Studies of Microspheres

Production yield: Production yield was found in the range between 40–60% (Table 2). These relatively low values may be due to 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 in the collecting chamber.

Particle size analysis: Average particle size of microspheres ranged from 20–50 mm, such particles are considered to be suitable for nasal administration. It was also noted that increasing drug-to-polymer ratio slightly increased the size of microspheres (Table 2).

Scanning electron microscopy (SEM): The optimized formulation F1 was analyzed by SEM for studying particle shape and surface structure (Figure 1). The microspheres were spherical in shape and possessed a slight rough surface.

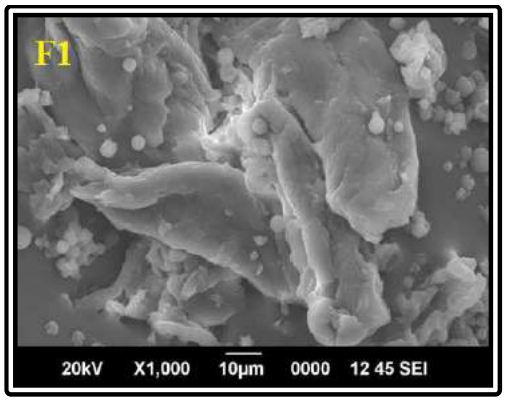

Fig. 1: SEM images of F1 Formulation

Drug loading and incorporation efficiency: Incorporation efficiency was high since it always exceeded 90%. It was found that with increasing the ratio of drug-to-polymer, the incorporation efficiency was also increased (Table 2).

Swelling index: Swelling capacity of the microspheres was mostly shown because of HPMC content in the preparation. The maximum degree of swelling was observed at 0.4607 for formulations F6. (Table 2).

In vitro drug release studies:

The in vitro release study of carbamazepine loaded microspheres was carried out using nasal diffusion cell (Figure 2). The release profile of carbamazepine from various batches of HPMC K4M microspheres after 6 hr at pH 6.6 phosphate buffers is shown in Table 3 and Figure 3

Figure 2 Assembly and specification of nasal diffusion cell

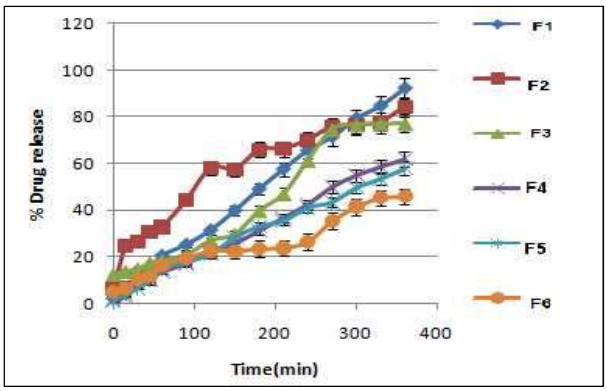

Fig. 3: Release profile of HPMC K4M microspheres (Formulations).

Drug release kinetics: The release constant was calculated from the slope of the appropriate plots and

the regression coefficient (R2) was determined. The in vitro drug release of F1 was best explained by Korsmeyer peppa's equation, the plot showed the highest linearity (R2=0.987) (Table 3).

Ex-vivo evaluation

Mucoadhesion studies: 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 adhered 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 HPMC K4M microspheres

Formulation code	Production yield (%)*	Average particle size (µm) #	Drug loading (%) *	Incorporation efficiency (%) *	Swelling index (%) *	Mucoadhesion (%) *
F1	41.75 ± 1.56	22.12±1.53	41.15± 0.79	82.31±1.24	0.3131 ±1.24	78.59 ± 1.05
F2	42.66 ± 1.96	24.34±2.14	28.93±1.24	86.79±0.87	0.3458 ±1.82	79.64 ± 2.56
F3	45.12 ± 0.87	28.52±2.09	21.89±1.45	87.56±0.55	0.3625 ±0.69	82.13 ± 0.69
F4	49.80 ± 0.79	33.69±0.96	17.99±1.36	89.95±0.96	0.3972 ±0.47	83.99 ± 0.97
F5	55.83 ± 1.43	39.14±1.78	14.93±2.01	89.61±0.67	0.4235 ±1.54	86.93 ± 1.43
F6	59.64 ± 1.56	42.66±1.99	12.90±1.89	90.33±1.29	0.4607 ±2.06	89.06 ± 1.97

*Value expressed as Mean ± SD, n=3, # Denotes average of 100 particles SD

Table 3: Model fitting of the release profile using four different models

Formulation Code	Kinetic models							
	Zero order	First order	Higuchi matrix	Korsmeyer peppa's	Best fit model	K	*n*	Mechanism
F1	0.858	0.948	0.932	0.987	Korsmeyer peppa's	1.688	0.612	Non-fickian diffusion

Histopathological study: It is necessary to examine histological changes in nasal mucosa caused by formulations. The histological study was performed staining the normal mucosa with hematoxylin solution (Figure 4A) and treated with the formulation F1 for 8hr. There was no change in mucosal structure (Figure 4B).

Ex vivo drug release study: The release study was performed using sheeps nasal mucosa. The sample obtained were analysed by UV spectrophotometer at 285nm. The Fig.6 shows the release pattern of optimized formulation F1. The release data of formulation from sheep nasal mucosa was found similar to that performed with dialysis membrane in vitro.

Differential scanning calorimetry (DSC): The thermogram of carbamazepine exhibited a sharp endothermic peak at 185.35°C, indicated melting point which was reported in the literature. The characteristic peak of carbamazepine was well recognized in the drug-loaded microspheres. Thus, there was no interaction between carbamazepine and HPMC. Further, the decrease in peak length and shift of peak of carbamazepine loaded microsphere curve revealed encapsulation of drug with some sort of energy minimization (Figure 5).

X-ray diffraction study: The X-ray diffraction spectra's were recorded for carbamazepine, blank microspheres and drug loaded microspheres for investigating the crystallanity of the drug in the polymeric microspheres (Figure 7). The X-ray diffractogram of carbamazepine has sharp peaks at diffraction angle 8.95°, 12.48°, 19.53°, 24.33°, 29.32° and 57.55° which shows a typical crystalline pattern. Blank microspheres show less intense peaks, however carbamazepine-loaded microspheres show peaks, but of low intensity, indicating that some amount of drug converts to amorphous form.

Infrared spectroscopic study (IR): IR spectra's were recorded for pure carbamazepine drug, physical mixture and drug-loaded microspheres (Figure 7). Pure carbamazepine showed sharp characteristic peaks at 537, 1115, 1307, 1672, 3156, 3466 cm¯1. All the above characteristic peaks of drug appear in the spectra of physical mixture at the same wave number, indicating no modification or interaction between the drug and the polymer which disappear in drug-loaded microspheres, which confirms encapsulation of drug into the polymer.

Stability of the microspheres: 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 particle size, % mucoadhesion and %drug release. The average particle size remained relatively unchanged, drug loss was minor and there was no change in mucoadhesive strength. From the stability studies of the optimized batches it was found that the microspheres remained stable even after exposing to stress conditions of temperature (Table 6).

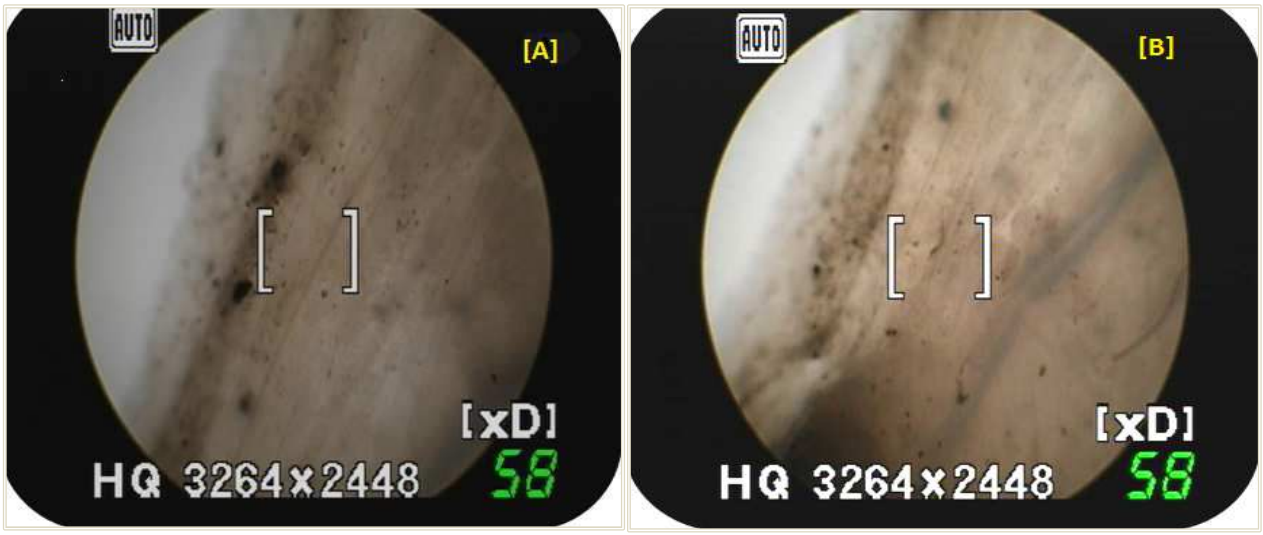

Figure 4: Microscopic images of nasal mucosa. (A) Control (Normal) nasal mucosa

(B) F1 formulation treated nasal mucosa.

Table 6: Results of optimized batch after stability period

Formulation code	Parameters	Storage Time			
		0 Month	1 Month	2 Month	3 Month
F1	Particle size (µm)	22.12	21.51	23.78	22.97
	Mucoadhesion (%)	78.59	79.32	77.21	78.71
	Release (%)	92.13	89.98	89.14	88.92

Statistical analysis by design expert software

The linear model for release was found to be significant. The increase in the concentration of HPMC K4 resulted in decrease in the release of carbamazepine. The Model F value of 186 for HPMC K4M is indicative of significant model. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise (Table 4 and 5).

Table 4: Analysis of variance for response surface linear model

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	p-value	Model Significance
For HPMC K4M						
Model HPMC K4M	2572.836	1	2572.836	186.2788	< 0.0001	Significant
Residual	69.05873	5	13.81175			
Lack of Fit	68.85373	3	22.95124	223.9146	0.0044	Significant
Pure Error	0.205	2	0.1025			
Correlation Total	2641.894	6				

Table 5: R-squared and final equation in terms of coded factor values by design expert software

Polymer	Std. deviation	Mean	C.V (%)	Press	R^2	Adjusted R^2	Predicted R^2	Adequate precision	Final equation in terms of coded factor	Final equation in terms of actual factor
HPMC K4M	***3.716***	***68.128***	***5.455***	***99.613***	***0.973***	***0.968***	***0.962***	***24.07***	68.12-23.91	101.60-0.04

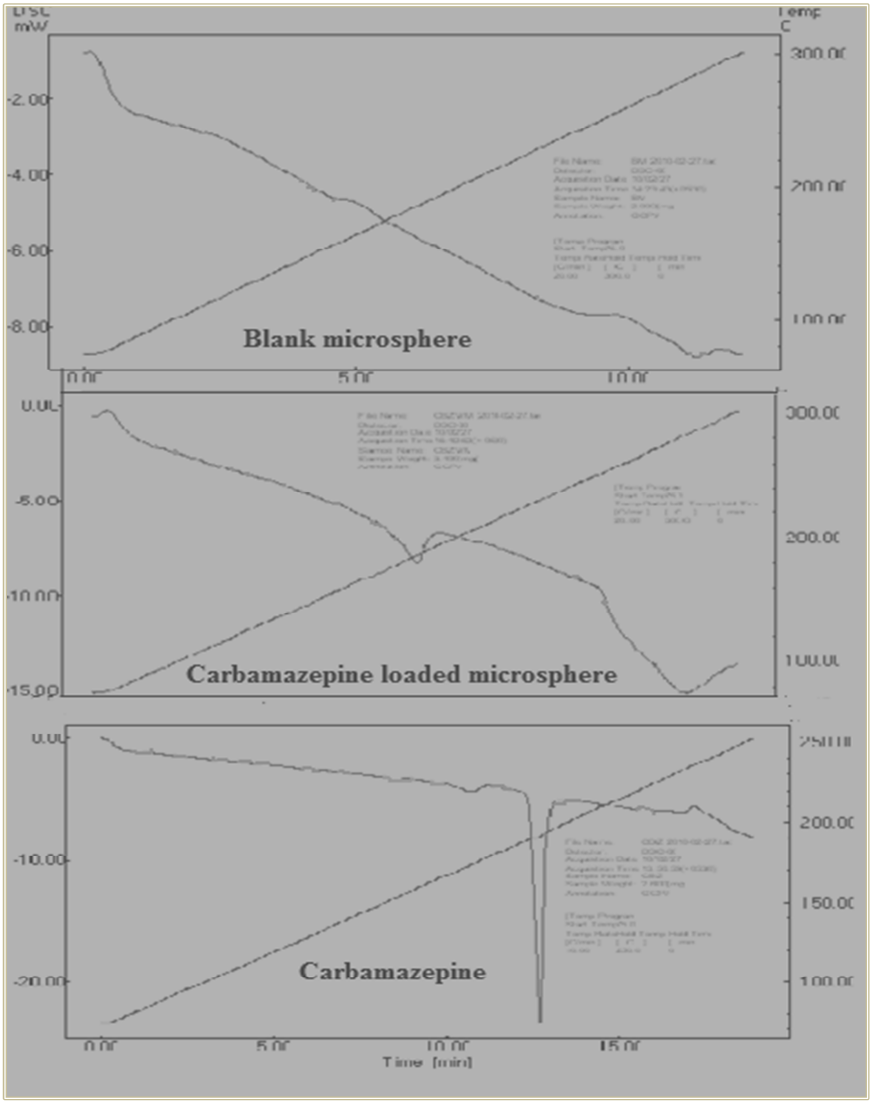

Figure 5 DSC curves of blank microsphere carbamazepine loaded microsphere and carbamazepine

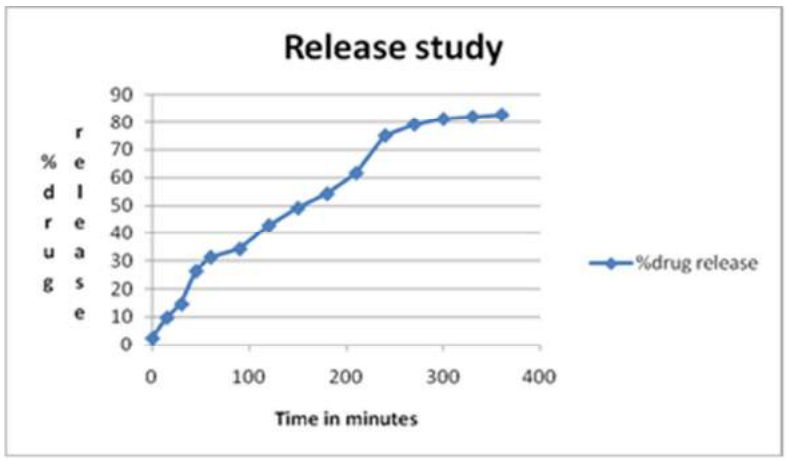

Fig.6 Ex vivo drug release study

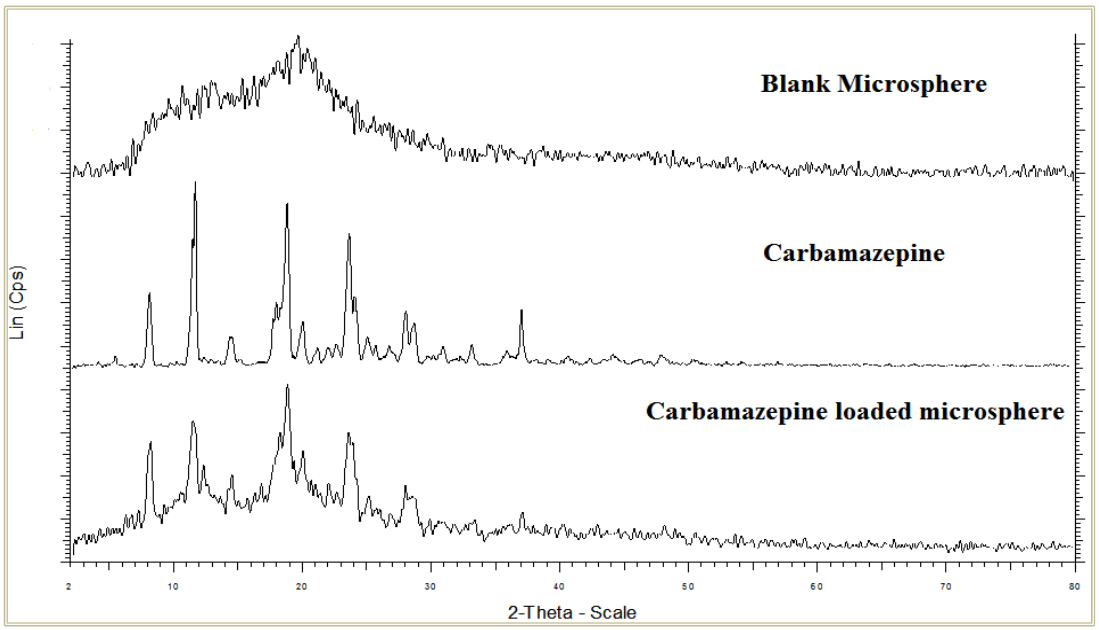

Fig. 7: X ray diffractogram of blank microsphere carbamazepine loaded microsphere and carbamazepine

Results & Discussion

The spray-drying technique appears to be a suitable method for the preparation of HPMC K4M microspheres loaded with carbamazepine. It is the one-step process, easy and rapid. The particle size, swelling ability, incorporation efficiency of the microsphere increases with the increase in the drug to polymer ratio. By increasing the proportion of the HPMC K4M polymer we can sustain the drug release from the polymer. The drug release of the optimized formulation followed the Korsmeyer peppa's equation. The release exponent (*n*) of F1 was 0.612 i.e. the exponent is in range of 0.5-1.0 depicted the Non fickian diffusion or anomalous transport i.e. diffusion with polymer relaxation. When the microspheres absorb water from the mucous and swell, the epithelial cells get hydrated and cause the tight junctions to separate [15]. Since the process is reversible, an increase in absorption of drugs via paracellular pathway will take place during the short period when the tight junctions are separated [16], during the in vitro release study the release pattern of high polymer ratio microspheres shows the sustain release and required extensive pharmacokinetic and pharmacodynamic studies for the correlation. The XRD study showed that some amount of drug is converted to amorphous form after its entrapment into the microspheres. IR study confirms the encapsulation of carbamazepine while DSC study shows that there is no interaction but slight energy minimization because of decrease in the peak length and slight shift of peak. From histological study it has been observed that there is no destructive effect to the nasal mucosa hence can be use safely for nasal drug delivery. Finally the stability has been shown by the optimized formulation in accelerated and stress condition which is performed according to the ICH stability guideline.

Conclusion

Spray drying is a suitable technique for preparation of carbamazepine mucoadhesive microspheres of carbamazepine based on HPMC K4M. The parameters evaluated reveal a promising nasal delivery system for the administration of carbamazepine. The designed

drug and polymer system also holds promise to further study i.e. in vivo studies leading to IVIVC for commercialization.

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Declaration of Interest
The authors report no conflicts of interest.

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