INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

FORMULATION AND EVALUATION OF MUCOADHESIVE MICROSPHERES OF PHENYLEPHRINE HYDROCHLORIDE FOR NASAL DRUG DELIVERY

Santhosh Raj M[1*] and Jayesh Dwivedi[2]

[1]Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur.

[2]Department of Pharmaceutics, Alwar Pharmacy College, Alwar.

ABSTRACT

Nasal drug delivery has been recognized as a very promising route for delivery of drugs used for maintenance of therapy of nasal allergy, nasal congestion, sinusitis and nasal infection. In the present work mucoadhesive microspheres of Phenylephrine Hydrochloride are designed by two different methods Emulsification-cross-linking method using Chitosan as polymer, Emulsification-solvent evaporation method using HPMC, Carbopol, Sodium CMC as a polymer in different Drug/Polymer ratio concentration. The prepared microspheres of all the formulations were evaluated for particle size, percentage yield, Encapsulation Efficiency, Drug Loading, In vitro Mucoadhesion Studies, shape and surface properties, In-vitro drug release studies, drug polymer interaction, stability. Compatibility studies by FTIR proved that there was no interaction between Phenyephedrine HCL and the polymers used. The mean particle size of microspheres of each batch ranged between 21.7 to 53.5 µm which ensured good handling characteristics of all batches. The percentage drug Encapsulation Efficiency of all formulations was found to be between 61.8% to 83.0%. The percentage drug loading of all the formulations were found to be between 9.2%. to 31.2%.The percentage Mucoadhesion were found to be in range 63.2% to 91.8%. All the formulations were subjected to in vitro release studies with phosphate buffer pH 6.6, microspheres exhibited controlled drug release upto 12 hrs. The data obtained suggest that mucoadhesive microsphere prepared by both techniques are very promising nasal delivery system for sustained delivery of drug and to improve patient compliance

Keywords: Chitosan, HPMC, Carbopol, Sodium CMC, Mucoadhesive Microspheres, Phenylephrine HCl

INTRODUCTION

Nasal drug delivery has frequently been proposed as the most feasible alternative to parenteral injections. This is due to the high permeability through nasal epithelium, allowing a higher molecular mass cut-off at approximately 1000 Da, and rapid drug absorption rate and plasma drug profiles sometimes almost identical to those from intravenous injections[1] .Conventionally, the nasal route has been used for delivery of drugs for treatment of local diseases such as nasal allergy, nasal infections and nasal congestion .Recent years have shown that the nasal route can be exploited for the systemic delivery of polar drugs having low molecular weight peptides and proteins that are not easily administered via other routes than by injection. From the pharmacokinetic standpoint, absorption is rapid which provides a faster onset of action compared to oral and intramuscular administration. Hepatic first-pass metabolism is

also avoided, allowing increased, reliable bioavailability[2]

Mucoadhesive Drug Delivery System

Mucoadhesive are synthetic or natural polymers, which interact with the mucus layer covering the mucosal epithelial surface and mucin molecules constituting a major part of mucus. The concept of mucoadhesive has alerted many investigators to be possibility that these polymers can be used to overcome physiological barriers in long-term drug delivery. They render the treatment more effective and safe, not only for topical disorders but also for systemic problems[3]. Mucoadhesive controlled release devices can improve the effectiveness of a drug by maintaining the drug concentration between effective and toxic levels, inhibiting the dilution of a drug at a specific site. Mucoadhesion also increases the intimacy and duration of contact between a drug containing polymer and a mucous surface. The combined effects of the direct drug absorption and decrease in excretion rate (due to prolong residence time) allow for an increased bioavailability of the drug with a smaller dosage and less frequent administration. Bioadhesive system can prevent the first pass metabolism of certain protein drugs by liver through the introduction of the drug via route bypassing the digestive tract. Drugs that are absorbed through the mucosal lining of tissues can enter directly into the blood stream and prevented from enzymatic degradation in the GIT[4].

Nasal Mucoadhesive Drug Delivery System

Nasal therapy known as “Nasaya Karma” has been recognized in Ayurvedic medicine since ages.

However, the potential of nasal drug delivery was recognized in year 1992. Conventionally the nasal route has been used for the delivery of drugs in the treatment of local diseases; however the last decade has recognized the importance of the nasal cavity as potential route for drug delivery.

There are an increasing number of research and review articles addressing topics on nasal drug delivery. This interest arises from the different possible advantages presented by the nasal cavity[5].

Mucoadhesive Microspheres

Mucoadhesive microspheres include micro particles and microcapsules (having a core of the drug) of 1-1000µm in diameter and consisting either entirely of a bioadhesive polymer or having an outer coating of it, respectively. Microspheres in general are investigated for targeted and controlled release drug delivery. A polymeric device allows for slow, controlled, and predictable drug release over a period of time and hence reduces the overall amount of drug needed. In nasal drug delivery, coupling of bioadhesive properties to microspheres is of great importance because of additional advantages: efficient absorption and enhanced bioavailability of the drug, a much more intimate contact with mucus layer and reduction in frequency of drug administration due to the reduction in mucociliary clearance of drug delivery system adhering to nasal mucosa[6]

Phenylephrine is a synthetic sympathomemetic agent chemically related to ephedrine and epinephrine indicated for the symptomatic relief of sinusitis, bronchitis and other symptoms associated with the common cold[7] In the present work microspheres are prepared by using 2 different technique.

EXPERIMENTAL

Materials

Phenylephrine hydrochloride was obtained as a gift sample from Remidex Pharma, Bangalore, Chitosan were purchased from Central Institute of Fisheries Technology, Cochin / Mumbai, Glutaraldehyde solution 25%, Dioctyl sodium sulfo succinate, Acetic Acid, Liquid paraffin (Heavy & Light), Sodium chloride, Sodium hydroxide, Potassium dihydrogen phosphate and Hydrochloric acid were purchase from SD Fine Chemie Pvt. Ltd. Mumbai & Carbopol 934, HPMC, Sodium CMC were of pharmaceutical grade.

Method

Preparation of Chitosan Microspheres

Chitosan microspheres were prepared by simple w/o emulsification-cross-linking process describe by Thanoo et al., 1992 using liquid paraffin (heavy and light, 1:1) as external phase. The hardened microspheres were separated by Remi centrifuge and washed several times with hexane to remove oil. Finally, microspheres were washed with distilled water to remove unreacted GA. The microspheres were air dried for 8 hrs and then stored in vacuum desiccator until further use. (Ref: Table-01)

Preparation of HPMC Microspheres

Accurately weighted amount of the polymers Carbopol, HPMC and Sodium CMC as shown in Table-1 were dissolved in 50ml of acetone to

form a homogenous polymers solution. Phenyephedrine HCl was then dispersed in it and mixed thoroughly. This organic phase containing drug was slowly poured at 150°C into liquid paraffin (50 ml) containing 1% (w/w) of Span-80 with stirring at 1000 rpm to form a uniform emulsion. Thereafter, it was allowed to attain room temperature and stirring was continued until residual acetone evaporated and smooth-walled, rigid and discrete microspheres were formed. The microspheres were collected by decantation and the product was washed with petroleum ether or n- hexane and stored in desiccators over fused calcium chloride. (Ref:Table 02)

Measurements

Determination of λ max of Phenylephrine hydrochloride

Accurately weighed 25mg of Phenylephrine hydrochloride was dissolved in 25ml of pH 6.6 phosphate buffer to give a solution of 1 mg/ml (1000 µg/ml) concentration and this solution was served as the first standard stock solution. From this stock solution 1 ml was taken and diluted to 10 ml using pH 6.6 phosphate buffers to get a solution of 100 µg/ml concentration and served as the second standard solution. From the above solution (10 µg/ml) aliquots of 0.5ml, 1ml, 1.5ml, 2ml and 2.5 ml were pipetted out into a series of 10 ml volumetric flasks. The volume was made up to 10 ml using phosphate buffer of pH 6.6 to get final concentration of 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, and 25 µg/ml respectively. One of the above solutions i.e., 15µg/ml was selected for the determination of λmax. This solution was scanned between the range of 200-400nm.The absorbance of each concentration was measured at λmax of 237 nm using UV Visible spectrophotometer against reagent blank. Standard curve was plotted with concentration on x-axis and absorbance on y-axis.

Characterization of Microspheres

Particle Size Analysis

Particle size[8]

Determination of average particle size of Mucoadhesive microspheres loaded with Phenyephedrine HCl was carried out by using optical microscopy. A minute quantity of microspheres was spread on a clean glass slide and average size of microspheres was determined in each batch.

Percentage yield[9 - 11]

The measured weight was divided by total amount of all non-volatile components which were used for the preparation of microsphere. Percentage yield can be calculated using the formula

% yield = Total weight of excipient and drug / Actual weight of product x 100

Encapsulation efficiency and Drug loading[12,13]

To determine the amount of drug encapsulated in Mucoadhesive microspheres, a weighed amount (50 mg) of microspheres was suspended into 50 ml of ethanol and sonicated for 15 min in order to extract the entrapped drug completely. The solution was filtered and 1 ml of this solution was withdrawn and diluted to 50 ml with pH 7.4 phosphate buffer solution. This solution was assayed for drug content by UV spectrophotometer at 247 nm.

Calculating this concentration with the dilution factor we get the percentage drug content.

A) Encapsulation efficiency was calculated as[14]

EE (%) = Actual Drug Content / Theoretical Drug Content X 100

B) Drug loading was calculated as[15, 16]

DL (%) = Actual Drug Content / Weight of Powdered Microspheres X 100

In vitro Mucoadhesion Studies [17 - 19]

A small portion of the sheep intestinal mucosa was mounted on a glass slide and accurately weighed microspheres were sprinkled on the mucosa. This glass slide was kept in desiccator for 15 min to allow the polymer to interact with the membrane and finally placed in the cell that was attached to the outer assembly at an angle of 45º. Phosphate buffer solution pH 7.4, previously warmed to 37 ± 5 ºC was circulated all over the microspheres and membrane at the rate of 1 ml/min. Washings were collected at different time intervals and microspheres were collected by centrifugation followed by drying at 50 ºC. The weight of washed out microspheres was determined and percentage mucoadhesion was calculated by following formula:

% Mucoadhesion = (Wa-Wl) X 100 / Wa

Where, Wa = weight of microspheres applied; Wl = weight of microspheres leached out.

Scanning electron Microscopy[20]

Dry microspheres are kept in a brass stub coated with gold in an ion sputter. Then picture of microspheres were taken by random scanning of the stub. The SEM analysis of the mucoadhesive microspheres was carried out by using JEOL–6360A analytical scanning electron microscope.

In- vitro release studies[21, 22]

The use of natural membranes is very important to predict the drug release characteristics. The modified Franz diffusion cell was used for permeation studies. It consists of two compartments, one is donor compartment and another is receptor compartment of 10 ml capacity. Within 1.5 h, a piece of nasal mucosa was mounted as flat sheet in between the donor and receptor compartment of Franz diffusion cell. Receptor compartment was filled with 10 ml of phosphate buffer of pH 6.6. A magnetic bead was placed in the receptor compartment, and the whole assembly was placed on the magnetic stirrer. The optimized formulation containing drug equivalent to 10mg was placed in the donor compartment. At predetermined time, aliquot of 1 ml was withdrawn from the acceptor compartment and equal amount of fresh buffer solution was replaced and were suitably diluted and analyzed spectrophotometrically at 237 nm. The study was continued for 12 hours.

Stability Studies[23, 24]

The microspheres were placed in screw capped glass container and stored at ambient humidity conditions, at various temperatures like 25± 2°C (60± 5RH), 30± 2°C (65± 5RH), 40± 2°C (75± 5RH) for a period of 60 days. The samples were analyzed for physical appearance and for the drug content at regular interval of 15 days.

RESULTS AND DISCUSSION

Preformulation Study

A. Description

It is white or almost white crystalline powder in appearance. It has some odour or almost odorless.

B. Spectroscopic study

1. UV spectrophotometric study

Calibration Curve of Phenylephrine Hydrochloride

Standard plot of Phenylephrine hydrochloride was done as per the procedure in experimental methods. The curve was found to be linear in the concentration range of 5- 30 µg/ml at λmax of 237 nm with correlation coefficient of 0.996 which indicates that it obeys Beer's – Lambert's Law (fig. 1). The calculation of the drug content, *in vitro* release, and stability studies are based on this calibration curve.

FTIR Studies

The FTIR studies revealed no chemical interaction between the drug molecule and polymers.

Mechanical properties

The mean particle size of microspheres of each batch ranged between 21.7 to 53.5 µm which ensured good handling characteristics of all batches. The percentage drug Encapsulation Efficiency of all formulations was found to be between 61.8% to 83.0%. The percentage drug loading of all the formulations were found to be between 9.2%. to 31.2%.The percentage Mucoadhesion were found to be in range 63.2% to 91.8%, Ref: Table 3.

Scanning electron Microscopy

The surface morphology of Phenyephedrine HCl microsphere using SEM is shown in the Figure-02 to 05. microsphere had a mean particle size range of 21.7 to 53.5 µm and spherical in shape. The surface of the microspheres was also shown to be a porous with rough surface. Close inspection of the electron microsphere revealed no drug particles adhering to the surface that had mean removed by washing and filtration of the microsphere during the recovery process. Drug loading didn't cause any change in the shape or surface morphology of the microspheres.

In vitro Mucoadhesion

In vitro mucoadhesive studies of Chitosan microspheres (CM-1 to CM-5) revealed that concentration of polymer in the microspheres, amount of cross linking agent and time of cross linking affect the in vitro mucoadhesion. It was concluded that as concentration of polymer increased, the in vitro mucoadhesion was also increased. It was concluded that as the time of cross linking and the volume of cross linking

agent increases, the mucoadhesive strength of microspheres was decreased. This may be due to that, increase in cross linking of free – amino groups of Chitosan results in decrease in the degree of freedom after some extent and hence reduction in the degree of entanglement (Table 3).

In vitro mucoadhesive studies of HPMC microspheres (pH-1 to pH-6) Adhesion of polymer with the mucus membrane is mediate by hydration in the case of hydrophilic polymer. Upon hydration these polymers becomes sticky and adhere to mucus membrane. Formulation pH1 containing SCMC showed the highest mucoadhesivity. The greater mucoadhesivity of SCMC microspheres were due to anionic nature of the polymer which is desirable characteristics of adhesion to the mucus layer (Table 3).

In vitro release studies

Was performed by using modified Franz diffusion cell with phosphate buffer pH:6.6 Microspheres exhibited controlled release rate upto 12hrs. In Chitosan microspheres CM-1 to CM -5 Release rate decrease as the concentration of polymer increases (Table – 4) (Fig.5).

Drug release form pH 1- pH 6 microspheres were slow, extended and dependent on the type of polymer and concentration of polymer used. Formulation pH1 containing SCMC showed the maximum release due to rapid swelling property and high dissolution of SCMC in dissolution environment. Dissolution medium permeation in to the microspheres is facilitated due to high swelling action of the SCMC which leads to more medium for the transport of the drug is available. While HPMC microspheres showed the least drug release. Drug release is also affected by the size of microspheres. (Table – 5) (Fig.6).

Stability studies

Stability studies of the formulations were carried out to determine the effect of contents on the stability of the drug at 25°C/60% RH, 30 C/65% RH and 40°C/75% RH for 60 days. There was no significant change in the drug content.

CONCLUSION

By studying all the experimental results it was conclusively demonstrated that microspheres prepared by both techniques would become the promising candidate for delivery various drugs in sustained release manner. Dosing frequency and loss of drug also reduced by use of such type of formulations.

Table 1: Formulation of Chitosan Microspheres

Formulation Code	Drug : Polymer Ratio	Volume of GA	Cross Linking time (Hrs)
CM-1	1:1	1	2
CM-2	1:2	1	2
CM-3	1:3	1	2
CM-4	1:4	1	2
CM-5	1:5	1	2

Table 2: Formulation of HPMC, CP, SCMC Microspheres

Formulation Code	Drug (mg)	Sodium CMC (mg)	HPMC (mg)	Carbapol (mg)	Span 80 (%)	Liquid paraffin (ml)	Acetone (ml)
PH-1	200	800	--	--	1	50	50
PH-2	200	--	800	--	1	50	50
PH-3	200	--	--	800	1	50	50
PH-4	200	400	400	--	1	50	50
PH-5	200	400	--	400	1	50	50
PH-6	200	--	400	400	1	50	50

Table 3: Mechanical properties of Microspheres

Formulation code	Particle Size (µm)	% yield	%Encapsulation Efficiency	%Drug Loading	% mucoadhesion
CM-1	21.75	80.35%	62.4	31.2	63.25
CM-2	31.13	86.40%	74.4	24.8	71.92
CM-3	26.80	79.22%	61.86	15.46	73.89
CM-4	38.55	74.32%	56.01	11.2	75.12
CM-5	48.46	72.13%	55.2	9.2	84.30
PH-1	39.53	86.22%	72.3	21.2	92.4
PH-2	37.32	90.44%	65.4	23.8	89.4
PH-3	53.54	86.46%	82.6	21.46	86.86
PH-4	44.64	87.42%	79.4	19.2	86.01
PH-5	49.22	87.72%	68.6	18.2	85.2
PH-6	47.85	85.12%	63.2	20.2	87.01

Table 4: In vitro release study of CM-1 to CM-5 Formulation

Time (hrs)	CM-1	CM-2	CM-3	CM-4	CM-5
01	14.4	15.6	15.3	12.9	12.0
02	18.4	22.1	19.7	17.1	16.8
03	25.7	31.1	25.6	23.1	22.8
04	33.1	40.5	33.8	31.3	29.7
05	41.1	50.1	40.1	38.2	37.9
06	55.6	59.7	48.3	44.9	43.9
07	66.1	67.0	59.1	54.7	55.2
08	75.2	73.2	70.2	66.3	64.1
09	83.1	81.4	78.4	73.4	71.4
10	88.5	89.2	83.2	81.2	79.3
11		90.2	89.4	88.4	88.1
12			91.4	92.5	91.1

Table 5: In vitro release study of pH-1 to pH-6 Formulation

Time (hrs)	pH-1	pH-2	pH-3	pH-4	pH-5	pH-6
01	15.6	14.4	11.0	12.9	12.0	15.4
02	22.1	18.4	16.8	17.1	16.8	19.4
03	31.1	25.7	21.8	23.1	22.8	25.7
04	40.5	33.1	29.7	31.3	29.7	34.1
05	50.1	41.1	36.9	38.2	37.9	43.1
06	59.7	55.6	42.9	44.9	43.9	55.6
07	67.0	66.1	55.2	54.7	55.2	63.1
08	73.2	75.2	63.1	66.3	64.1	71.4
09	81.4	83.1	71.4	73.4	71.4	78.3
10	89.2	88.5	79.3	81.2	79.3	84.1
11	90.2		87.4	88.4	88.1	90.4
12	92.5		91.2	92.5	91.1	92.1

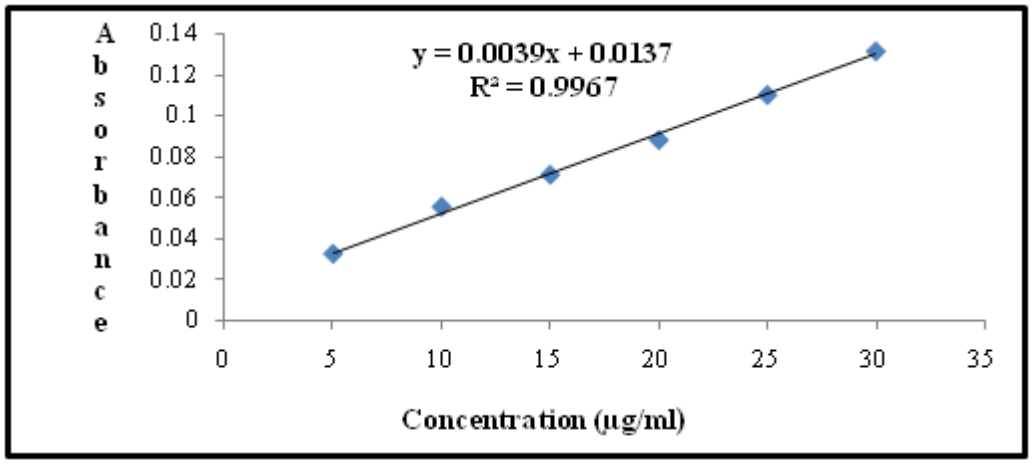

Fig. 1: Calibration curve of Phenylephrine Hydrochloride at 237 nm

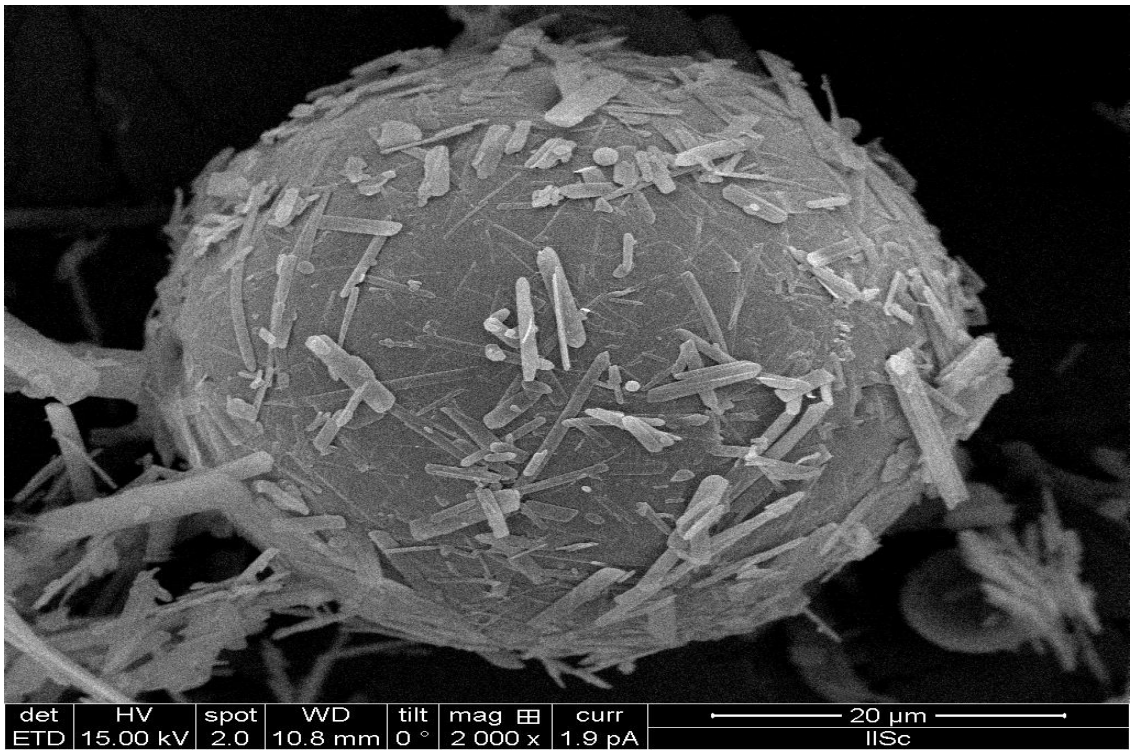

Fig. 2: Scanning electron microscopy of Microspheres

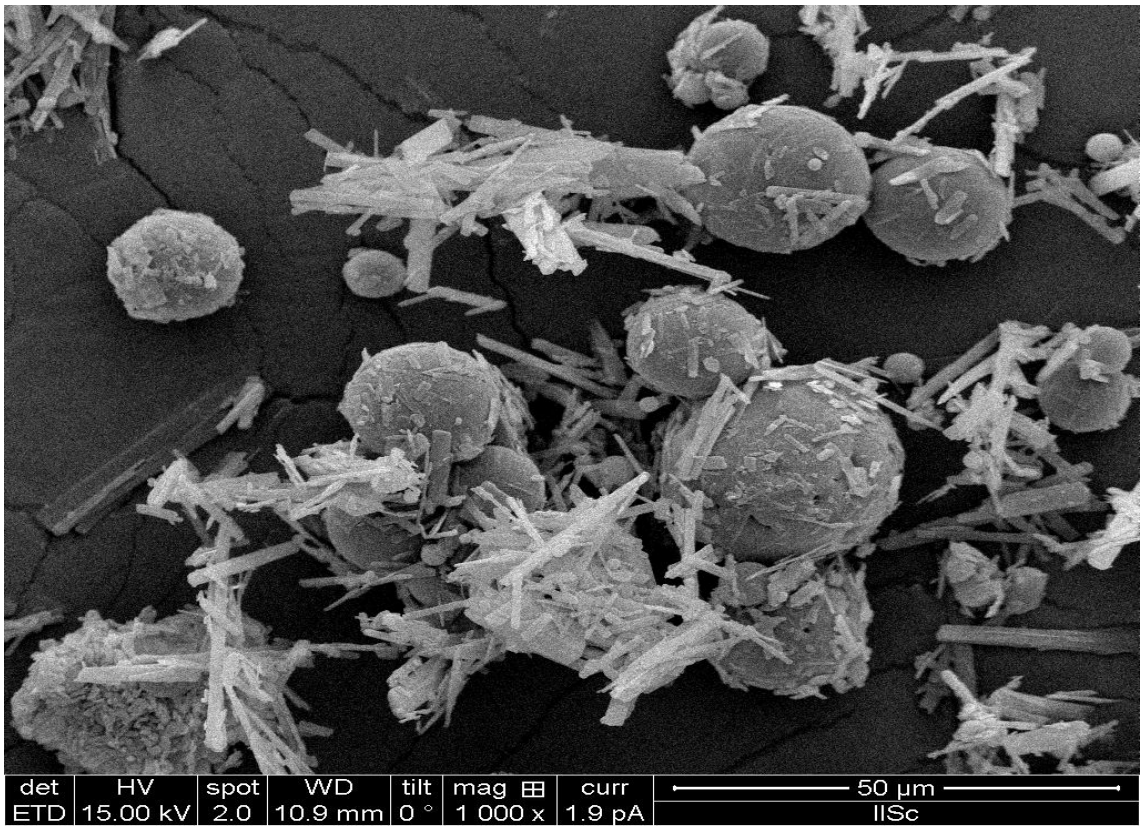

Fig. 3: Scanning electron microscopy of Microspheres

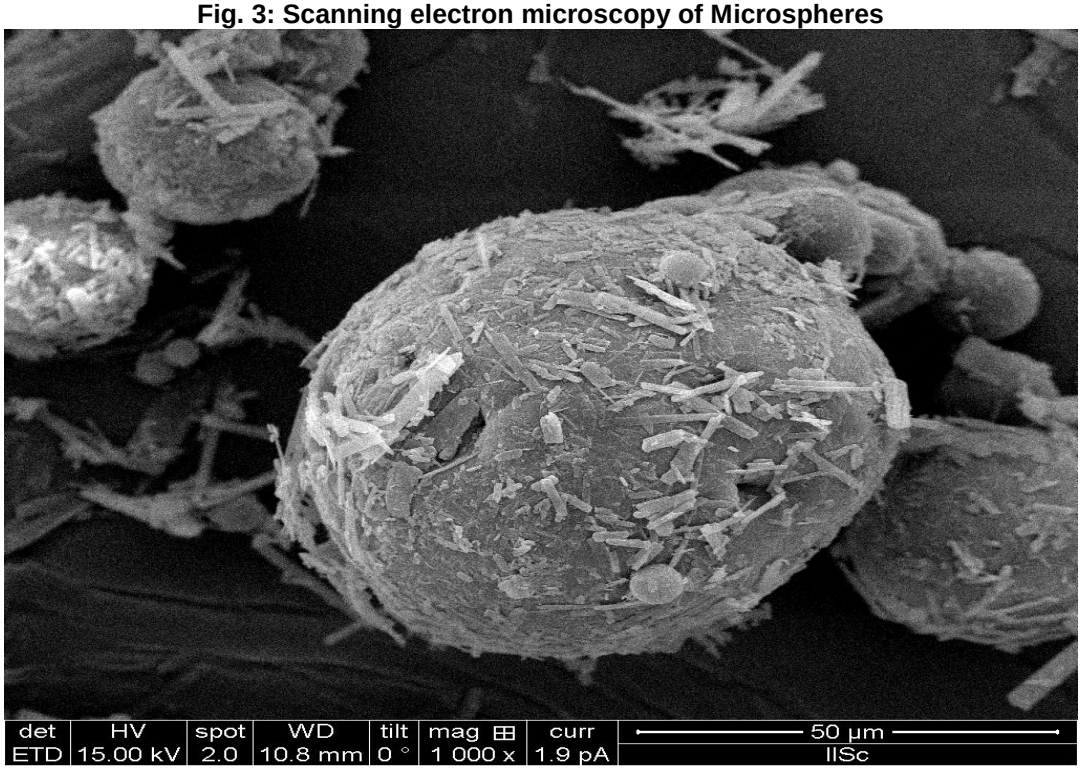

Fig. 4: Scanning electron microscopy of Microspheres

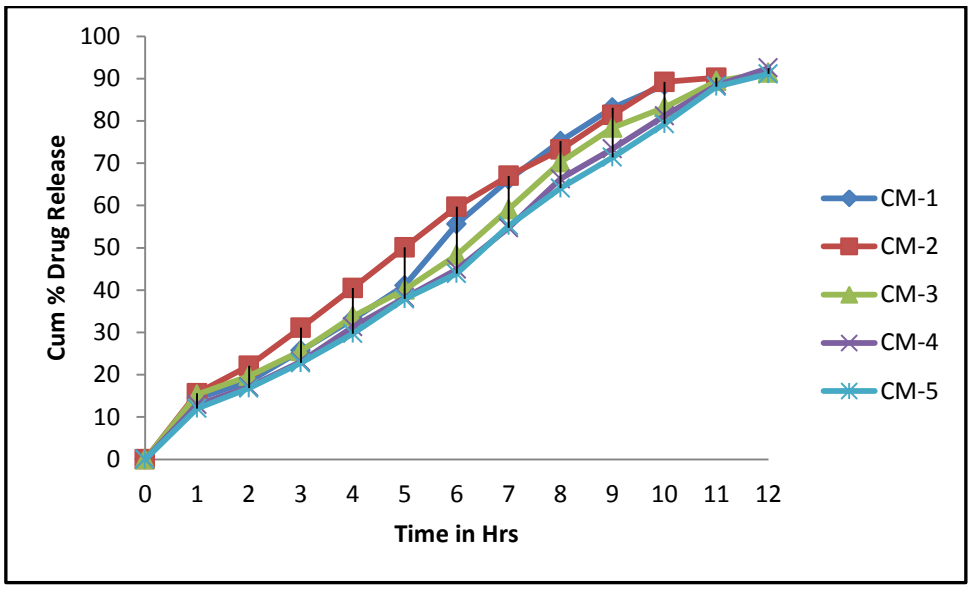

Fig. 5: In vitro release study of CM-1 to CM-5 Formulation

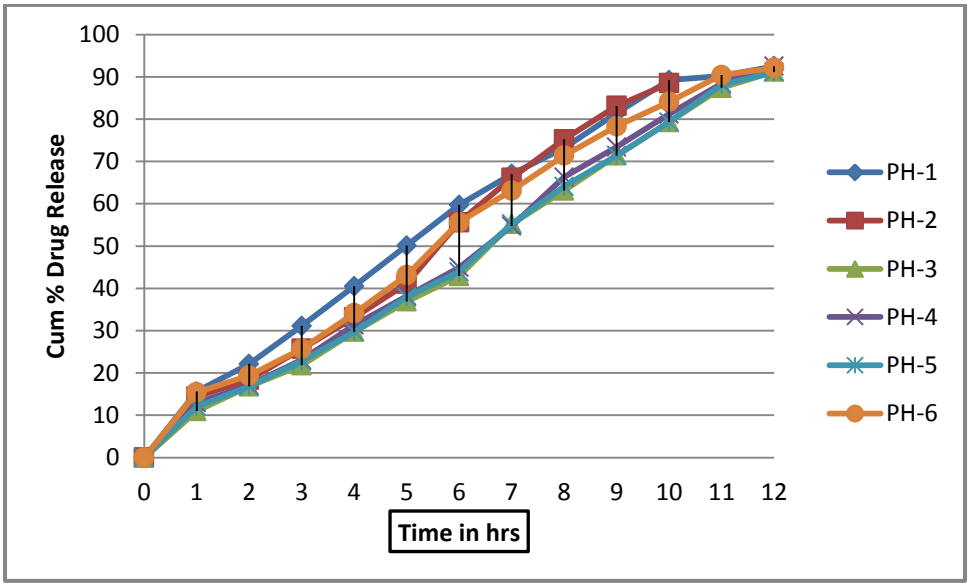

Fig. 6: In vitro release study of pH-1 to pH-6 Formulation

REFERENCES

1. Ugwoke MI, Verberke N and Kigent R. The biopharmaceutical aspects of nasal mucoadhesive drug delivery. J Pharm Pharmacol. 2001;53:3-22.
2. Lisbeth Illum. Nasal drug delivery-Possibilities, Problems and Solutions. J Control Release. 2000;87:187-198.
3. Ahuja A, Kar RK and Ali J. Mucoadhesive drug delivery system-Review. Drug Dev Ind Pharm. 1997;23(5):489-515.
4. Chowdary KPR and Srinivas L. Mucoadhesive drug delivery system-A review of current status. Indian Drugs. 2000;37(9):400-406.

5. Alagusundaram M, Chengaiah B, Gnanaprakash K, Ramkanth S, chetty CM and Dhachinamoorthi D. Nasal drug delivery system - an overview. Int J Res Pharm Sci. 2010;1(4):454-65.
6. Soane RJ, Frier M and Perkins AC. Evaluation of the clearance characteristics of bioadhesive system in humans. Int J Pharm. 1999;178:55-65.
7. Bodmeier R, Chen H, Tyle P and Jarosz P. Pseudoephedrine HCl microsphere formulated into an oral suspension dosage form. J Control Rel. 1991;15:65-77.
8. Manavalan Ramasamy. Physical pharmaceutics, India. 2nd ed., Vignesh publisher, 2004.
9. Patel A, Ray S and Thakur RM. DARU. 2006;14(2):57-64.
10. Kothawade KB, Gattani SG and Surana SJ. Indian Drugs. 2009;46(11):67-70.
11. Cevher E, Orhan Z, Mulazimoglu L, Sensoy D, Alper M and Yildiz A. Int J Pharm. 2006;317:127-35.
12. Streubel A, Siepmann J and Bodmeier R. J Microencapsulation. 2003;20:329-347.
13. Jayvadan patel K, Rakesh Patel P, Avani Amin F and Madhabhai Patel M. AAPS Pharmscitech. 2005;6:49-55.
14. Mathew ST, Gayathri Devi S and Sandhya KV. AAPS Pharm Sci Tech. 2007;8(1):1-9.
15. Trivedi P, Verma A and Garud N. Asian J Pharm. 2008;2:110-5.
16. Jain SK, Nitin K Jain, Gupta Y, Jain A, Jain D and Chaurasia M. Ind J Pharm Sci. 2007;69(4):498-504.
17. Dandagi PM, Masthiholimath VS, Gadad AP and Iliger SR. Ind J Pharm Sci. 2007;69(3):402-07.
18. Ascentiis AD, Grazia JL, Bowman CN, Colombo P and Peppas NA. J Control Release. 1995;33:197-201.
19. Ping He, Stanley S, Davis and Lisbeth Illum. Int J of Pharmaceutics. 1998;166:75-68.
20. Saravanan M, Bhaskar K, Srinivasa Rao G and Dhanaraju MD. J Microencapsulation. 2003;20(3):289-302.
21. Matiholimath VS, Dandagi PM, Jain SS , Gadad AP and Kulkarni AR. Int J Pharm. 2007;328:49-56.
22. Vasir JK, Tambwekar K and Garg S. Bioadhesive microspheres as a controlled drug delivery system. Int J Pharm. 2003;255:13-32.
23. Shah P, Rajshree M and Rane Y. J Pharm Res. 2007;6(1):1-9.
24. Prajapati RK, Mahajan HS and Surana SJ. Ind J of Novel Drug Delivery. 2011;3(1):9-16.