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Formulation Development and Evaluation of Nasal Mucoadhesive Microspheres of Nifedipine

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ABSTRACT

Objective: Nasal administration is an ideal alternative to the parenteral and oral route for systemic drug delivery and to avoid first pass metabolism. Objective of present study was to develop and evaluate Nasal mucoadhesive microspheres of Nifedipine. Nifedipine is widely used for the treatment of hypertension and angina. **Method:** The Nifedipine loaded mucoadhesive microspheres were prepared by emulsion solvent evaporation method employing two different mucoadhesive polymers, viz. Carbopol 974P NF and HPMC K15M. Ethyl cellulose was used as a rate controlling polymer using ethanol as a solvent. **Result:** Optimized formulation was selected mainly on the basis of drug release mechanism and time of drug release. In that, the optimized batch showed maximum *in-vitro* drug release of 93.78 % in 8 hrs. **Conclusion:** Mucoadhesive microspheres showed good controlled release properties. The result of the present study demonstrated that nifedipine can be considered for mucoadhesive drug delivery containing HPMC K15M and Carbopol 974P NF as mucoadhesive polymers for controlled release of the drug over a period of 8 hrs which depend on concentration of polymer for the management of hypertension. No interaction was found between the drug and excipients.

INTRODUCTION

Nifedipine, a systemic calcium channel blocker, is a practically water insoluble and light sensitive drug used in angina pectoris and hypertension[1]. As its biological half-life is about 2 h and is eliminated rapidly, repeated daily administrations are needed to maintain effective plasma levels[2]. It shows a low and irregular bioavailability of about 50% after oral administration with a high first pass metabolism[3]. Oral bioavailability of Nifedipine is about 50% of administered dose and Nasal mucosa consists of a rich vasculature and a highly permeable structure for systemic absorption.[4,5]

The microspheres were prepared by emulsion-solvent evaporation method from Nifedipine by using different polymers like Carbopol 974P NF and HPMC K15M along with film forming polymer ethyl cellulose and sorbitan monooleate. Carbopol (acrylic acid homopolymer) is an anionic polymer that has been used in mucoadhesive systems by several researchers[7]. Carbopol has been selected as a polymer in the preparation of mucoadhesive microspheres because of good mucoadhesive properties and is not absorbed by body tissues and being totally safe for human oral consumption. The objective of this study was to develop, characterize, and evaluate mucoadhesive microspheres of Nifedipine employing mucoadhesive polymers for prolonging gastrointestinal absorption.[6]

MATERIALS AND METHOD:

Nifedipine was procured from Yarrow chemicals, HPMC K15M LV from Loba Chemie and Carbopol 974 NF from Qualingens. All reagent and chemical used were of analytical grade.

Optimized Method for Preparation of Microspheres:

The Nifedipine loaded mucoadhesive microspheres were prepared by emulsion solvent evaporation method employing two different mucoadhesive polymers, viz. Carbopol 974P NF and HPMC K15M. Ethyl cellulose was used as a rate controlling polymer.

a) Preparation of Carbopol 974P NF Microspheres and HPMC K15 M Microspheres :[7]

0.9 g of Ethyl cellulose and Carbopol 974P NF with two different Carbopol/Ethyl cellulose ratio (1:5, 1:3 w/w) were dissolved in 20 ml of ethanol using magnetic stirrer; Weighed amount of

Nifedipine was added to the Ethyl cellulose–Carbopol solution under magnetic stirring. Then the suspension was quickly injected using a 5 ml syringe into 120 ml of light liquid paraffin contained in a 250 ml beaker, which contains 2.5% (v /v) of Span 80, while stirring using a mechanical stirrer. Stirring rate was kept at 2000 rpm for 1 min to form a w/o emulsion. Stirring speed was then lowered and continued for 2 h at room temperature until ethanol evaporated completely and microspheres were formed. The formed microspheres were vacuum filtered through Whatman filter paper. The residue was washed 2-3 times with 50 ml portions of n-hexane. The product was then dried for 24 h at room temperature.

The procedure employed for the preparation of HPMC K15M microspheres was same as above. However, the internal solvent used was a mixture of ethanol and methanol (1:1). This was due to the insolubility of HPMC K15M in ethanol. Literature shows that it is getting solubilized in the mixture of ethanol and methanol.

Table 1: Formulation of Nifedipine microspheres

Mucoadhesive Polymer	Sr. No.	Formulation code	Amount of polymer(0.9g)		Stirring rate (X2) (rpm)	Amount of Drug (mg)
			Mucoadhesive polymer (mg)(X1)	Film forming Polymer(mg)		
Carbopol 974 NF	1	F1	0.225	0.675	700	100
	2	F2	0.225	0.675	1200	100
	3	F3	0.150	0.750	700	100
	4	F4	0.150	0.750	1200	100
HPMC K15M	5	F5	0.225	0.675	700	100
	6	F6	0.225	0.675	1200	100
	7	F7	0.150	0.750	700	100
	8	F8	0.150	0.750	1200	100

Characterization of Prepared Mucoadhesive Microspheres:[8,9,10,11]

1. Production Yield (%):[8]

The production yield of microspheres of various batches 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 % production yields were calculated as per the formula mentioned below.

% PY = WO / WT X 100

PY = Production Yield;

WO = Practical mass (microspheres);

WT = Theoretical mass (Polymer + Drug)

2. Encapsulation efficiency:[8,9,10,11,12]

To determine encapsulation efficiency, 100 mg of accurately weighed drug loaded bioadhesive microspheres were added to 100 ml of methanol. The resulting mixture was kept shaking on a mechanical shaker for 24 h. Then solution was filtered and 1 ml of this solution was appropriately diluted with methanol and analyzed with spectrophotometrically at 241.2 nm using a UV-Visible spectrophotometer (2450 Shimadzu with U.V Prob 2.2.1 software)mThe drug encapsulation efficiency was calculated using the following formula:

Encapsulation efficiency = (Practical drug content/Theoretical Drug content) × 100

3. Particle size analysis:[12]

Particle size of different batches of microspheres was determined by optical microscopy. The projected diameter of microspheres from each batch was determined using ocular micrometer and stage micrometer equipped with optical microscope. Analysis was carried out by observing the slide containing microspheres under the microscope. The average particle size of the microspheres was expressed as diameter.

4) Scanning electron microscope (SEM):[8,9,11]

A scanning electron microscope was used to characterize the surface topography of the microspheres. The microspheres were placed on a metallic support with a thin adhesive tape and were coated with gold under vacuum. The surface was scanned and photographs were taken at 30kV accelerating voltage for the drug loaded microspheres.

5) Swelling index: [12]

The swelling ability of the microspheres in physiological media was determined by swelling them to their equilibrium (Jain et al. 2004). Accurately weighted amounts of microspheres were immersed in a little excess of Phosphate buffer (pH 6.8) and kept for 24 h. The following formula was used for calculation of percentage of swelling:

$$Ssw = (Ws\text{-}Wo/Ws) \times 100$$

Where, Ssw = Percentage swelling of microspheres,

Wo = Initial weight of microspheres, and

Ws = Weight of microspheres after swelling.

6) Measurement of *in-vitro* mucoadhesion:[11,12]

The *in-vitro* mucoadhesion of microspheres was carried out by modifying the method described by Ranga Rao and Buri (1989) and others (Majithiya and Murthy 2005, Patil and Murthy 2006) using sheep nasal mucosa. The microspheres were placed on sheep nasal mucosa after fixing to the polyethylene support. The mucosa was then placed in the desiccator to maintain at 480% RH at room temperature for 30 min to allow the polymer to hydrate and to prevent drying of the mucus. The mucosa was then observed under a microscope and the number of particles attached to the particular area was counted. After 30 min, the polyethylene support was introduced into a plastic tube cut in circular manner and held in an inclined position at an angle of 45. Mucosa was washed thoroughly at flow rate of l mL per min for 5 min with phosphate buffer pH 6.2. Tissue was again observed under a microscope to see the number of microspheres remaining in the same field area.

The adhesion number was determined by the following equation:

$$Na=N/No\ 100$$

where Na is adhesion number,

N0 is total number of particles in a particular area and

N is number of particles attached to the mucosa after washing.

7) Differential scanning calorimetry (DSC):[8,11]

Differential scanning calorimetry (DSC) scans of Drug, blank microspheres and drug loaded microspheres were performed using DSC-PYRIS-1. The samples were heated from 50- 300^{o}C and a rate of 10^{o}C min-1.

8) *In-vitro* Drug Release Studies:[8,9,10,11,12]

Drug release from the microspheres was carried out using a beaker method incorporating phosphate buffer solution of pH 6.4 as the release medium. A weighed amount of microspheres, equivalent to 30 mg of Nifedipine, were suspended in 50 ml of the dissolution medium in 250 ml beaker and stirred on a magnetic stirrer at 50 rpm at 37°C. 2 ml sample was withdrawn at appropriate time intervals and centrifuged at 5000 rpm. Supernatants were diluted suitably and absorbance of the resulting solution was measured at 341 nm in a double-beam UV spectrophotometer using the dissolution medium as blank.

The residue was redispersed in 2 ml of the fresh dissolution medium and replaced back into the vials. The mechanism of Nifedipine released from the microspheres was studied by fitting the dissolution data in different kinetic models.

9. Stability Studies:[8,9,10,11,12]

Stability is defined as the ability of particular drug or dosage form in a specific container to remain with its physical, chemical, therapeutic and toxicological specifications. Stability tests are the series of tests designed to obtain information on the stability of the pharmaceutical product in order to define its shelf life and utilization period under specified packaging and storage conditions. The purpose of stability testing is to provide information on how the quality of a drug product varies with time under the influence of variety of environmental factors such as temperature, humidity and light, and to establish a half life for the drug product at recommended storage conditions.

Procedure:

From the eight batches of Nifedipine loaded microspheres, formulation F1 were tested for stability studies. The formulations were divided into 3 sample sets and stored at:

- ✓ 4 ± 1°C
- ✓ 25± 2°C and 60 ± 5% RH.
- ✓ 37± 2°C and 65 ± 5% RH.

After 30 days, the drug release of selected formulations was determined by the method discussed previously for entrapment efficiency and an in vitro drug release study was also carried out for the same formulation.

RESULT AND DISCUSSION

❖ **Spectroscopic studies :**

Table 2: Calibration curve of Nifedipine in methanol

Nifedipine Conc. (μg/ml)	Absorbance
2	0.075
4	0.123
6	0.176
8	0.226
10	0.285

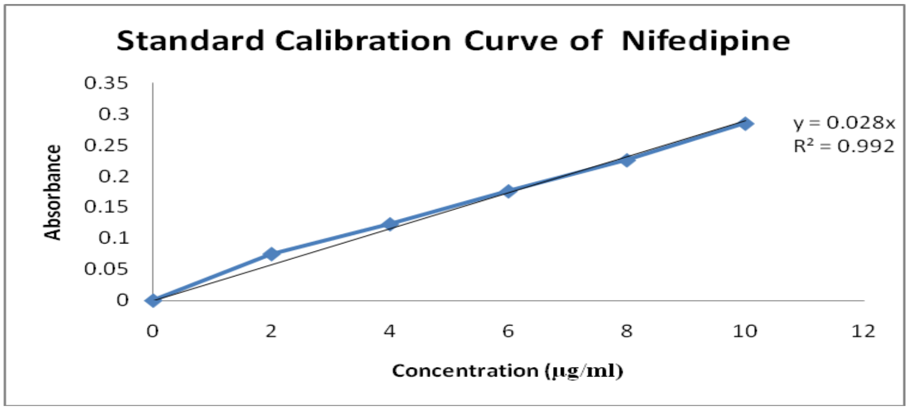

Figure 1: Calibration Curve of Nifedipine

❖ **Preformulation Studies :**

a) IR Spectroscopy:

The IR spectrum of the pure Nifedipine sample recorded by FTIR spectrometer is shown in Figure 2.

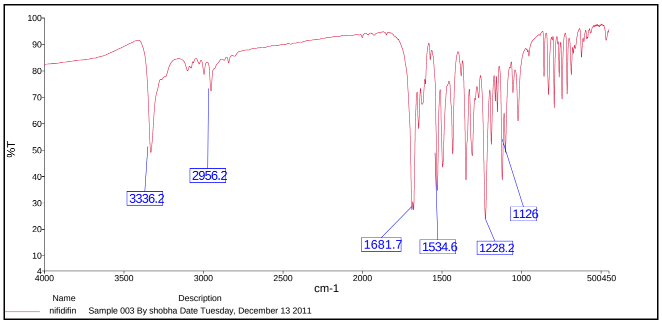

Figure 2: IR Spectra for Nifedipine

Table 3: InfraRed Spectral Data of Nifedipine

Compound Code	IR Bands (cm^{-1})	Types of Vibrations
Nifedepine	3332	- ArCH. str.
	2956	-me-ch. Str
	1681	-C=O str.
	1530	-C=C str.
	1227	-C-O str.
	1122	-C-N str.

Table 3 showed that functional group frequencies of Nifedipine were in the reported range which indicates that the obtained sample was of Nifedipine and was pure.

b) Solubility Analysis:

Results of solubility analysis showed that Nifedipine was insoluble in cold water, hot water. Soluble in ethanol, dimethyl sulfoxide, acetone, chloroform, methanol.

- **Compatibility Studies by IR-Spectroscopy**

Preformulation studies were carried out to study the compatibility of pure drug Nifedipine with the polymers Carbopol 974P NF, HPMC K15M and Ethyl Cellulose prior to the preparation of mucoadhesive microspheres of Nifedipine. The individual IR spectra of the pure drug and polymers as well as the combination spectra of the drug and polymer are shown in **Figure 3 (a) and 3 (b)**

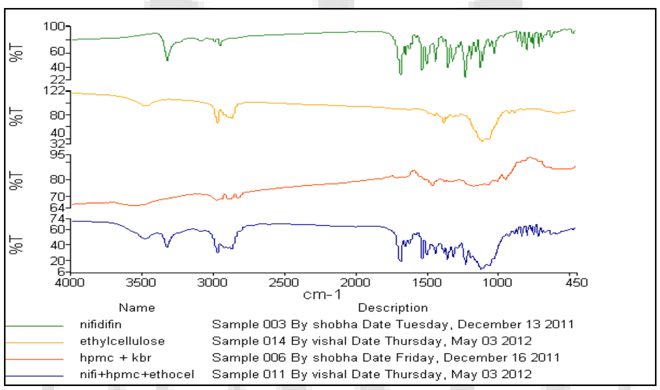

Figure 3 (a): IR spectra of the Nifedipine + Ethocel + HPMC+ combination

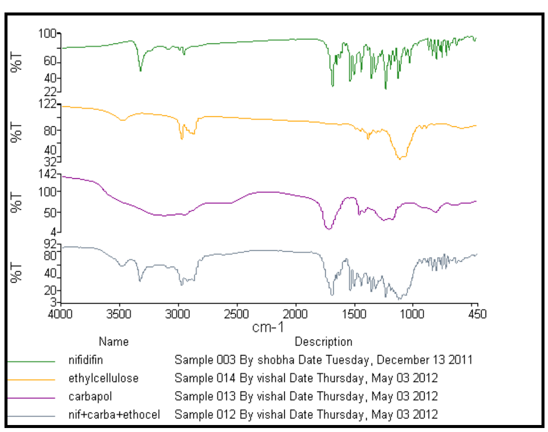

Figure 3(b): IR spectra of the Nifedipine + Ethocel + Carbopol + combination

❖ **Physiochemical Characterization of Microspheres**

Production Yield:

The production yields of microspheres prepared by emulsion-solvent evaporation method were found to be between 75.3 to 86.1% as shown in **Table 4.** It was found that production yield of microspheres prepared by HPMC K15M was greater than Carbopol 974P NF.

Table 4: The Production Yield of Microspheres of Nifedipine.

Sr. No	Formulation	Production Yield (%)
1	F1	86.1
2	F2	75.3
3	F3	78.7
4	F4	79.1
5	F5	80.4
6	F6	82.9
7	F7	85.3
8	F8	82.1

2. Particle Size Analysis:

The size of all the eight batches of microspheres prepared in this study was in the range of 178–262 µm. It is clear that as the stirring rate increases, the particle size decreases both at higher and lower level of mucoadhesive polymer. While concentration of mucoadhesive polymer had opposite effect in particle size.

Table 5: The Arithmetic Mean Sizes of Microspheres of Nifedipine

Sr. No.	Formulation	Particle size (µm)
1	F1	262±5.18
2	F2	248±5.38
3	F3	255±5.69
4	F4	242±6.22
5	F5	210±4.32
6	F6	184±5.43
7	F7	201±5.34
8	F8	178±4.22

3) Encapsulation efficiency (EE)

The values for entrapment efficiency are shown in Table 6. For carbopol based microspheres, they were in the range of 62% to 75% and. While for HPMC based microspheres, they were in the range of 62 to 70 %.

Table 6: The Encapsulation efficiency (%) of Microspheres of Nifedipine

Sr. No.	Formulation	Encapsulation efficiency (%)
1	F1	75.74
2	F2	72.94
3	F3	68.34
4	F4	67.70
5	F5	70.27
6	F6	68.94
7	F7	64.47
8	F8	62.00

4) Surface morphology

The microspheres were found to be discrete and spherical in shape and had nearly smooth surfaces. No difference in the morphology was observed between placebo and drug loaded microspheres Figures 4 and 5, suggesting that the drug may be present in the bulk of the microspheres and not surface associated.

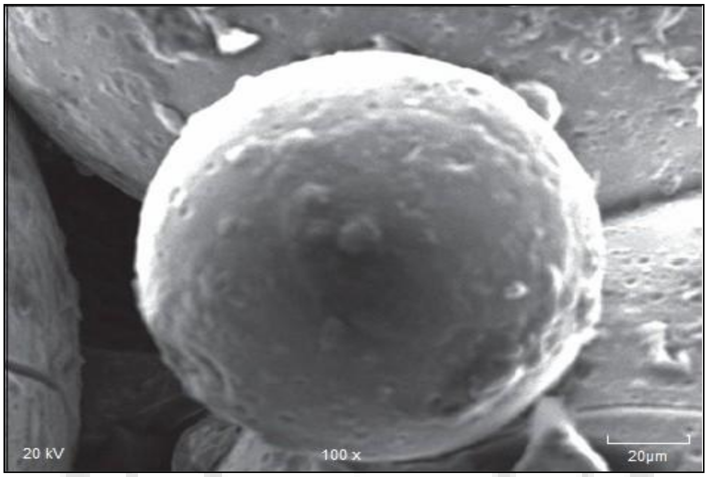

Figure 4: Placebo microspheres

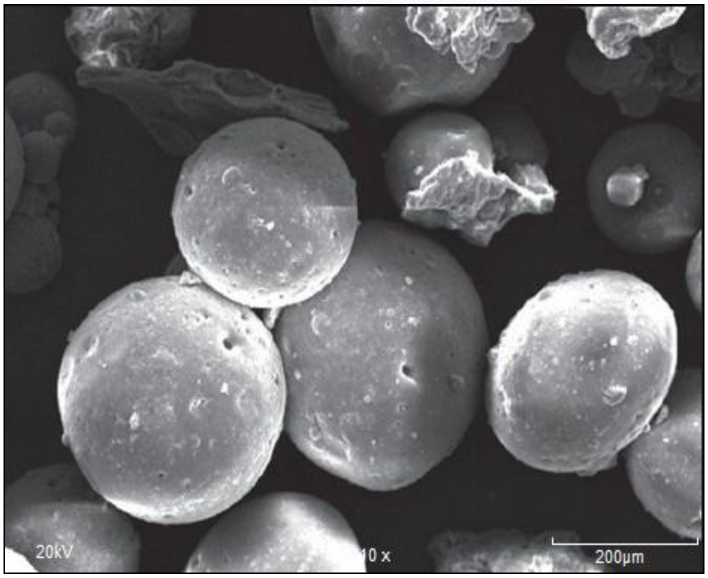

Figure 5: Drug loaded microsphere

5) Degree of Swelling*:*

The degree of swelling of all the formulations are shown in **Table 7**. From results, it is known that the degree of swelling increases marginally as the concentration of mucoadhesive polymer increases from 0.93 to 1.63. From this, it may be concluded that when the microspheres are in contact with mucus layer, they swell rapidly and take up liquid from the mucus layer.

Table 7: The Degree of Swelling of Microspheres of Nifedipine

Sr. No.	Formulation	Degree of Swelling
1	F1	1.63
2	F2	1.61
3	F3	1.56
4	F4	1.54
5	F5	1.16
6	F6	1.10
7	F7	1.03
8	F8	0.98

6. *In-vitro* Mucoadhesion Studies:

The results of the *in-vitro* mucoadhesion studies are shown in **Table 8** mucoadhesion increased with the increase in concentration of mucoadhesive polymer. The higher mucoadhesion of carbopol microspheres may be attributed to the higher molecular weight of carbopol than HPMC.

Table 8: The *in-vitro* Mucoadhesion Studies (%) of Microspheres of Nifedipine

Sr. No	Formulation	*In-vitro* mucoadhesion (%)
1	F1	98
2	F2	94
3	F3	90
4	F4	90
5	F5	85
6	F6	84
7	F7	81
8	F8	82

Table 9: % Drug release from Nifedipine microspheres

Time (hours)	Formulation			
	F1	F2	F3	F4
1	37.43781095	23.88059701	21.51741294	32.960199
2	49.87562189	28.10945274	26.61691542	38.43283582
3	56.46766169	34.95024876	34.95024876	51.11940299
4	65.04975124	48.75621891	45.89552239	58.08457711
5	74.75124378	55.34825871	60.07462687	63.68159204
6	81.96517413	62.31343284	63.09999999	74.75124378
7	86.94029851	74.50248756	71.64179104	86.06965174
8	93.78109453	86.94029851	78.85572139	92.53731343

Time (hour)	Formulation			
	F5	F6	F7	F8
1	35.44776119	23.38308458	25.24875622	25.24875622
2	38.43283582	29.10447761	27.6119403	36.94029851
3	56.46766169	35.94527363	34.95024876	56.46766169
4	62.06467662	44.02985075	45.89552239	59.32835821
5	66.79104478	56.71641791	58.08457711	66.79104478
6	74.75124378	63.68159204	61.06965174	73.25870647
7	85.07462687	73.75621891	67.41293532	85.07462687
8	91.91542289	85.69651741	75.37313433	89.55223881

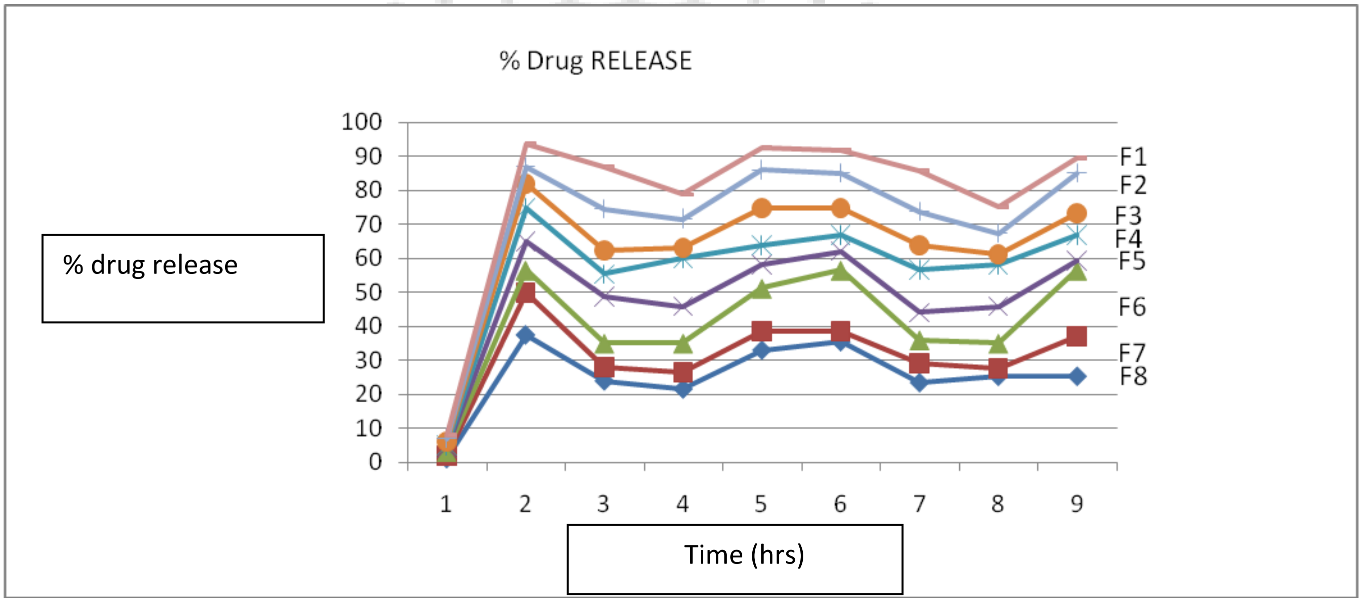

Figure 6: *In-vitro* drug release profile of nifedipine microspheres of formulation F1-F8

To obtain the values of the release constant and to understand the release mechanism the *in-vitro* release data was fitted to various mathematical models. The correlation coefficients for the different drug release kinetic models are shown in **Table 10**. Models with the highest correlation coefficient were judged to be the most appropriate model for the *in-vitro* release study.

Table 10: Model fitting for the Release Profile of Formulations

Formulation Code	Zero Order	First Order	Peppas		Hixon-Crowell	Best Fit Model
	R	R	R	N	R	
F1	0.9731	0.9764	0.9936	1.6472	0.9753	Peppas
F2	0.9941	0.9941	0.9776	0.9143	0.9940	Zero Order
F3	0.9964	0.9964	0.9851	0.8430	0.9964	First order
F4	0.9873	0.9888	0.9840	1.3500	0.9883	First order
F5	0.9943	0.9938	0.9764	0.9177	0.9940	Zero Order
F6	0.9942	0.9931	0.9802	0.9119	0.9940	Zero Order
F7	0.9925	0.9932	0.9748	0.9671	0.9930	First order
F8	0.9894	0.9910	0.9968	1.1414	0.9905	Peppas

Differential scanning calorimetry (DSC) analysis:

DSC thermograms of pure Nifedipine, placebo ethyl cellulose and carbopol microspheres and Nifedipine-loaded microspheres are displayed in Figure 7. It was used to determine the existence of possible interaction between the polymer and drug. From DSC data it was concluded that there is no interaction between polymer and drug.

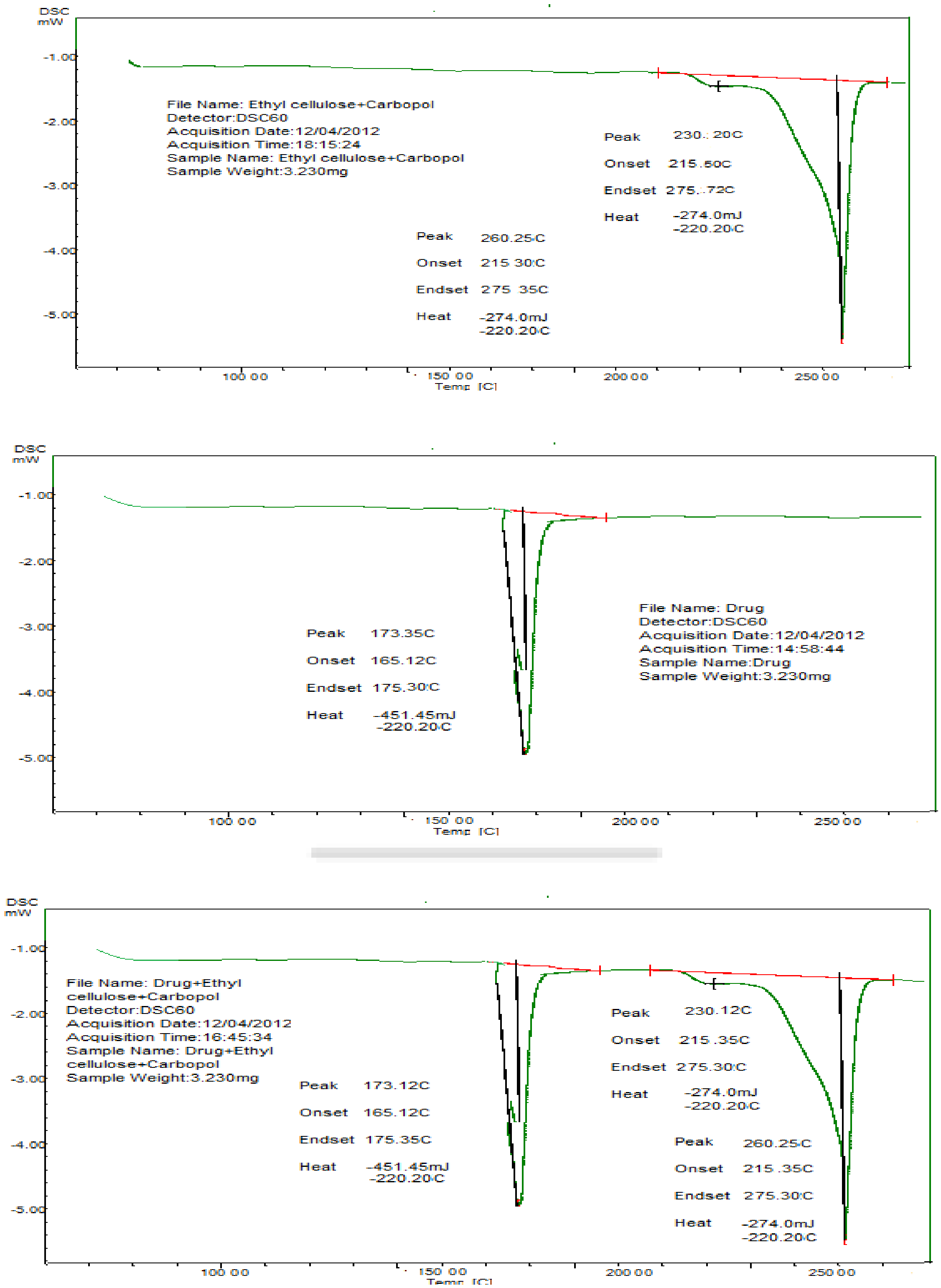

Figure 7: DSC thermograms of (a) Placebo microspheres, (b) pure Drug (c) Drug-loaded microspheres

Stability Studies:

Table 11: Stability Studies – % Entrapment Efficiency and *in-vitro* drug release after 30 Days Storage

Formulation Code	P1		P2		P3	
	% EE	% DR (up to 8 h)	% EE	% DR (up to 8 h)	% EE	% DR (up to 8h)
F1	73.25	93.25	71.91	95.25	68.32	98.78

P1: % drug release for formulation stored at 4 ± 1^{o}C

P2: % drug release for formulation stored at 25 ± 2^{o}C and 60 ± 5% RH

P3: % drug release for formulation stored a37 ± 2^{o}C and 65 ± 5% RH.

Figure 8: *In-vitro* drug release after 30 Days Storage of optimized batch

CONCLUSION

Mucoadhesive microspheres showed good controlled release properties. The result of the present study demonstrated that Nifedipine can be considered for mucoadhesive drug delivery containing Carbopol 974P NF and HPMC K15M as mucoadhesive polymers for controlled release of the drug over a period of 8 hrs which depend on concentration of polymer for the management of hypertension. After evaluating all the formulation, F1 batch which contains combination of polymers showed good entrapment efficiency, mucoadhesion and drug release profile and therefore it can be considered as best formulation.

Formulation F1 showed best results among the formulations made. Particle size of formulation F1 was found to be 262 ± 5.18µm, which is appropriate for nasal administration. Production yield of F1 was found to be 80.1 %. The encapsulation efficiency, swelling index and mucoadhesion values for formulation F1 were 75.74 %, 1.63 % and 98 % respectively. Formulation F1 showed maximum *in-vitro* drug release of 93.78 % in 8 hrs. The release kinetics best fitted Peppas model. From the interaction study of formulation using FTIR, no interaction was found between the drug and excipients.

REFERENCES

1. K .R. Chowdary, K.S. Ramesh, Controlled nifedipine release from microcapsules of its dispersions in PVP-MCC and HPC-MCC, Drug Dev . Ind. Pharm.21 (1995)1183-1192.
2. D.Murdock,R.N.Brogden, Sustained release nifedipine formulation, Drug 41(1991) 737-779.
3. L.Z.Benet, S.øie, J.B.Schwartz,Design and optimization of dosage regimens; Pharmacokinetic data, in J.G.Hardman, A. Goodman Gilman, L.E.Limbird (Eds.), Goodman and Gilmans the Pharmacological Basis of Therapeutics, 9th ed., McGraw-Hill, New York, USA,1996,p.1765.
4. Martindale, The Extra Pharmacopoeia, 30th ed., 374, (1993).
5. Tripathi KD. Essentials of medical pharmacology. 5th ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2003.
6. Henriksen L, Green KL, Smart JD, Smistad G and Karlsen .j.Bioadhesion of hydrated chitosans :An in vitro and in vivo study.Int.J.Pharm.1996;145:231-240
7. Tao Y, Lu Y, Sun Y, Gu B, Lu W, Pan J. Development of mucoadhesive microspheres of acyclovir with enhanced bioavailability. Int J Pharm 2009;378:6-30
8. Duygu Gürcan1, AlperOkyar, BernaÖzbek, GülBaktır, AlevGerçeker, YıldızÖzsoy.Nasal administration of ciprofloxacin HCl loaded chitosan microspheres: in vitro and in vivo evaluation, ActaPharmaceuticaSciencia (2010)52: 5-18
9. E. Gavini, A.B. Hegge, G. Rassu, V. Sanna, C. Testa, G. Pirisino, J. Karlsen, P. Giunchedi, Nasal administration of Carbamazepine using chitosan microspheres: In vitro/in vivo studies, International Journal of Pharmaceutics (2006);307: 9–15
10. Baby h. Dandge1,and m h g dehghan1, formulation and evaluation of nasal mucoadhesive microparticles of diltiazem hydrochloride international journal of chemtech research vol.1, no.4, oct-dec 2009. pp 1036-1042
11. Patil, Sanjay B. and Sawant, Krutika K.(2009) 'Development, optimization and in vitro evaluation of alginate mucoadhesive microspheres of carvedilol for nasal delivery', Journal of Microencapsulation,26:5,432 — 443.
12. Desai Sapna,G.Vidyasagar, Desai Dhruv, Brain targeted nasal midazolam microspheres, Int J Pharm Biomed Sci2010, 1(2), 27-30.
13. Desai Sapna,G.Vidyasagar, Desai Dhruv, Brain targeted nasal midazolam microspheres, Int J Pharm Biomed Sci2010, 1(2), 27-30.
14. Chein Y W. Novel drug delivery systems. 2nd ed. New York: Marcel Dekker, Inc.; 1992. p. 229.
15. N.K. Jain, “Advances in controlled and Novel Drug Delivery”, CBS publications, Pp 268-269.
16. Chein Y W, Chang JF. Nasal systemic drug delivery, 2nd Ed; vol 39, Marcel Dekker Inc, New York, Basel. 1989; 1-77.
17. Ansil’s pharmaceutical dosage forms and Drug Delivery System, loyd V. Allen, Jr, Nchols G. Popovich, Howard C. Ansel, 8th edition, p 260-275.
18. http://en.wikipedia.org/wiki/Nasal_cavity
19. Mr.Amolchaudhari, mr.K.r.jadhav, Dr.V.j.kadam. An overview: microspheres as a nasal drug delivery system. International Journal Of Pharmaceutical Sciences Review And Research, Volume 5, Issue 1, November – December 2010; article-003.
20. Michael I. Ugwoke, Remigius U. Agu, Norbert Verbeke, RenaatKinget. Nasal mucoadhesive drug delivery: Background, applications, trends and future perspectives, Advanced Drug Delivery Reviews 57 (2005) 1640– 1665.