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Original Article

# FORMULATION AND EVALUATION OF NEVIRAPINE MUCOADHESIVE MICROSPHERES

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**ABSTRACT**

**Objective:** The objective of the present research was to formulate and evaluate HPMC K4M, HPMC K100, and Carbopol 940 Mucoadhesive microspheres in combination with sodium alginate for controlling release of Nevirapine.

**Methods:** Nevirapine microspheres were prepared by an Ionotropic gelation method using aluminium sulfate as a cross linking agent. The developed Nevirapine microspheres were characterized for Micromeritic properties, morphology, drug entrapment efficiency, *in vitro* wash off test, *in vitro* drug release, and interaction studies (Fourier transfer infrared spectroscopy (FTIR) & Differential scanning calorimetry (DSC).

**Results:** The Nevirapine mucoadhesive microspheres were free-flowing and discrete. The mean particle size ranged from 705.21±2.00 to 935.45±2.07 µm and the entrapment efficiencies ranged from 63.50 to 96.42 %. All the nevirapine microsphere batches showed good *in vitro* mucoadhesive property ranging from 03-68 % in the *in-vitro* mucoadhesive test after 8 h. FT-IR studies indicated the lack of nevirapine-polymer interactions in the nevirapine microspheres formulation. There were no compatibility issues and the crystallinity of nevirapine was found to be reduced in prepared mucoadhesive microspheres, which were confirmed by DSC and X-ray diffraction studies (XRD). Among different formulations, the nevirapine microspheres of batch F8 had shown the optimum percent drug entrapment and the controlled release of the nevirapine for about 12 h (98.65%). The Release pattern of nevirapine from microspheres of batch F8 followed the Korsmeyer- peppas and zero-order release kinetic model. The value of 'n' was found to be 1.402, which indicates that the drug release was followed super case II transport type. Stability studies were carried out for F8 formulation at 4 °C/Ambient, 25±2 °C/60±5 %, 40±2 °C/75±5 % RH revealed that the drug entrapment and mucoadhesive behavior were within permissible limits.

**Conclusion:** The results obtained in this present work demonstrate the potential use of HPMC K100 polymer for preparation of controlled delivery nevirapine mucoadhesive microspheres and prolonged residence at the absorption site.

**Keywords:** HPMC K4M, HPMC K100M, Carbopol 940, Sodium alginate, Mucoadhesive microspheres, Nevirapine.

## INTRODUCTION

Microcarriers technology offers an novel approach for drug delivery by coupling the drug to microcarrier particles, such as microspheres, microcapsules, liposomes, etc., which modify the release and absorption characteristics of the drug [1-3]. Microspheres possess important features among the particulate drug delivery systems by virtue of their small size and efficient carrier characteristics; however, the success of microspheres delivery system is limited due to their short residence time at the site of absorption [4]. It would therefore be advantageous to have the means for providing an intimate contact of the microspheres delivery system with absorbing biological membranes. This can be accomplished by coupling mucoadhesion characteristics to microspheres by using mucoadhesive polymer and developing mucoadhesive microspheres [5]. Mucoadhesive microspheres have advantages like efficient absorption, enhanced bioavailability of drugs, maximum utilization of drug, a much more intimate contact with mucus membrane, better patience compliance and drug targeting to absorption site can be executed by using suitable mucoadhesive polymers on the surface of microspheres [6]. The rationale behind the use of a mucoadhesive microsheres system is that, the application of bioadhesive microspheres with good bioadhesive properties would permit such microspheres to swell in contact with GIT mucosa to form a gel and control the rate of clearance from the GIT, thereby giving poorly absorbed drugs a longer time to be available at the absorptive surface.

Nevirapine is an anti retroviral bioactive used in treatment of HIV/AIDS and viral diseases belongs to class II under BCS classification and exhibits low and variable oral bioavailability due to poor water solubility [7]. Nevirapine is a peptidomimetic inhibitor of both the HIV-1 and HIV-2 proteases [8]. Nevirapine requires control release due to its short biological half life (3-5 hrs), low bioavailability 65%, narrow therapeutic index and moreover it is primarily absorbed from stomach [9]. All the drawbacks necessitated the development of mucoadhesive microspheres for improving residence of dosage form in GIT which could utilize all the efficacy of Nevirapine, thereby reduce dosing frequency and enhance bioavailability. Therefore, mucoadhesive microspheres are promising candidate for delivery of Nevirapine for treatment of HIV/AIDS patients. The novelty of this work is in combining the advantage of particulate system (microsphere) and mucoadhesive drug delivery system by taking Sodium alginate and Mucoadhesive polymers i.e. HPMC K4, HPMC K100 and Carbopol 940.

## MATERIALS AND METHODS

### Materials

Nevirapine was a gift sample from Aurobindo Pharma Ltd, Hyderabad. HPMC K4M, HPMC K100M, Carbopol 940 and Sodium alginate polymers were obtained from Karnataka Fine Chem Pvt. Ltd (Bangalore).

### Preparation of nevirapine mucoadhesive microspheres

The Nevirapine mucoadhesive microspheres were prepared by Ionotropic external gelation technique [10, 11]. Nevirapine and mucoadhesive polymers were individually passed through sieve ≠ 80. The weighed quantity of the nevirapine was added to purified water containing the mucoadhesive polymers and thoroughly mixed with a stirrer at 400 rpm to form a homogenous polymer solution. The resulting dispersion was sonicated for 30 min to remove any air bubbles. For the formation of mucoadhesive microspheres the dispersion was extruded drop wise from a needle of 24 G in diameter from a height of about 5 cm into aqueous aluminium sulfate solution (10%) and stirred at 400 rpm. The added droplets were retained in the aluminium sulfate solution for 30 min to complete the curing reaction and to produce spherical rigid nevirapine mucoadhesive microspheres [10]. Then the solution containing formed nevirapine microspheres was filtered by using Whatman filter paper. The mucoadhesive microspheres were

allowed to dry at 45 °C for 12 h. The composition of various nevirapine microspheres formulations was mentioned in Table1.

**Percentage yield**

The percentage yield of Nevirapine microspheres of various batches was calculated by using the weight of the final product after drying with respect to the initial total weight of the nevirapine and polymer used for preparation of nevirapine mucoadhesive microspheres.

**Particle size**

Particle size and size distribution of the Nevirapine mucoadhesive microspheres were calculated by sieve analysis method [12]. The Nevirapine mucoadhesive microspheres were separated into different size fractions (% weight fraction) by sieving for 10 min using standard sieves having nominal mesh opening of 1.4 mm, 1.2 mm, 1.0 mm, 0.85 mm and 0.71 mm and the mean particle size was determined.

**Morphology of microspheres**

The surface morphology and shape of the Nevirapine microspheres were determined by scanning electron microscopy (SEM). The sample was mounted on a copper sample holder and sputter coated with platinum in an argon atmosphere.

**Drug entrapment efficiency**

The drug entrapment efficiency of nevirapine microspheres was estimated by dispersing the microspheres in 100 ml of 0.1N HCl buffer by vigorous shaking on mechanical shaker for 24h in order to extract the entrapped nevirapine completely.

The solution was filtered and the nevirapine content in the filtrate was measured after suitable dilution by UV spectrophotometer at 284 nm (LABINDIA UV-3092 PC).

**Table 1: Composition of nevirapine mucoadhesive microspheres**

| Formulation code | Drug: polymer ratio | Polymer ratio |
|---|---|---|
| F1 | 1:0.5 | 0.25:0.25 (Sodium alginate: HPMC K4) |
| F2 | 1:1 | 0.5:0.5 (Sodium alginate: HPMC K4) |
| F3 | 1:1.5 | 0.75:0.75 (Sodium alginate: HPMC K4) |
| F4 | 1:2 | 1:1 (Sodium alginate: HPMC K4) |
| F5 | 1:0.5 | 0.25:0.25 (Sodium alginate: HPMC K100) |
| F6 | 1:1 | 0.5:0.5 (Sodium alginate: HPMC K100) |
| F7 | 1:1.5 | 0.75:0.75 (Sodium alginate: HPMC K100) |
| F8 | 1:2 | 1:1 (Sodium alginate: HPMC K100) |
| F9 | 1:0.5 | 0.25:0.25 (Sodium alginate: Carbopol 940) |
| F10 | 1:1 | 0.5:0.5 (Sodium alginate: Carbopol 940) |
| F11 | 1:1.5 | 0.75:0.75 (Sodium alginate: Carbopol 940) |
| F12 | 1:2 | 1:1 (Sodium alginate: Carbopol 940) |

**Mucoadhesive test**

The mucoadhesive property of nevirapine microspheres was evaluated by an *in vitro* adhesion testing method known as wash-off method**.** The freshly excised piece of the goat intestinal mucosa was mounted on the glass slide with cotton thread. About 100 microspheres were spread onto each prepared glass slide and immediately thereafter the support was hang onto the arm of the USP disintegration machine.

When the test apparatus was operated, the intestinal mucosa was given a slow, regular up and down movement in the test fluid (0.1N HCL buffer) at 37±0.5 °C. At an interval of 60 min up to 8 h the equipment was stopped and the number of nevirapine microspheres still sticking onto the intestinal mucosa was counted and percent mucoadhesion was calculated [13].

***In vitro* dissolution**

The release of nevirapine from the mucoadhesive microspheres was studied in 0.1N HCl buffer pH 1.2 as medium using dissolution test apparatus paddle type at 37±0.5 °C and a rotating speed of 50 rpm. A sample of mucoadhesive microspheres equivalent to 100 mg of nevirapine was used in each test. 5 ml Aliquots were withdrawn through a filter (0.45 µ) from the dissolution apparatus hourly for 12 h, and replaced with an equal volume of fresh dissolution medium. The samples were analyzed at 284 nm for nevirapine content using a UV spectrophotometer (LABINDIA UV-3092 PC) [14].

**Release kinetic studies**

The rate and the mechanism of release of Nevirapine from the prepared mucoadhesive microspheres were analyzed by fitting the dissolution data into various kinetic models like zero order; first order, korsemeyer peppas, Higuchi's model and Coefficient of correlation (r) values were calculated for the liner curves by regression analysis of the above plots.

**FTIR studies**

Fourier transforms infrared spectroscopy (FTIR) spectra of the pure Nevirapine, best formulation were produced using the KBr disk method. The samples were analyzed between wave numbers 4000 to 400 cm-1 resolution.

**DSC studies**

The thermal behavior of pure nevirapine and nevirapine loaded microspheres were studied using a differential scanning calorimeter Shimadzu DSC 60 at a heating rate of 10 °C/min. 5 mg Samples were accurately weighed into aluminum pans and then sealed. The measurements were performed at a heating range of 50 °–400 °C under nitrogen atmospheres.

***X-ray* diffraction study (XRD)**

The crystallinities of nevirapine, and nevirapine loaded mucoadhesive microspheres were evaluated by XRD measurement using an X-ray diffractometer (Brucker). All samples were measured in the 2ø angle range between 1–90 ° and with an interval of 0.1 by exposing them to Cuk α1 radiation (40 KV, 30 MA) and the scanning rate was 5 °/min.

**Stability study**

Stability studies were carried out for Nevirapine formulation as per ICH guidelines. The best mucoadhesive microspheres formulation (F8) was sealed in high density polyethylene bottles and stored at 4 °C/Ambient, 25±2 °C/60±5 %, 40±2 °C/75±5 % RH in closed for 90d. The samples (F8) were evaluated for entrapment efficiency and percentage mucoadhesion for every one month up to three months [15].

**RESULTS AND DISCUSSION**

**Percentage yield and Micromeritic studies**

The production yields of nevirapine microspheres prepared by the ionotropic gelation method were found to be between 83.33 % to 93.23 % as shown in table 2. It was found that production yield of microspheres prepared by Carbopol 940 was greater than HPMC grades. All Nevirapine microspheres formulations were evaluated for micrometric properties. Results are shown in table 3.

Angle of repose of all microspheres batches varied from 22.72 to 30.61. Compressibility index varies from 9.76 % to 14.08 %. Hausner's ratio varies from 1.10 to 1.16. All formulations results revealed excellent flow property and compressibility.

**Table 2: Physicochemical properties of nevirapine mucoadhesive microspheres**

| Formulation code | Percentage yield | Entrapment efficiency | Particle size [µm] | Angle of Repose | Compressibility index | Hausner's ratio |
|---|---|---|---|---|---|---|
| F1 | 83.33 | 63.50±0.75 | 705.21±2.00 | 22.72 | 09.76 | 1.10 |
| F2 | 87.15 | 72.27±1.11 | 723.33±2.42 | 24.40 | 10.66 | 1.11 |
| F3 | 89.80 | 78.42±1.62 | 749.38±1.25 | 25.73 | 11.42 | 1.11 |
| F4 | 91.03 | 85.66±1.67 | 785.80±2.61 | 27.49 | 11.86 | 1.11 |
| F5 | 85.47 | 71.72±0.75 | 774.58±1.70 | 23.45 | 10.53 | 1.11 |
| F6 | 88.85 | 79.06±1.00 | 816.49±1.10 | 24.92 | 12.04 | 1.13 |
| F7 | 91.04 | 84.45±1.54 | 863.13±2.54 | 27.80 | 12.70 | 1.14 |
| F8 | 92.37 | 91.48±1.67 | 901.08±1.91 | 28.79 | 13.89 | 1.16 |
| F9 | 86.13 | 77.87±0.79 | 799.58±1.91 | 25.17 | 10.97 | 1.12 |
| F10 | 89.35 | 85.88±1.00 | 837.52±2.21 | 26.85 | 11.77 | 1.12 |
| F11 | 91.56 | 91.52±1.18 | 883.92±2.09 | 28.79 | 12.78 | 1.14 |
| F12 | 93.23 | 96.42±1.67 | 935.45±2.07 | 30.61 | 14.08 | 1.16 |

**Particle size**

The average particle size of Nevirapine microspheres ranged from 705.21±2.00 to 935.45±2.07 µm. The mean particle size was significantly increases with increasing mucoadhesive polymer concentration this may be attributed to the high viscosity of the mucoadhesive polymer solution [16].

**Morphology of microspheres**

The morphology of the mucoadhesive microspheres of best formulation F8 was examined by scanning electron microscopy. The SEM photographs revealed that nevirapine microspheres were discrete (Fig.1) and spherical shape with a rough surface morphology [17].

**Entrapment efficiency**

The percentage entrapment efficiency ranged from 63.50 to 96.42%. (table 2). The entrapment efficiency of the nevirapine microspheres prepared with Carbopol 940 was higher than those of microspheres prepared with HPMC grades. This may be attributed to higher molecular weight of Carbopol 940 than HPMC grades. An increase in the molecular weight of the polymer increases the entrapment efficiency of the mucoadhesive microspheres due to the formation of the more intact matrix network [18].

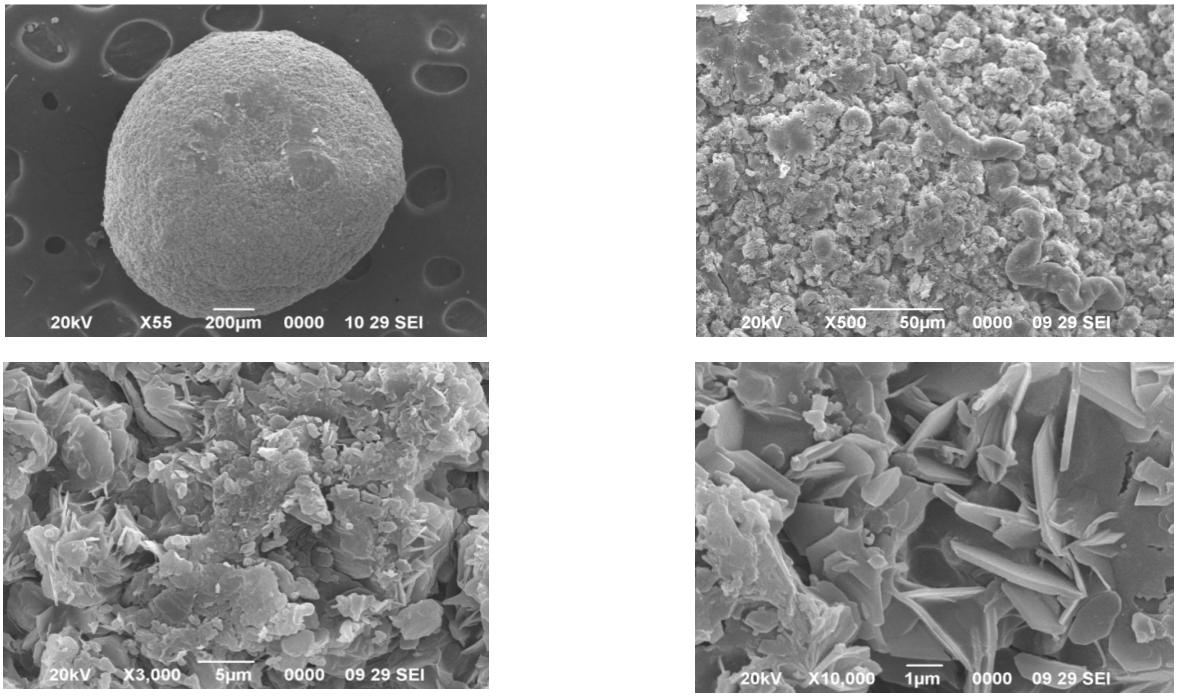


**Fig. 1: Scanning electron photomicrographs of the Formulation (F3): a) 55 X, b) 500 X, c) 3000 X, d) 10000 X**

**Mucoadhesive test**

The results of the in-vitro mucoadhesion studies of all nevirapine microsphere formulations were shown in table 4. Percentage mucoadhesion of nevirapine formulations increased with the increase in concentration of mucoadhesive polymers. The higher mucoadhesion of Carbopol 940 based mucoadhesive microspheres may be attributed to higher molecular weight of Carbopol 940 than HPMC K4 and HPMC K100 based microspheres. Our result is supported by the report of Robinson *et al.* [19].

The solubility, hydration and mucoadhesiveness of the polymers depend on the pH of the *in-vitro* wash off medium.

The results of the *in-vitro* wash-off test indicated that the nevirapine microspheres had fairly good mucoadhesive properties.

The developed nevirapine mucoadhesive microspheres would adhere to the Gastro intestinal walls, thus resisting gastric emptying and prolong the residence time at the absorption site, thereby improve and enhance the bioavailability [20, 21].

**Table 4: Results of *in vitro* wash off test In 0.1 M HCL (pH 1.2)[a]**

| Hours | 1 | 2 | 4 | 6 | 8 |
|---|---|---|---|---|---|
| F1 | 95±1.52 | 85±2.08 | 53±0.57 | 32±1.15 | 03±0.57 |
| F2 | 97±1.15 | 87±1.52 | 61±0.57 | 39±1.00 | 12±1.15 |
| F3 | 100±0.57 | 93±1.52 | 67±1.15 | 47±1.52 | 22±1.73 |
| F4 | 100 | 98±1.15 | 71±1.00 | 56±0.57 | 34±1.52 |
| F5 | 97±1.00 | 89±0.57 | 71±1.52 | 57±0.57 | 36±1.15 |
| F6 | 100 | 97±0.57 | 80±1.00 | 64±1.15 | 45±1.52 |
| F7 | 100 | 99±1.53 | 88±1.00 | 71±1.15 | 57±0.57 |
| F8 | 100 | 100±0.57 | 90±1.52 | 77±1.15 | 64±0.57 |
| F9 | 100 | 96±0.57 | 83±1.15 | 66±1.52 | 45±1.55 |
| F10 | 100 | 100±0.57 | 87±1.15 | 72±1.00 | 54±1.52 |
| F11 | 100 | 100 | 93±0.57 | 79±1.00 | 62±1.53 |
| F12 | 100 | 100 | 95±0.57 | 82±1.52 | 68±1.00 |

### *In vitro* dissolution studies

The *in vitro* Nevirapine release profiles for all batches was shown in fig. 2-3. Drug release from these mucoadhesive microspheres were slow, controlled release and dependent upon the nature and concentration of mucoadhesive polymers used [22,23]. It was found that there was decrease in nevirapine release with increase in mucoadhesive polymer content.

This could be attributed to the greater degree of swelling upon hydration with greater mucoadhesive polymer content in the microspheres which leads to increase in the diffusional path length that slows down drug release. Among all the formulation F8 was chosen as the best formulation which containing HPMC K100M in combination with sodium alginate (1:2) showed good drug release profile in 12h (98.65%). Hence it is considered as the best microsphere formulation which seems to be a good candidate for controlled release.

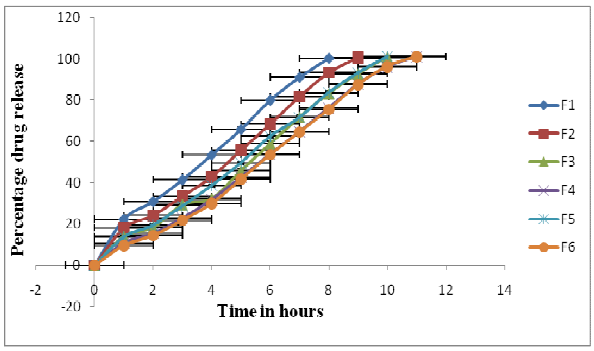


**Fig. 2: Comparative release profile of formulation F1 to F6**

### Release kinetic studies

Drug release kinetic data for Nevirapine mucoadhesive microspheres were shown in table No.5. All the microsphere formulations (F1 to F12) followed the Korsmeyer-peppas model and zero-order release kinetic with regression values ranging from 0.928 to 0.996. Korsmeyer-Peppas plots, 'n' value ranges from 0.388 to 1.402 indicating that the Nevirapine release mechanism followed Anomalous and super case-II transport mechanism.

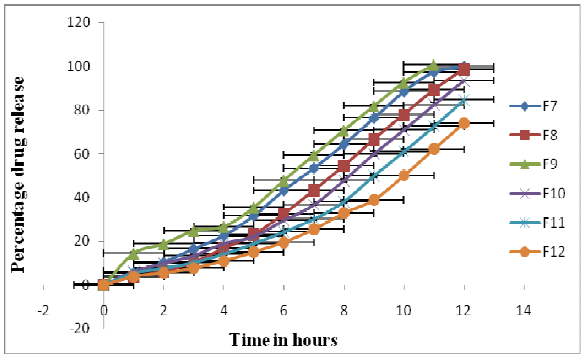


**Fig. 3: Comparative release profile of formulation F7 to F12**

### FTIR studies & DSC studies

IR Spectra of pure drugs sample of nevirapine were compared with IR spectra of nevirapine loaded Microspheres, as there was no significant change in the pattern of peaks of pure drug and nevirapine loaded Microspheres (Fig.4). Hence there was no interaction seen in between nevirapine and Mucoadhesive polymers.

The thermal behavior of prepared nevirapine microspheres was studied in comparison with thermo grams of both pure nevirapine and drug loaded microspheres as shown in fig. 5. The thermogram of pure nevirapine showed a sharp endothermic peak at 245.6 ℃, which corresponds to its melting point. The characteristic peak of nevirapine was well recognized in the nevirapine-loaded microspheres. Thus, there was no incompatibility between nevirapine and mucoadhesive polymers used in the formulation of microspheres.

**Table 3: Release kinetic parameter of nevirapine from mucoadhesive microspheres**

| Formulation code | Zero order | First Order | Higuchi | Korsmeyer peppas | n-value |
|---|---|---|---|---|---|
| F1 | 0.996 | 0.868 | 0.969 | 0.978 | 0.388 |
| F2 | 0.992 | 0.780 | 0.954 | 0.970 | 0.835 |
| F3 | 0.987 | 0.767 | 0.944 | 0.964 | 0.952 |
| F4 | 0.992 | 0.800 | 0.956 | 0.980 | 1.017 |
| F5 | 0.996 | 0.778 | 0.964 | 0.983 | 0.924 |
| F6 | 0.992 | 0.799 | 0.955 | 0.982 | 1.066 |
| F7 | 0.988 | 0.778 | 0.940 | 0.990 | 1.239 |
| F8 | 0.974 | 0.697 | 0.905 | 0.979 | 1.402 |
| F9 | 0.975 | 0.725 | 0.912 | 0.932 | 0.870 |

| | | | | | |
|---|---|---|---|---|---|
| F10 | 0.956 | 0.773 | 0.877 | 0.958 | 1.129 |
| F11 | 0.938 | 0.805 | 0.849 | 0.938 | 1.151 |
| F12 | 0.928 | 0.826 | 0.837 | 0.945 | 1.225 |

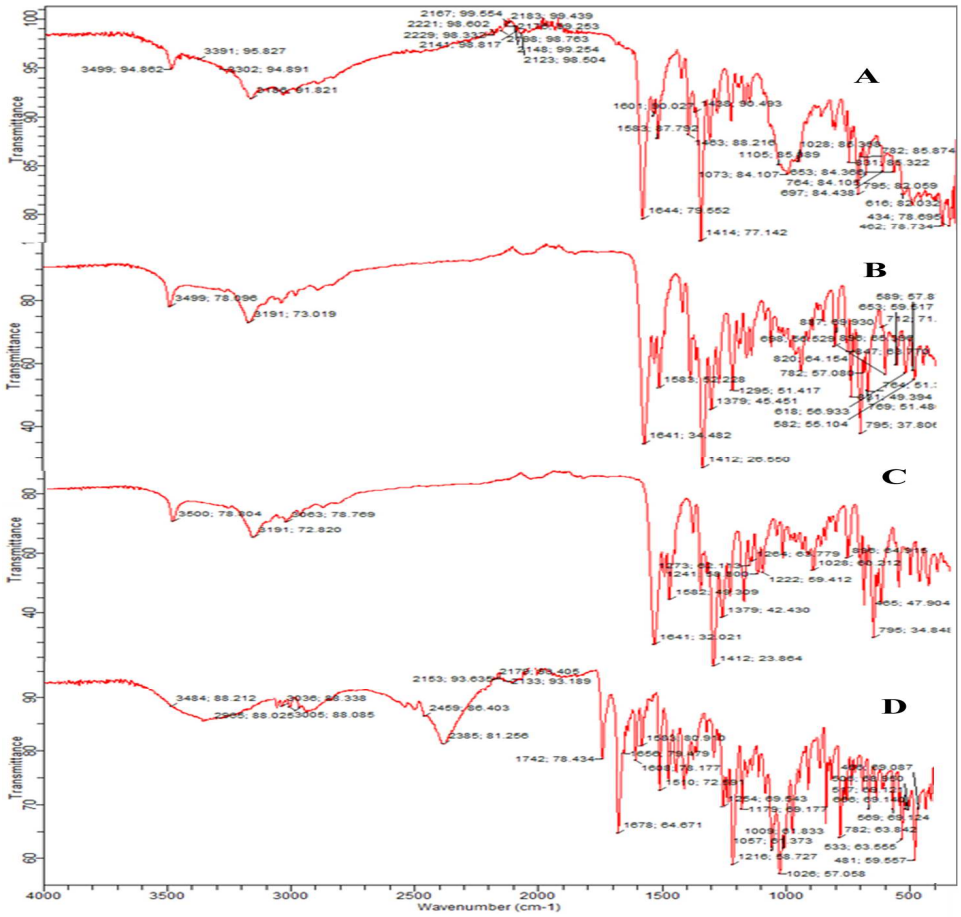


**Fig. 4: FTIR spectra of, (A): Pure Nevirapine; (B): Formulation containing K4M; (C): Formulation containing HPMC K100M; (D): Formulation containing Carbopol 940**

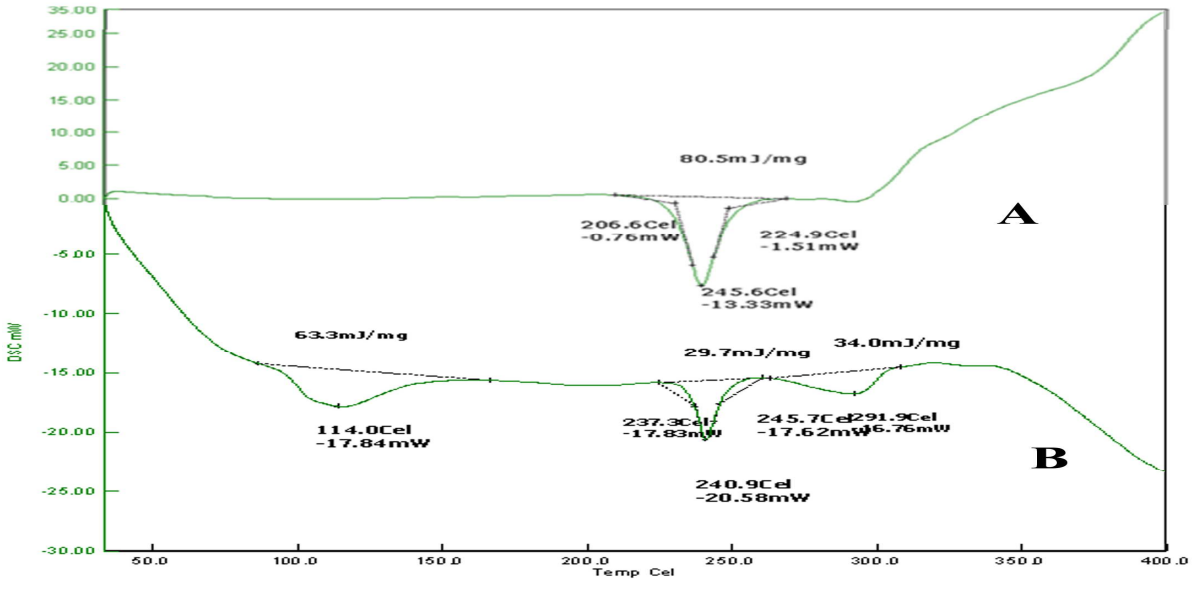


**Fig. 4: DSC Thermogram of, (A): Nevirapine; (B): Best formulation F8**

***X-Ray* diffraction study (XRD)**

The X-ray diffraction spectra's was recorded for nevirapine and nevirapine loaded microspheres (F8) for investigating the crystalainity of the nevirapine in the mucoadhesive microspheres. The X-ray diffractogram of nevirapine showed sharp peaks at 6000 depicting a typical crystalline pattern (fig. 5). However, nevirapine loaded mucoadhesive microspheres showed peaks, but of low intensity (3000), revealing that some amount of nevirapine was changed to amorphous form [24].

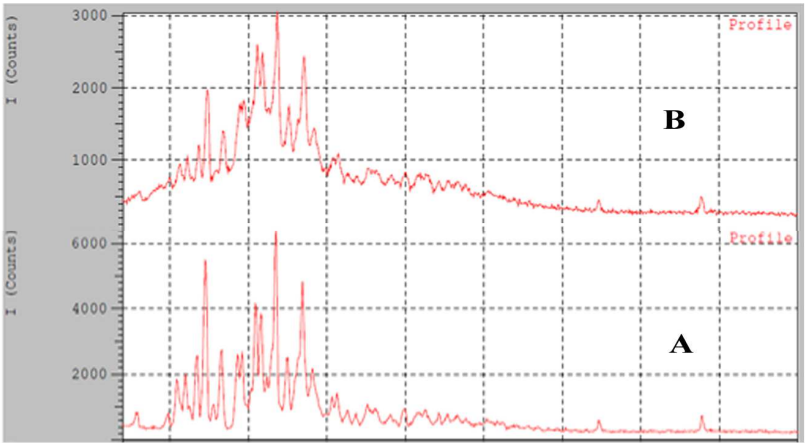


**Fig. 5**: **XRD pattern of, (A): Pure Nevirapine; (B): Best formulation F8**

**Table 6**: **Percentage entrapment efficiency and mucoadhesion of the F8 formulation**

| Stability condition | Sampling Day | Percentage Entrapment efficiency [a] | Percentage Mucoadhesion [a] |
|---|---|---|---|
| 4 °C/Ambient | 30 | 96.42±0.79 | 67.33±1.15 |
| | 60 | 96.05±0.67 | 65.67±1.53 |
| | 90 | 95.47±1.11 | 63.00±1.73 |
| 25 °C/60 RH | 30 | 96.13±1.00 | 68.00±1.53 |
| | 60 | 95.75±1.18 | 65.33±0.58 |
| | 90 | 95.14±1.54 | 61.67±1.53 |
| 40 °C/75RH | 30 | 96.36±1.67 | 67.33±0.58 |
| | 60 | 96.11±0.79 | 63.67±1.53 |
| | 90 | 95.43±1.62 | 60.00±1.73 |

[a]mean±SD, *n* = 3.

**Stability study**

Stability studies of the prepared nevirapine mucoadhesive microspheres were carried out by storing the optimized formulation F8 at 4 °C/Ambient, 25±2 °C/60±5 %, 40±2 °C/75±5 % RH for 3 mo. The Best batch F8 show negligible change in entrapment efficiency and percentage mucoadhesion as shown in table 6. So it can be said that nevirapine mucoadhesive microspheres prepared with HPMC K100M is stable.

**CONCLUSION**

The present study has been satisfactorily attempted to formulate a mucoadhesive system of Nevirapine for oral administration with a view of enhancing bioavailability of the drug. From the experimental results, it can be concluded that, The IR & DSC spectra revealed that there was no interaction between polymers and Nevirapine, hence they are compatible. The prepared mucoadhesive microspheres of Nevirapine gave good micromeritic results. The particle size analysis revealed that all formulations gave particles in the range of 705.21 to 933.45 µm which is suitable for oral administration of the formulation.

Among all the formulation F8 was chosen as best formulation which containing HPMC K100M in combination with sodium alginate (1:2) showed the good entrapment efficiency (91.48%) mucoadhesion about (64 after 8h) and good drug release profile in 12h (98.65%). The drug release was erosion controlled as the plot of Pappas model was found to be linear. SEM analysis of the F8 microspheres revealed that the formulation was rough and spherical with ideal surface morphology. The prepared muco adhesive microspheres of Nevirapine will increase therapeutic efficacy by increasing patient compliance and provide controlled release which will overcome the drawbacks associated with the conventional therapy.

**CONFLICT OF INTERESTS**

Declared None

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