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Formulation and evaluation of nevirapine mucoadhesive microspheres

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ABSTRACT

The aim of the present research was to formulate and evaluate Sodium alginate, Pectin and Sodium alginate in combination with Pectin mucoadhesive microspheres for controlled release of Nevirapine. The mucoadhesive microspheres were formulated by Ionotropic gelation technique, using Sodium alginate, Pectin and Sodium alginate in combination with Pectin as mucoadhesive polymer in various proportions in combination. Further, the prepared Nevirapine mucoadhesive microspheres were characterized for particle size, morphology, entrapment efficiency, mucoadhesion, *in vitro* drug release, Nevirapine release kinetics and compatability studies (FTIR & DSC). The Nevirapine Microspheres were free-flowing and discrete. The mean particle size ranged from 643.26 ± 5.93 µm to 892.34 ± 5.64 µm and the entrapment efficiencies ranged from 54.51 to 91.60 %. The Nevirapine entrapment efficiency was found to be dependent on type and concentration of mucoadhesive polymer used for formulation. Scanning electron microscopy revealed the surface morphology of Nevirapine mucoadhesive microspheres .The FTIR and DSC confirmed stable character of Nevirapine in the drug-loaded mucoadhesive microspheres. The crystallinity of Nevirapine was found to be reduced in prepared mucoadhesive microspheres (F12), which were confirmed by XRD studies. The mechanism of Nevirapine release from the mucoadhesive microsphere was found to be anomalous and super case-II transport type. Stability studies were done for the best formulation F12 indicates that there is no change in entrapment efficiency of the formulation.

Keywords: Sodium alginate, Pectin, Mucoadhesive Microspheres, Nevirapine.

1. INTRODUCTION

Oral controlled drug delivery systems continue to be the most popular drug delivery systems as it offers several advantages over the conventional drug delivery systems like; Improving patient's compliance due to reduction of frequency of administration. [1] The common problem encountered with controlled release system is the lack of ability to restrain and localize the dosage form at gastro-intestinal tract, due to gastric emptying phenomenon. [2] In order to overcome this drawbacks, it has been proposed, to coupling the drugs to Microparticulate delivery systems an important part of novel drug delivery. [3] However the success of microparticulated system is limited due to their limited residence time at the site of absorption. [4] It can be accomplished by coupling mucoadhesion characteristics to Microparticulated system by using mucoadhesive polymers and developing mucoadhesive microspheres. [5] Mucoadhesive microspheres have advantages like localization of drug action of the delivery system at a given target site, Increased residence time combined wit h controlled drug release may lead to lower admin istration frequency, cost reductions may be achieved and dose-related side effects may be reduced . [6]

Nevirapine, the first ARV member of non-nucleoside reverse transcriptase inhibitor approved by the Food and Drug Administration (FDA) for HIV and an important component of HAART, is typically the primary choice for efficient viral suppression. [7,8] Nevirapine belongs to class II under BCS and exhibits low and variable or al bioavailability due to poor aqueous solubility. B ecause of its low biological half life more over it is primarily absorbed from stomach make it suitable candidate for administration by gastro-rentensive dosage form.[9] The US FDA has issued a

'black box label' on nevirapine due to its hepatotoxicity. The use of nevirapine has been restricted except in cases where the benefit to the patient exceeds the risk. Therefore the development of a delivery system for controlled and targeted release of Nevirapine which could utilize all the efficacy of Nevirapine thereby reduced dosing frequency and to improve the quality of HIV infected patients. In the present study, mucoadhesive Nevirapine microspheres were developed using a hydrophilic polymer, Sodium alginate, Pectin and Sodium alginate in combination with Pectin.[10,11]

2. MATERIAL AND METHODS

2.1. Materials

Nevirapine was a gift sample from Hetro Pharma Ltd, Hyderabad. Sodium alginate and Pectin polymers were received as gift sample from Aurobindo Pharma Ltd, Hyderabad. All other ingredients and solvents used were of analytical grade.

2.2. Formulation of Nevirapine mucoadhesive microspheres

The composition of the various Nevirapine mucoadhesive microspheres formulations were mentioned in Table1. Nevirapine and mucoadhesive polymers were individually passed through sieve ≠ 60. The required quantities of mucoadhesive polymers were dissolved in purified water to form a homogenous solution. Nevirapine was added to the polymer solution and mixed thoroughly with magnetic stirrer at 400 rpm to form a homogeneous dispersion and resulting dispersion was sonicated for 30 min to remove entrapped air bubbles. For the formation of mucoadhesive microspheres the bubble free homogeneous dispersion was then extruded manually drop wise into 10% cross linking solution (aluminum sulphate) using syringe (needle size 22 G). The extruded droplets were cured in the aluminium sulphate (cross linking) solution for 30 minutes to complete the reaction and to produce spherical rigid microspheres. [12] The obtained Nevirapine microspheres were collected by decantation, washed repeatedly with distilled water and dried at 45°C for12 hour. The final products were stored in well closed container for further use.

2.3. Percentage yield

The percentage yield was calculated by dividing weight of dried Nevirapine microspheres (W1) by initial weight of the Nevirapine and mucoadhesive polymers (W2) used for the formulation and converting the weight ratio into percent. [13]

2.4. Particle Size

Particle size and size distribution of the Nevirapine microspheres were measured by sieve analysis method. The Nevirapine microspheres were separated into different size fractions (% weight fraction) by sieving for 10 min using standard sieves having nominal mesh aperture of 1.4 mm, 1.2 mm, 1.0 mm, 0.85 mm and 0.71 mm and the mean particle size of the Nevirapine microspheres was determined. [14]

2.5. Morphology of Microspheres

The surface morphology and shape of the Nevirapine mucoadhesive microspheres was examined by scanning electron microscopy (JEOL JSM-5200). The microsphere sample was mounted on to an aluminum stub and sputter-coated with platinum particles in an argon atmosphere. [15]

2.6. Drug Entrapment Efficiency

The amount of Nevirapine entrapped was estimated by method of extraction of drug present

Table - 1: Composition of Nevirapine mucoadhesive microspheres

Formulation code	Drug: Polymer ratio	Drug : Polymer ratio
F1	1:0.5	Drug : Sodium alginate
F2	1:1	Drug : Sodium alginate
F3	1:1.5	Drug : Sodium alginate
F4	1:2	Drug : Sodium alginate
F5	1:0.5	Drug : Pectin
F6	1:1	Drug : Pectin
F7	1:1.5	Drug : Pectin
F8	1:2	Drug : Pectin
F9	1:0.5	Drug : Sodium alginate (0.25) : Pectin (0.25)
F10	1:1	Drug : Sodium alginate (0.50) : Pectin (0.50)
F11	1:1.5	Drug : Sodium alginate (0.75) : Pectin (0.75)
F12	1:2	Drug : Sodium alginate (1.0) : Pectin (1.0)

in mucoadhesive microsphere. The dried Nevirapine mucoadhesive microspheres (100mg) were taken and extracted in 100 ml of 0.1N HCl for 24 hours in rotary shaker. The solution was filtered through a 0.45 µm filter and the concentration of Nevirapine present in filtrate determined spectrophotometrically at 284 nm (LABINDIA UV-3092 PC) against 0.1 N HCl as a blank. [16]

2.7. Mucoadhesive Test

The mucoadhesive property of Nevirapine microspheres was evaluated by *in vitro* wash off test. A Piece of goat intestinal mucosa was mounted on the glass slide using cyanoacrylate glue. About 100 microspheres were spread onto each wet rinsed intestinal mucosa specimen and immediately the support was hung onto the arm of USP disintegration apparatus. Now intestinal mucosa was given a slow regular up and down movement in test fluid (0.1N HCL buffer at 37±0.5^{0}C) by operating the disintegration test apparatus. Every one hour intervals up to 8 hrs the equipment was stopped and the number of Nevirapine mucoadhesive microspheres still sticking onto the intestinal mucosa was counted and percent mucoadhesion was calculated. [17]

2.8. *In Vitro* Dissolution

Mucoadhesive Microspheres containing equivalent to 100 mg of Nevirapine were introduced into dissolution medium of 0.1N HCl (900ml) for 12 hrs at 37±0.5°C at a rotation speed of 50 rpm by using USP type II dissolution test (Labindia ,Disso-2000,Mumbai,India). Samples of 5ml were withdrawn through a filter (0.45 µ) at every one hour intervals up to 12th hrs and replaced with equal volume of 0.1N HCl buffer. The samples were analyzed at 284 nm for Nevirapine content using spectrophotometer. All dissolution runs were carried out in triplicate. [18]

2.9. Release kinetic and mechanism of Nevirapine release

In order to understand the mechanism and kinetic of Nevirapine release from the prepared microspheres were analyzed by fitting the dissolution data into various kinetic models like zero order; first order, korsemeyer peppas and Higuchi's model and Coefficient of correlation (r) values were calculated for the liner curves by regression analysis of the above plots. [19]

2.10. FTIR Studies

Compatibility study of Nevirapine with different mucoadhesive polymers was determined by I.R. Spectroscopy (FTIR) using Shimadzu FT-IR spectrometer model. The pellets were prepared with IR grade KBr using Nevirapine, mucoadhesive polymers and mucoadhesive microspheres formulations containing both Nevirapine and polymer and the scanning were done between wave numbers 4000 to 400 cm^{-1} at 4 cm^{-1} resolution.

2.11. Thermal Analysis (DSC)

Differential scanning calorimetries were carried out on pure drug Nevirapine and Nevirapine loaded microspheres using a Shimadzu DSC 60 to evaluate any possible Nevirapine - mucoadhesive polymers interaction. Samples (5mg each) were accurately weighed into aluminum pans and sealed. DSC run were conducted over a temperature range 40-300 °C at a heating rate of 10 °C / min under nitrogen atmospheres. [20]

2.12. *X-Ray* Diffraction study (XRD)

The crystallinities of Nevirapine and Nevirapine loaded mucoadhesive microspheres were evaluated by XRD measurement using an X-ray diffractometer. XRD studies were performed on the prepared samples by exposing them to Cuk α1 radiation (40 kV, 30 mA) and the scanning rate was 5° /min over a range of 4-90° and with an interval of 0.1. [21]

2.13. Stability Study

To assess the Nevirapine and mucoadhesive formulation stability, stability studies were carried out as per ICH guidelines. The best mucoadhesive microspheres formulation (F12) was selected for stability study on the basis of; drug entrapment efficacy and *in vitro* wash off test and *in vitro* drug dissolution results. In the investigation, best formulation were stored at 4 ^{0}C± 1^{0}C / Ambient ,25 ± 2^{0}C/ 60 ± 5 % RH, 40 ± 2^{0}C/ 75 ± 5 % RH in closed HDPE bottles for 90days. The samples were pulled out from stability chamber every month interval up to 3 months and evaluated for entrapment efficiency and percentage mucoadhesion. [22, 23]

3. RESULT AND DISCUSSION

3.1. Percentage yield and Micromeritics studies

The purpose of this study was to formulate mucoadhesive microspheres of Nevirapine by ionotropic gelation method, using Sodium alginate, Pectin and Sodium alginate in combination with Pectin as a polymer, Pectin / Sodium alginate microspheres are used to provide controlled release of Nevirapine and to enhance the uptake of drug across epithelial layer.

The prepared Nevirapine microsphere gave good percentage yield. The percentage yield of Nevirapine mucoadhesive microspheres ranged from 84.12 to 95.62 %. All Nevirapine

microspheres formulations were evaluated for micrometric properties. Results are shown in table 3. Angle of repose of all microspheres batch varied from 17.24 to 27.35.Compressibility index varies from 8.30 % to 16.00 %. Hausner's ratio varies from 1.21 to 1.30. Here all these formulations results revealed good flow property and compressibility.

3.2. Particle Size

The average particle size of Nevirapine mucoadhesive microspheres ranged from 643.26 ± 5.93 to 892.34 ± 5.64 µm, and such particles are considered to be suitable for oral administration. The results also revealed that with the increase in the Nevirapine: polymer ratio there was an increase in the size of mucoadhesive microspheres (Table 2). [24]

3.3. Morphology of Microspheres

The morphology of the Nevirapine microspheres of optimized formulation F12 was examined by scanning electron microscopy and depicted in the Figure 1. The SEM photographs revealed that microspheres were discrete and spherical shape with a rough surface morphology

Table - 2 : Physico chemical properties of Nevirapine mucoadhesive microspheres

Formulation code	Percentage yield [a]	Theoretic drug content (mg)	Entrapment efficiency [a]	Particle size [µm] [a]
F1	90.81	66.6	54.51 ± 1.19	643.26 ± 5.93
F2	92.34	50	66.43 ± 1.32	695.69 ± 6.91
F3	94.41	40	74.40 ± 0.88	741.48 ± 8.46
F4	95.62	33	83.33 ± 0.73	793.25 ± 2.64
F5	88.12	66.6	57.40 ± 0.61	675.33 ± 2.10
F6	90.72	50	68.78 ± 0.99	703.60 ± 1.38
F7	92.51	40	76.54 ± 1.69	753.70 ± 0.172
F8	94.35	33	87.70 ± 0.72	808.60 ± 4.65
F9	84.12	66.6	59.10 ± 0.85	703.51 ± 3.23
F10	86.72	50	72.70 ± 0.77	774.31 ± 7.91
F11	89.51	40	84.62 ± 1.00	837.18 ± 4.46
F12	91.35	33	91.60 ± 0.91	892.34 ± 5.64

[a] Mean ± SD, *n* = 3

Table - 3: Micromeritics properties of Nevirapine mucoadhesive microspheres.

Formulation code	Bulk density [a]	Tapped density [a]	Compressibility index [a]	Hausner's ratio [a]	Angle of Repose [a]
F1	0.381 ± 0.010	0.440 ± 0.020	12.50 ± 1.17	1.21 ± 0.01	18.43 ± 0.99
F2	0.382 ± 0.010	0.440 ± 0.010	13.84 ± 0.84	1.25 ± 0.06	21.80 ± 1.05
F3	0.382 ± 0.020	0.451 ± 0.030	14.60 ± 1.01	1.25 ± 0.09	24.90 ± 1.12
F4	0.382 ± 0.010	0.463 ± 0.020	16.30 ± 0.90	1.30 ± 0.07	26.57 ± 0.69
F5	0.451 ± 0.010	0.490 ± 0.020	8.30 ± 1.15	1.24 ± 0.13	17.24 ± 1.07
F6	0.463 ± 0.010	0.520 ± 0.010	12.40 ± 1.16	1.25 ± 0.11	20.77 ± 1.32
F7	0.452 ± 0.010	0.524 ± 0.010	12.40 ± 0.58	1.25 ± 0.11	22.90 ± 0.88
F8	0.562 ± 0.010	0.546 ± 0.020	14.20 ± 1.01	1.30 ± 0.12	24.57 ± 1.08
F9	0.563 ± 0.010	0.632 ± 0.020	11.40 ± 1.02	1.19 ± 0.01	20.56 ± 1.15
F10	0.567 ± 0.020	0.640 ± 0.020	12.20 ± 1.18	1.25 ± 0.11	22.17 ± 1.09
F11	0.561 ± 0.020	0.650 ± 0.010	14.40 ± 1.04	1.25 ± 0.10	25.17 ± 0.85
F12	0.553 ± 0.010	0.656 ± 0.010	16.00 ± 0.72	1.27 ± 0.08	27.35 ± 0.93

[a] Mean ± SD, *n* = 3.

which could be due to the surface association of the Nevirapine with mucoadhesive polymer. [25]

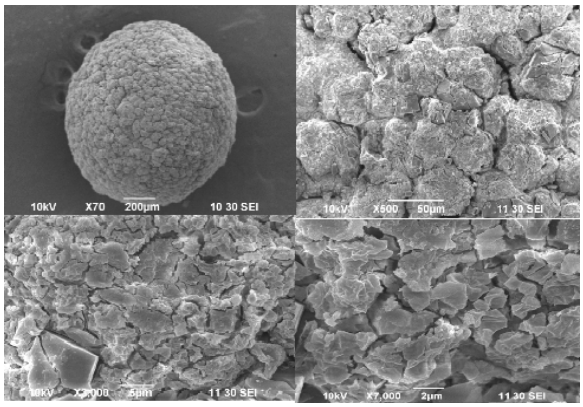

Figure- 1: Scanning electron photomicrograp--hs of the Formulation F12: a) 70 X, b) 500 X, c) 3000 X, d) 7000 X

3.4. Entrapment Efficiency

The percentage entrapment efficiency ranged from 54.51 to 91.60 % (Table 2). The entrapment efficiency of the Nevirapine microspheres prepared with Sodium alginate in combination with pectin was higher than those of microspheres prepared with Sodium alginate and Pectin. The results revealed that increase in the concentration of the mucoadhesive polymer increase the entrapment efficacy of Nevirapine. This can be due to increase in the viscosity of the mucoadhesive polymeric solution, which increases the strength of formed matrix. [26]

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	85	46	19	03	0
F2	90	62	39	17	4
F3	95	74	51	30	18
F4	97	86	69	49	28
F5	91	56	23	6	0
F6	96	75	55	31	6
F7	97	86	64	45	25
F8	98	91	82	63	41
F9	83	46	19	03	0
F10	90	62	39	17	4
F11	95	74	51	30	18
F12	98	80	61	43	35

[a] Mean ± SD, *n* = 3.

3.5. Mucoadhesive Test

The results of the in-vitro mucoadhesion studies of all Nevirapine formulations were shown in Table 4. Percentage mucoadhesion of batches increased with the increase in amount of mucoadhesive polymers. The higher mucoadhesion of Sodium alginate in combination with pectin based mucoadhesive microspheres may be attributed to the higher molecular weight of Sodium alginate with pectin than Sodium alginate and Pectin based microspheres. 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 Gastric mucosa, thus resisting gastric emptying and extend residence time at the absorption site thereby improve and enhance the bioavailability of drug. [28, 29]

3.6. *In Vitro* Dissolution studies

The *invitro* Nevirapine release profiles for all batches were shown in Figure 2. The Nevirapine release behaviors depend upon the nature and concentration of mucoadhesive polymers in polymer matrix. [30,31] Sodium alginate and Pectin microspheres (F1 and F8) were able to control the Nevirapine release up to 10 hours where as Sodium alginate in combination with Pectin microspheres were able to control the drug released more than 11 hours. It has been observed that Sodium alginate and Pectin based mucoadhesive microsphere showed comparatively rapid Nevirapine release as compared to Sodium alginate with Pectin based formulations.

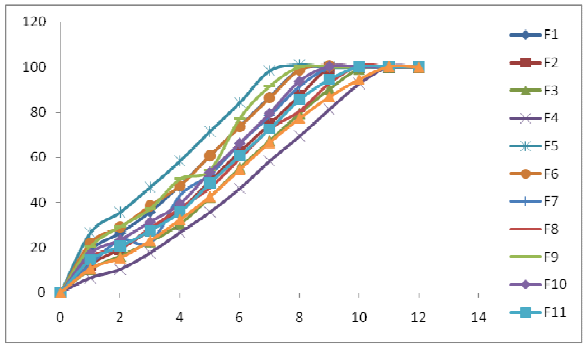

Figure - 2: Comparative release profile of formulation F1 to F12

3.7. Release kinetic and mechanism of Nevirapine release

Drug release kinetic data for Nevirapine microspheres was shown in Table No. 5. All the formulations (F1 to F12) follow zero order release kinetics with regression values ranging from 0.684 to 0.994. Korsmeyer-Peppas plots, 'n' value ranges from 0.684 to 1.216 indicating that the Nevirapine release mechanism followed anomalous and super case-II transport mechanism.

Table - 5: Release Kinetic parameter of Nevirapine from mucoadhesive microspheres

Formulation code	Zero order	First order	Higuchi	Korsmeyer peppas	n-value
F1	0.994	0.728	0.955	0.975	0.826
F2	0.991	0.637	0.945	0.980	0.986
F3	0.989	0.710	0.941	0.976	1.037
F4	0.990	0.839	0.939	0.984	1.216
F5	0.991	0.825	0.972	0.979	0.684
F6	0.968	0.835	0.938	0.947	0.806
F7	0.990	0.637	0.946	0.960	0.890
F8	0.991	0.755	0.960	0.963	0.891
F9	0.993	0.770	0.958	0.974	0.798
F10	0.986	0.766	0.940	0.992	0.961
F11	0.990	0.790	0.951	0.970	0.985
F12	0.992	0.869	0.953	0.979	1.017

3.8. FTIR Studies and DSC studies

FT-IR spectra of pure Nevirapine and Nevirapine loaded microspheres were compared and shown in figure 3. The FT-IR spectra of Nevirapine loaded Microspheres showed the characteristic peaks of the pure Nevirapine indicating that there was no interaction between the drug and mucoadhesive polymers. The thermogram of Nevirapine exhibited a sharp endothermic peak at 245.6°C shown in figure 4, which corresponds to its melting point. The characteristic peak of Nevirapine was well recognized in the drug-loaded mucoadhesive microspheres. Thus, there was no interaction between Nevirapine and mucoadhesive polymers.

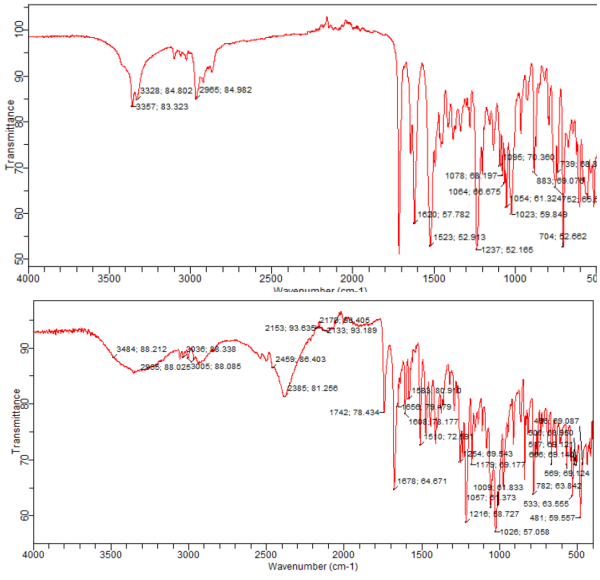

Figure - 3: FTIR spectra of, (A): Pure Nevirapine; (B): Best Formulation (F12).

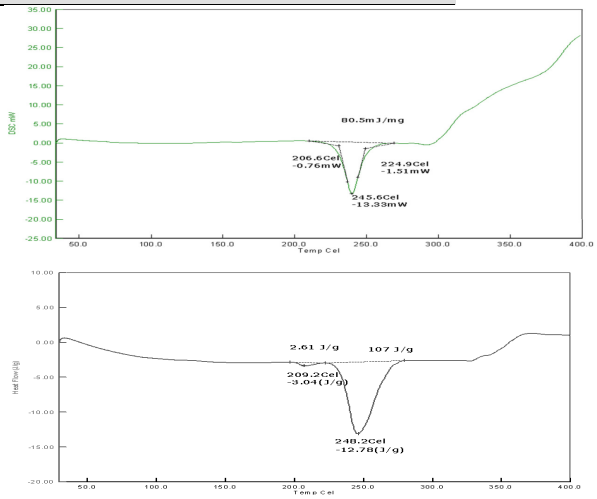

Figure - 4: DSC Thermograms of, (A): Pure Nevirapine (B): Best Formulation (F12)

3.9. *X-Ray* Diffraction study (XRD)

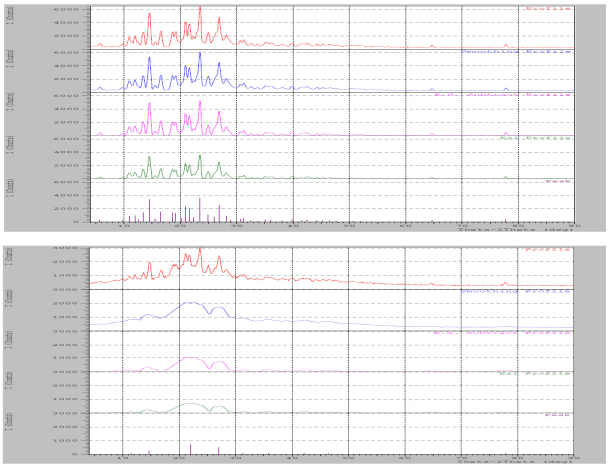

Figure - 5 : XRD pattern of, (A): Pure Nevirapine; and (B): Best formulation 12

The X-ray diffractograms of Nevirapine and formulation F12 are shown in figure 5. Pure Nevirapine has shown characteristic intense peaks due to its crystalline nature. Whereas, in case of formulation F12 showed less intense peak of low intensity, revealing amorphous dispersion of the Nevirapine after entrapment into mucoadhesive microspheres. [32]

3.10. Stability Study

Stability studies of the prepared Nevirapine microspheres were carried out by storing the best formulation F12 at 4 ^{0}C± 1^{0}C / Ambient ,25 ± 2^{0}C/ 60 ± 5 % RH, 40 ± 2^{0}C/ 75 ± 5 % RH for 90 days. The best formulation F12 show insignificant change in entrapment efficiency and physical appearance as shown in table 6. So it can be concluded that Nevirapine mucoadhesive microspheres prepared with Sodium alginate / Pectin is stable.

Table - 6: Percentage entrapment efficiency and mucoadhesion of the F12 formulation

Stability condition	Sampling Day	Percentage Entrapment efficiency [a]
4 ^{0}C / Ambient	0	87.70 ± 0.724
	30	87.17 ± 0.516
	60	86.92 ± 0628
	90	86.29 ± 0.337
25°C/ 60 RH	0	87.70 ± 0.724
	30	87.42 ± 0.519
	60	87.27 ± 0.437
	90	87.05 ± 0.295
40°C/ 75RH	0	87.70 ± 0.724
	30	87.13 ± 0.637
	60	86.57 ± 0.548
	90	86.07 ± 0.439

4. CONCLUSION

The Sodium alginate in combination with Pectin mucoadhesive microspheres containing Nevirapine can be successfully prepared by ionotropic technique. The present method was quite simple, rapid and does not imply the use of toxic organic solvent. The method also achieves good micrometric properties and better encapsulation efficiency. The prepared mucoadhesive microspheres were spherical and free flowing. The entrapment efficiencies ranged from 54.51 to 91.60 % and mean size was in the range of 643.26 ± 5.93 to 892.34 ± 5.64 µm. The Nevirapine release depends upon the mucoadhesive polymer type and concentration in the polymer matrix. Thus the results demonstrate the potential use of Sodium alginate in combination with Pectin polymers for preparation of controlled delivery Nevirapine mucoadhesive microspheres and prolonged residence at the absorption site. Further in-vivo activities are required to confirm the claim of beneficial effect of Nevirapine in the form of Sodium alginate in combination with Pectin mucoadhesive microspheres.

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