Vol 5|Issue 3| 2015 |276-281.

International Journal
of
Pharmacy Review & Research
www.ijprr.com

e-ISSN: 2248 – 9207
Print ISSN: 2248 – 9193

FORMULATION AND EVALUATION OF NEVIRAPINE LOADED CHITOSAN AND EUDRAGIT NANOPARTICLES FOR ANTIRETROVIRAL THERAPY

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ABSTRACT

The goal of the present investigation was to formulate and evaluate chitosan and Eudragit® nanoparticles of nevirapine for antiretroviral therapy. Nanoparticles of nevirapine were prepared using chitosan, Eudragit® S 100, liquid paraffin and Tween-20 using Emulsion droplet coalescence method. The concentration of the polymers Chitosan and Eudragit® S 100 were selected based on the results on preliminary screening. The nanoparticles prepared were evaluated for morphology, loading efficiency, in-vitro release and in-vitro antiretroviral activities. The particle shape and morphology of the prepared nevirapine nanoparticles were determined by SEM analysis. The amount of nevirapine entrapment in the nanoparticles was calculated by the difference between the total amount of drug added to the nanoparticles and the amount of non entrapped drug remaining in the aqueous supernatant. A Franz diffusion cell was used to monitor nevirapine release from the nanoparticles. The formulations F2, F5, F6 and F8 showed good drug release from the polymer. The percentage cumulative drug release after 12 hours was 98, 99.06, 98.3 and 99.6% respectively. Formulations 6 out of 8 showed good drug release from the polymer, the percentage cumulative drug release after 12 hours were in the range of 97-99.6%. Among the six formulations F5 (2.5% Chitosan & 1 % Eudragit® S 100) showed maximum drug release in 12 hours diffusion study and good entrapment efficiency.

Key words: Emulsion droplet coalescence, nanoparticles, chitosan, Eudragit® S 100, Nevirapine.

INTRODUCTION

Novel drug delivery systems present an opportunity for formulation scientists to overcome the many challenges associate with antiretroviral (ARV) drug therapy. The currently available anti–HIV drugs classified into the nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors and recently fusion, integration inhibitors. Most of these drugs bear some significant drawbacks such as relatively short half-life, low bioavailability, poor permeability and undesirable side effects. So the efforts have been made to design drug delivery systems for antiretroviral therapy as reducing dosing frequency, increase bioavailability, decrease degradation/metabolism in GIT, improve CNS penetration and inhibit CNS efflux, delivery them to target cells and selectively minimal side effects. Antiviral drugs are a class of medication used specifically for treating viral infections. Like antibiotics for bacteria, specific anti-viral are used for specific viruses. Unlike most antibiotics, antiviral drugs do not destroy their target pathogen; instead they inhibit their development. In this trend nanoparticles show a fastest development from out of total novel drug delivery systems. Nanoparticles are stable, solid colloidal particles consisting of macromolecules materials and ranging in size from 10 to 1000nm. Drugs can be absorbed on the particle surface or can be entrapped or dissolved in the particle matrix. Nanoparticles are known to accumulate in the tissue because of phagocytosis by MO/Mac. Thus using nanotechnology, engineering researchers have developed a small but powerful device capable of enhancing the targeted delivery of drugs to treat life-threatening illnesses [1-5].

Nevirapine (NVP), a non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immuno-deficiency virus type 1 (HIV-1), block polymerase activity after binding directly to the HIV-1 reverse transcriptase leading to disruption of the enzyme's catalytic site. NVP is a weak base with low water solubility, and belongs to BCS

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class II drug. NVP is a weak base (pKa= 2.8) with low intrinsic water solubility (0.06 mg/ml) which gives rise to difficulties in the formulation of dosage forms and leads to variable dissolution rates with a resultant decrease in bioavailability. Hence in the present work, NVP nanoparticles were tried, in order to achieve sufficient solubility along the whole gastro-intestinal tract, which is a crucial step in the development of NVP formulations [6-10].

MATERIALS AND METHODS

Nevirapine was a gift sample from Mylan Pharmaceuticals, Nasik, India. Chitosan was procured from Jinendra scientific, Jalgaon (MH), India. Eudragit S 100 was obtained from SD Fine Chemical Maharashtra, India. Liquid paraffin, tween20, sodium chloride, Sodium hydroxide, Sodium dihydrogen phosphate and disodium hydrogen phosphate [11-15].

Preparation of Drug Loaded Nanoparticles

Method: emulsion-droplet coalescence method

Chitosan was dissolved in 1% acetic acid and 50 mg of nevirapine in phosphate buffered saline. This solution was added to 10 ml of liquid paraffin containing 5%v/v tween 20. This mixture was stirred using a homogeniser 3 minutes to form water in oil (w/o) emulsion. Similarly, another w/o emulsion consisting of Eudragit S 100 in 3M sodium hydroxide solution was prepared. Then these two emulsions were mixed and stirred using homogenizer. As a result of coalescence of the droplets, chitosan in the system was solidified to produce nanoparticles. Eudragit S 100 producing second coating over chitosan nanoparticles. The resultant Nevirapine nanoparticles were centrifugated at 3000 rpm was 60 mts (REMI, India) and washed using ethanol and water, consecutively to remove the remaining surfactant and liquid paraffin. Later they were dried in air for 3 hour followed by hot air oven at 50° for 4 hour and stored in a dessicator (Table 1) [16-20].

Evaluation of Nanoparticles

Fourier Transformation Infra-red (FTIR) analysis

The infra red spectrum of the drug nevirapine was recorded using Fourier Transform Infra-Red spectrophotometer. The infra red spectra was determined by crushing the sample with KBr to make pellets under hydraulic pressure of 10 tons, and the FTIR spectra was recorded between 500 to 4000 cm^{-1} using Fourier Transform Infra-Red spectrophotometer.(graph 5,6 and 7).

Determination of particle size and zeta potential

Particle size of the prepared nanoparticles was measured by Photon Correlation Spectroscopy (PCS) with Zetasizer 3000 (Malvern Instruments, Malvern, UK). The Refrigerator dried powder was suspended in Milli-Q water (1mg/ml) at 25 °C and sonicated for 25 sec in an ice bath (VC 505, Vibracell Sonics, USA) before measurement. The mean particle diameter and size distribution of the suspension were carried out for three times for each batch of sample under identical conditions and mean values were reported. The Zeta potential value was also measured using same suspension and same equipment [21-25].

Detection of shape and morphology

The particle shape and morphology of the prepared nevirapine nanoparticles were determined by SEM analysis. The nanoparticles were viewed using a Jeol-5610 L V (Tokyo, Japan) for morphological examination. Powder samples of dried nanoparticles were mounted onto aluminium stubs using double side adhesive tape and then sputter coated with a thin layer of gold at 10 Torr for vaccum before examination. The specimens were scanned with an electron beam of 1.2 kv acceleration potential and images were corrected in secondary electron mode (Fig 1) [26-30].

Determination of loading/entrapment efficiency

The amount of nevirapine entrapment in the nanoparticles was calculated by the difference between the total amount of drug added to the nanoparticles and the amount of non entrapped drug remaining in the aqueous supernatant. The latter was determined following the separation of drug loaded nanoparticles from the aqueous medium by centrifugation at 5000 rpm for 30 min. The supernatant was collected and the particles were washed with water and then subjected to another cycle of centrifugation.12 The amount of free nevirapine in the supernatant was determined by UV-Visible spectro photometer.

In-vitro release study

A Franz diffusion cell was used to monitor nevirapine release from the nanoparticles. The receptor phase was phosphate buffered saline (PBS, pH 7.4) thermostatically maintained at 37°C, with each release experiment run in triplicate. Dialysis membrance with molecular weight cut off 12,000 to 14000 Daltons was used to separate receptor and donor phases. The latter consisted of a 2ml suspension of nanoparticles containing 10 mg of nevirapine, mixed for 5 seconds to aid re-suspension, in a 1% w/v Tween-80 solution in PBS. Samples (1ml) from the receptor phase were taken at time intervals and an equivalent volume of PBS replaced into the receiver compartment. Diffusion of nevirapine into the receptor phase was evaluated spectrophotometrically [31, 32].

RESULTS AND DISCUSSION

In total nine formulations of nevirapine loaded nanoparticles were prepared and evaluated for various parameters such as particle size, morphology, drug entrapment efficiency, *in-vitro* release antiretroviral activity.

Particle size and Zeta potential (ζ) measurement

The particle size has direct impact on the stability, cellular uptake, drug release and biodistribution. The mean particle sizes of the prepared nanoparticles as measured by the Photon Correlation Spectroscopy (PCS) were in size range of 492 to 576 nm and the size

distributions were monodispersed (0.214 to 0.842) in all the formulations. There were no noticeable differences between the sizes of nanoparticles obtained with different drug polymer ratio, as similar findings was reported earlier for the nanoparticles of anti hypertensive drugs with chitosan . The formulation showed the smaller particle size than the formulation, but exhibited less entrapment efficiency and low zeta potential. The zeta potential of the formulated nanoparticles measured in water, exhibited positive values of +8.9 to +19.8 mV. The positive zeta potential value is due to the quaternary ammonium group present in the chitosan polymer and suggested that the drug was encapsulated with the polymer. The positive zeta potential values can facilitate an effective adhesion of the nanoparticles with the negatively charged mucus of the gastro-intestinal tract, prolonging the effective residence time of the formulations.

Preliminary screening for encapsulation efficiency

The nanoparticle drug delivery system is prone for the delivery of drugs to the targeted site. In early stages of formula optimization studies, the w/o emulsion formation was the problem. It was overcome by replacing surfactant; the surfactant selected was tween-20, which had high encapsulation efficiency.

Preparation of nanoparticles

Nanoparticles were prepared by emulsion droplet coalescence method. It is a laboratory method proved for the preparation of nanoparticles. The concentration of the polymers Chitosan and Eudragit® S 100 were selected based on the results on preliminary screening. The surfactant used for the preparation was tween 20. The time taken to complete preparation was around 2 hours.

Detection of shape and morphology

The particle shape and morphology of the prepared nevirapine nanoparticles were determined by SEM analysis.

DSC studies

As DSC is useful tool to monitor the effect of additives on the thermal behaviour of materials, these techniques were used to derive qualitative information about the physicochemical status of drug in particles. The peak for nevirapine pure sample was obtained in 152.04°C. The peak in physical mixture and nanoparticles were 154.68°C and 157.51°C respectively.

Entrapment efficiency and loading capacity

The data of drug entrapment efficiency and drug loading capacity for drug loaded nanoparticles were as shown in the table **3**. The formulation EF1 1 showed around 35% of drug loading.

In-vitro diffusion study

The drug release profile from the nanoparticles was as shown in the graphs (Graph **4** & **5**). The formulations F2, F5, F6 and F8 showed good drug release from the polymer. The percentage cumulative drug release after 12 hours was 98, 99.6, 98.3 and 99% respectively.

Table 1. Formulation of Nevirapine Nanoparticles

Formulation	Conc. of Chitosan (%)	Conc. of Edragit® S 100 (%)	Amount of drug (mg) (NEVIRAPINE)	Conc.of Tween 20 (%)
F 1	0.5	1	50	0.5
F 2	1	1	50	0.5
F 3	1.5	1	50	0.5
F 4	2	1	50	0.5
F 5	2.5	1	50	0.5
F 6	1	O.5	50	0.5
F 7	1	1.5	50	0.5
F 8	1	2	50	0.5
F 9	1	2.5	50	0.5

Table 2. Particle size and Zeta potential (ζ) measurement

Batch	Drug-Polymer ratio	Particle size (nm) ± SD (n = 3)	Polydispersity index	Zeta potential (mV) ± SD (n = 3)
F1	1:1	492 ± 1.80	0.483 ± 0.053	+14.1 ± 0.47
F2	1:2	517 ± 3.26	0.356 ± 0.078	+17.6 ± 0.56
F4	1:3	526 ± 1.41	0.214 ± 0.007	+19.8 ± 0.81
F5	1:4	576 ± 2.21	0.842 ± 0.088	+8.9 ± 0.79

Table 3. Evaluation parameters

parameters	F1	F2	F3	F4	F5	F6	F7	F8
pH	**7.1**	**7.5**	**7.7**	**7.6**	**7.8**	**7.0**	**7.2**	**7.4**
Practical yield	**130**	**175**	**130**	**265**	**295**	**115**	**225**	**265**
Efficiency of particle recovery (mg)	**86.67**	**87.50**	**86.66**	**88.33**	**84.28**	**76.66**	**90.00**	**88.35**
Unencapsulated drug(mg out of 50 mg)	**8.5**	**10.9**	**11.8**	**13.0**	**14.35**	**14.5**	**12.3**	**9.8**
Entrapment efficiency(%)	**65**	**72**	**74.35**	**84.7**	**93.12**	**88.9**	**80.62**	**76.45**
Loading capacity(%)	**31.92**	**22.34**	**29.38**	**13.96**	**12.08**	**35.73**	**30.16**	**14.86**

Table 4. Invitro dissolution study

Time(Hrs)	%release F1	%release F_2	%release F_3	%release F_4	%release F_5	%release F_6	%release F_7	%release F_8
1	44.6	22.9	24.5	21	**8.6**	15	27	32
4	56.83	35.83	32.66	36.66	**16.66**	38.33	53.5	52.66
8	69.5	54	43.83	70.83	**38.33**	46.66	63.83	70.83
12	81.6	66.66	48	74.16	**54.66**	73.16	74.66	71.16
16	88.6	78.3	51	80.66	**69.16**	83.83	79.83	86.33
20	91.9	88.4	57.5	89.16	**84.33**	92.33	84.16	89.33
24	96	98	79.1	98	**99.6**	98.33	97	99.6

Fig 1. Mean particle size distributions of nevirapine nanoparticles prepared with the drug polymer ratio (1:4)

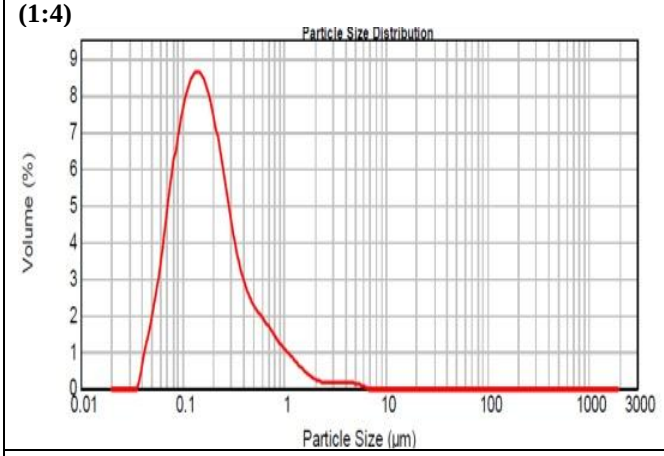


Fig 2. SEM of prepared nanoparticles

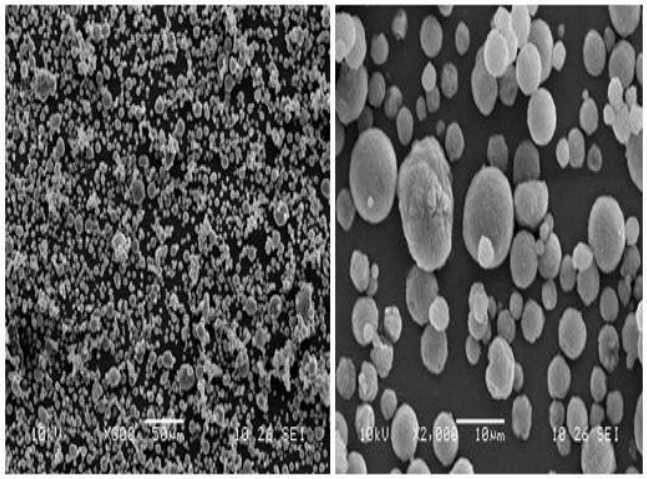


Fig 3. DSC graph of drug and polymer

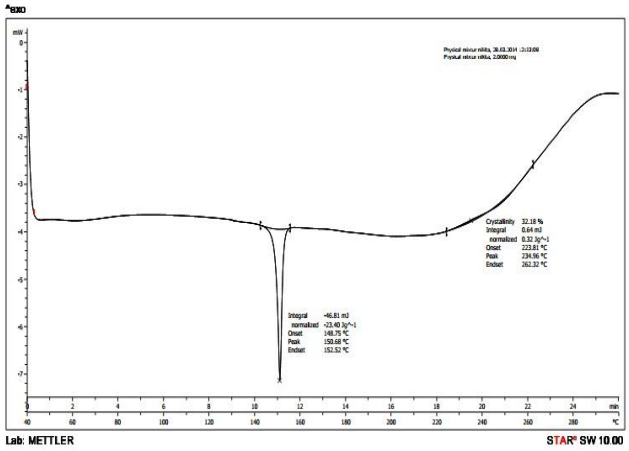


Fig 4. % drug entrapment of batch F1-F8

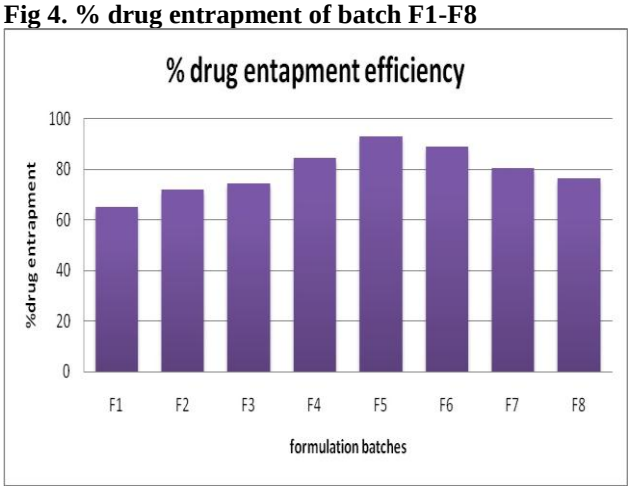


Fig 5. In vitro release profile of F1-F8

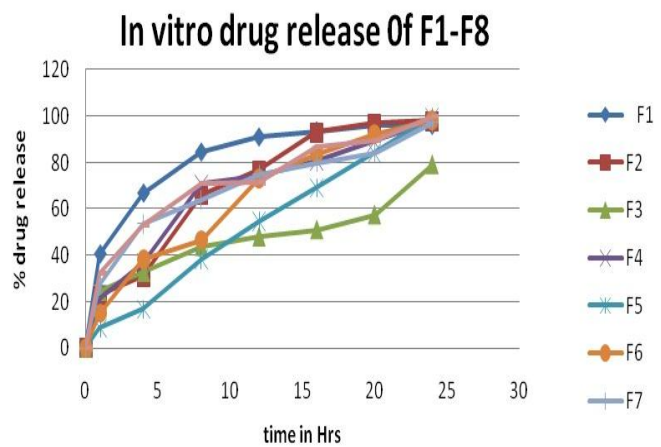


Fig 6. FTIR graph of nevirapine

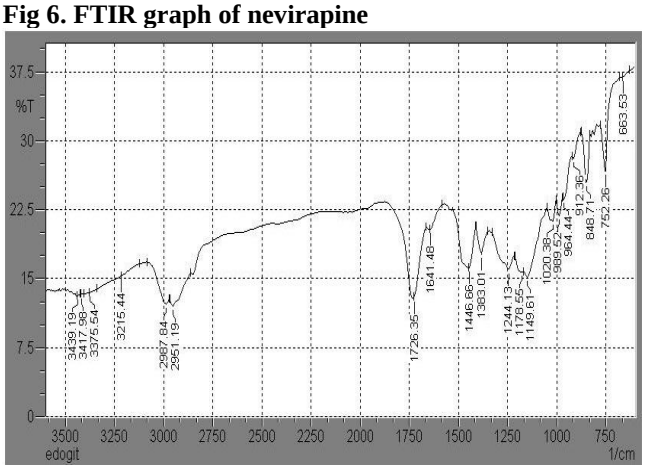


Fig 7. FTIR graph of Eudragit Rs100

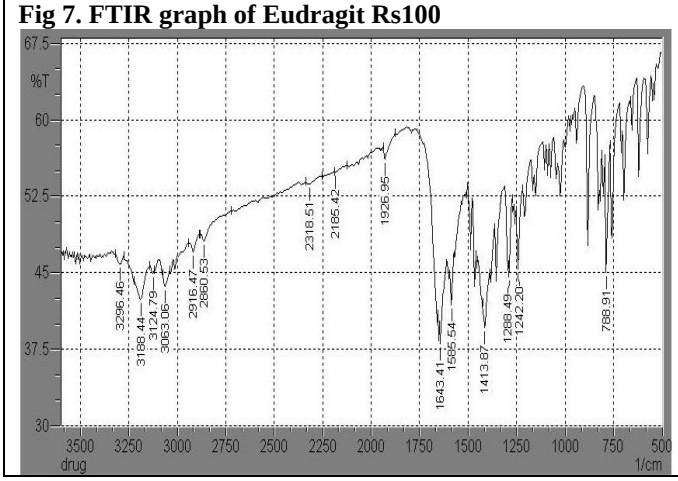
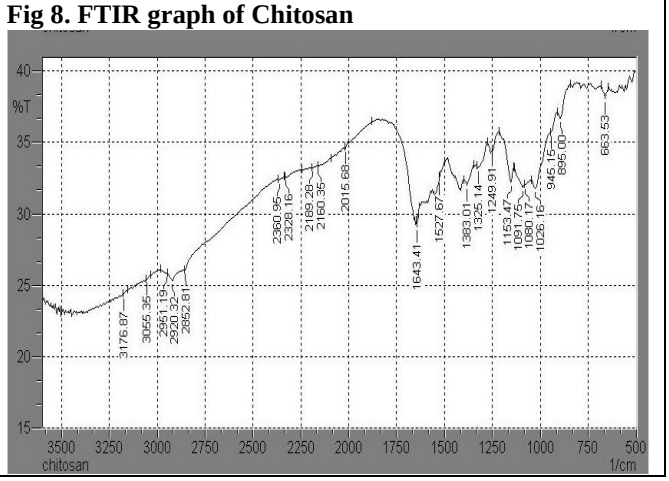


Fig 8. FTIR graph of Chitosan



CONCLUSION

On preliminary screening different formulations were developed with various ratios of polymers and different surfactants. It revealed that formulations with the polymer concentration (1.0-2.5%) and surfactant (tween-20) had better drug release and entrapment efficiency. So the formulations were designed with that polymer concentration and surfactant.

Eight formulations were evaluated and among them F2, F5, F6 and F8 were found to have good results. Among the four formulations F5 (2.5% Chitosan & 1% Eudragit® S 100) showed maximum drug release in 12 hours diffusion study and good entrapment efficiency. The work on formulation development of nevirapine nanoparticle was very much advantageous than the existing dosage forms in antiretroviral, hence better action.

ACKNOWLEDGMENT

The authors are highly obliged to Mylan Lab, Nasik. India for providing gift sample of Nevirapine. The authors are thankful to Dr. Surajj M. Sarode, Hod, smt. S.S.Patil college of pharmacy, chopda, for providing all necessary facilities.

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