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Preparation and evaluation of Nevirapine nanoparticle

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Abstract
The Nevirapine loaded nanoparticles were prepared by ionic gelation method and solvent evaporation method. Nanoparticles of different core: coat ratio were formulated and evaluated for process yield, loading efficiency, particle size, zeta potential, *in vitro* drug release, kinetic studies and stability studies there was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulations. The percentage drug entrapment efficiency is maximum for FC4 which was found to be 91.73% when compared to the formulation FE3 shows 85.46%. But, when comparing the two different methods, the formulation with Chitosan polymer shows better entrapment efficiency than with formulation with Eudragit L100 polymer. This is because Eudragit L100 contains higher amount of quaternary ammonium groups, which facilitates the diffusion of a part of entrapped drug to the surrounding medium during the preparation of nanoparticles. The *in vitro* release behavior from all the drug loaded batches were found to follow first order and provided sustained release over a period of 12 h. No appreciable difference was observed in the drug content of product during 90 d in which nanoparticles were stored at 5˚C and room temperature.

Keywords: nanoparticles; chitosan; Eudragit L 100; Nevirapine; ionic gelation technique: solvent evaporation technique

1. Introduction

Acquired Immunodeficiency Syndrome (AIDS) was first reported in 1981 in San Francisco and New York in USA [1]. The human immunodeficiency virus (HIV) is a retrovirus which infects the cells of immune system, destroying these cells and impairing the immune system's ability to fight with the invaders [2]. Acquired immunodeficiency syndrome (AIDS) is a serious disease afflicting several populations of the world. Several classes of the drugs are used in the treatment of AIDS. Of these, nonnucleoside reverse transcriptase inhibitors (NNRTIs) are a specific class of antiAIDS drugs [3]. Some of the NNRTIs use is limited due to low bioavailability resulting from dissolution rate-limited bioavailability. Their bioavailability can be improved by formulating as nanoparticle.

Nevirapine is available as oral tablets as well as oral paediatric suspension. These conventional formulations of nevirapine were approved during 1996-98 [4]. Later on, extended release formulations were also approved for clinical use. Clinical trials conducted during the beginning of this century proved that it is better than indinavir, nelfinavir and efavirenz. Because of its high potency, no food effect, low pill burden and low cost, combination of nevirapine with other anti AIDS drugs has been used as the first line therapy in developing countries. The innovator of this product is Boehringer Ingelheim Pharmaceuticals, USA and patents with conventional dosage form have already been expired and currently several generic products are available in the USA and other regulated markets. Challenges still remain in the area of research for developing products of nevirapine to further enhance its solubility and reduce fluctuations in bioavailability [5].

Chitosan possesses some ideal properties of a polymeric carrier for nanoparticles such as biocompatibility, biodegradability, non-toxicity, and low cost. It possesses a positive charge and exhibits an absorption enhancing effect. This characteristic can be employed to prepare cross-linked chitosan nanoparticles. Depending on the desired administration way, the size of the carriers should be optimized. Thus, if the carrier size is under 1 μm, an intravenous injection (the diameter of the smallest blood capillaries is 4 μm) is enabled and this carrier is also desirable for intramuscular and subcutaneous administration, minimizing any possible irritant reactions [6].

Eudragit l 100 is a white, free flowing powder with at least 95% of dry polymers. It is an anionic co- polymerization product of methyl methacrylate and methacrylic acid. It is soluble at pH.6. The ratio of free carboxyl groups to the ester is about 1:1 in Eudragit l 100. It is redily soluble in neutral to weakly alkaline conditions (pH6-7) and from salts with alkalis, thus affording film coats which are resistant to gastric media, but soluble in intestinal fluid. It is freely soluble in alcohol, acetone, and sodium hydroxide. It is unsolvable in dichloromethane, ethyl acetate, petroleum ether and water. Nevirapine and Eudragit l 100 were selected as core and coat material for the formulation of nanoparticles to achive controlled drug release [7].

Hence, the objective of the work was to formulate Eudragit l100 and chitosan nanoparticles containing Nevirapine by Solvent evaporation method and ionic gelation method, evaluate its physicochemical characteristics such as particle size, shape, zeta potential, drug loading capacity and *in vitro* release characteristics.

2. Materials and methods

Nevirapine used was a gift sample from Strides Arcolab Pvt. Ltd. Bangalore and chitosan from India sea foods, Cochin. Glacial acetic acid, tween 80, sodium tripolyphosphate

Dichloromethane and methanol were obtained from SD fine chemical ltd, Mumbai, India. All other chemicals used were of analytical grade.

2.1 Preparation of nanoparticles by ionic gelation method[8]

Chitosan nanoparticles were prepared by ionic cross linking of chitosan solution with TPP anions. Chitosan was dissolved in aqueous solution of acetic acid (0.25, v/v) at various concentrations such as 1.0, 2.0, 3.0, 4.0, 5.0 mg/ml. Under magnetic stirring at room temperature, 5 ml of 0.84% (w/v) TPP aqueous solution was added dropwise using syringe needle into 10 ml chitosan solution containing 10 mg of nevirapine. pH was adjusted to 6.0 by adding 0.1 M NaOH. The stirring was continued for about 30 min. The resultant nanoparticles suspensions were centrifuged at 12000 × *g* for 30 min using C24 centrifuge. The formation of the particles was a result of the interaction between the negative groups of the TPP and the positively charged amino groups of chitosan (ionic gelation)

2.2 Preparation of nanoparticles [9]

Nevirapine nanoparticle were prepared by solvent evaporation method. Drug and polymer were dissolved in 5 ml of methanol and 5 ml of dichloromethane and this solution was added to 5 ml of an aqueous PVA (2% w/v) solution. The resultant mixture was stirrered for 5 min to obtain an o/w emulsion was immediately added drop-wise to 125 ml of an aqueous PVA (2% w/v) solution. The contents were stirred at system allowing the formation of a turbid particulate suspension. The nanoparticles were separated by centrifugation 1000g for 30 min.

2.3 Characterization of prepared nanoparticles

2.3.1 Fourier transform infra-red spectroscopy (FT-IR) analysis

The IR spectra of the samples be recorded on an FTIR spectrophotometer (Perkin Elmer 1600 series) with KBr pellet (12 mm disc), compressed in a hydraulic press at 10 tons for 30 seconds.

2.3.2 Entrapment efficiency (EE%) [8]

The entrapment efficiency is also known as Association Efficiency. The nanoparticles of drug loaded were centrifuged at a high speed of 3500-4000 rpm for 30 min and the supernatant is assayed for non-bound drug concentration by UV spectrophotometer (Das *et al.*, 2005).

Efficiency (DEE) was calculated as follows:

$$DEE\ \% = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100$$

2.3.3 Scanning Electron Microscopy [10]

The shape and surface topography of nanoparticles were examined by means of Scanning Electron Microscopy (SEM) (JSM-T20. Tokyo, Japan). An appropriate sample of polymeric nanoparticles was mounted on metal stubs, using double- sided adhesive tapes. Samples were gold coated and observed for morphology, at acceleration voltage of 15KV.

2.3.4 Particle size distribution [11]

The size distributions along the volume mean diameters of the suspending particles were measured by dynamic scattering particle size analyser (Nanotrac Particle Analyzer 150, Microtrac Inc., PA, USA) (Alexis *et al*., 2008).

2.3.5 *In vitro* release studies [12]

The release of drug was determined by using the treated egg membrane mounted on the one end of open tube, containing drug equivalent to10 mg of formulation. The dialysis tube was suspended in 250 ml beaker, containing 200 ml PBS (pH 7.4). The solution was stirred at 200 rpm with the help of magnetic stirrer at 37±0.5 °C. Perfect sink conditions were maintained in the drug release testing. The samples were withdrawn at proper time interval at (1, 2, 3, 4, 6, 8, 12). The dissolution medium was replaced with same amount of fresh PBS (pH 7, 4) solution to maintain the volume 200 ml throughout the experiment. The drug content in the withdrawn samples (5 ml) were estimated at 282 nm and cumulative % of drug released was calculated and 2.3.6. Kinetic modeling [13].

In order to understand the kinetic and mechanism of drug release, the result of *in vitro* drug release study of nanoparticles were fitted with different kinetic equation like zero order (cumulative % release v/s time), first order (log % drug remaining v/s time), Higuchi's model (cumulative % drug release v/s square root of time), Peppas plot (log of cumulative % drug release v/s log time). R2 and 'n'values were calculated for the linear curve obtained by regression analysis of the above plots (Table No.2).

2.3.6 Stability study

The stability study was carried out using the batch FE-3 and FC-4. FormulationFE-3 and FC-4 was divided into 3 sets of samples and stored at 5±3℃ in refrigerator, room temperature and 45 ± 2℃, 75% RH in humidity control ovens. After 90 days drug content of all samples were determined through the method as in drug content (Figure No.7). *In vitro* release study of formulation FE-3 was also carried out after 90 days of storage (Table No.3 and Figure No.5).

3. Results and discussion

3.1 Entrapment efficiency of Nevirapine nanoparticles

The entrapment efficiency of Nevirapine nanoparticles formulation FC1 to FC5 containing Nevirapine: Chitosan prepared by ionic gelation method in various ratios of 1:1, 1:2, 1:3, 1:4, 1:5 ranged from 74.95%, 77.48%, 79.73%, 91.73%, 81.29 %. The highest entrapment efficiency was observed with FC4 (%) against the least % EE with FC1 (%). The results suggested that an increase in the Chitosan concentration resulting increased entrapment efficiency as shown in Table 5.9 & Fig 5.11 The entrapment efficiency of FE1 to FE5 which was prepared by solvent evaporation method in various ratios of 1:1, 1:2, 1:3, 1:4, 1:5 ranged

from73.84 %, 75.12%, 85.46%, 76.82%, 68.10% FE1-FE5. An increase in the concentration of Eudragit L100 in a fixed volume of solvent resulting increase entrapment as shown in Table 5.10 & Fig 5.12 this is probably because with increasing polymer content, more drug would be coated leading to higher entrapment efficiency.

But, when comparing the two different methods, the formulation with Chitosan polymer shows better entrapment efficiency than with formulation with Eudragit L100 polymer. This is because Eudragit L100 contains higher amount of quaternary ammonium groups, which facilitates the diffusion of a part of entrapped drug to the surrounding medium during the preparation of nanoparticles.

3.2 FTIR

The interaction study between the drug and polymer was evaluated using FT-IR spectrophotometer. There was no significant difference in the IR spectra of drug loaded nanoparticles

3.3 Surface morphology

Scanning Electron photomicrographs for the optimized formulation FC4 and FE3 was shown in Figure no 3 and 4. The SEM photomicrographs indicated that the nanoparticles were roughly spherical in shape and particles are uniform in size. The presence of aggregates might be attributed to a short redispersion time after centrifugation and drying at room temperature.

3.4 *In vitro* release studies

In vitro release study of Nevirapine from various formulations was conducted for 12 hrs by using dialysis membrane. Cumulative % drug release was plotted against time. All the formulation showed more than 20 % in the first 1 hr due to the presence of un-entrapped drug and the drug entrapped on the surface of polymer core which released faster showing dose dumping which is suitable to produce the initial effect of drug. It has been found that from the nanoparticles formulation, FC1-FC5 prepared by ionic gelation method shows FC1-86.3 %, FC2-82.5 %, FC3-78.2 %, FC4-66.8 % and FC5-70.6 was shown in Fig no 5. The increase in chitosan ratio from FC1 to FC5 causes decrease in the drug release and the release was more controlled by increasing the chitosan ratio. The nanoparticles of formulation FE1-FE5 prepared by solvent evaporation method shows FE1-81.9 %, FE2-78.2 %, FE3-68.5 %, FE4-71.7 % and FE5-73.2 %were shown in Fig no 6. The increase in Eudragit L100 ratio from FE1 to FE5 causes decrease in the drug release. But, when comparing the two different methods, the formulation with Chitosan polymer shows controlled release rate than with formulation with Eudragit L100 polymer, because

3.5 Stability studies

In vitro release profiles for the same formulation stored at different storage conditions were also showed in Table 3,4 and Fig 5.31, 5.32.

In vitro release studies revealed that the formulation stored at 5ºC ± 3ºC showed 78.46% & 66.82% for FC4 & FE3 respectively which was stored at room temperature showed 78.16% & 66.34% for FC4 & FE3 respectively and the formulation stored at 40°C ± 2°C showed 62.43% & 51.52% for FC4 & FE3 respectively. At higher temperature, there might be chances for drug degradation that decreased the drug release.

Table 1: Formulation and physicochemical characterization of Nevirapine nanoparticles by ionic gelation method

Sl no	Batch code	Drug: polymer ratio	% Drug entrapment efficiency
1	FC1	1:1	74.95
2	FC2	1:2	77.86
3	FC3	1:3	79.48
4	FC4	1:4	91.73
5	FC5	1:5	81.29

Table 2: Formulation and physicochemical characterization of Nevirapine nanoparticles by solvent evaporation method

S.No	Batch code	Drug: Polymer ratio	% Drug entrapment efficiency
1	FE1	1:1	73.84
2	FE2	1:2	75.12
3	FE3	1:3	85.46
4	FE4	1:4	76.82
5	FE5	1:5	68.10

Table 3: Stability studies – *in vitro* release study of a selected formulation FC-4 after three months storage at 5±3℃, Room temperature, 45℃±2℃/75%RH

S. No	Time in hrs		% cumulative Drug Release	
		5ºC±3ºC	**30ºC±2ºC/65% ±5% RH**	**40ºC±2ºC/75% ±5% RH**
1	0	0	0	0
2	1	19.23	19.16	15.33

3	2	27.37	27.31	24.02
4	3	30.91	29.85	27.18
5	4	38.04	37.96	34.17
6	6	47.37	47.29	43.51
7	8	57.89	57.38	52.44
8	12	67.96	66.40	56.25

Table 4: Stability studies – *in vitro* release study of a selected formulation FE-3 after three months storage at 5±3℃, Room temperature, 45℃±2℃/75%RH

Time in hrs	% cumulative Drug Release		
	5ºC±3ºC	30ºC±2ºC/65% ±5% RH	40ºC±2ºC/75% ±5% RH
0	0	0	0
1	23.36	23.25	18.97
2	30.60	29.34	26.89
3	38.79	37.73	35.06
4	42.66	42.58	38.79
6	50.34	49.87	46.08
8	58.26	56.15	54.12
12	66.52	66.06	58.92

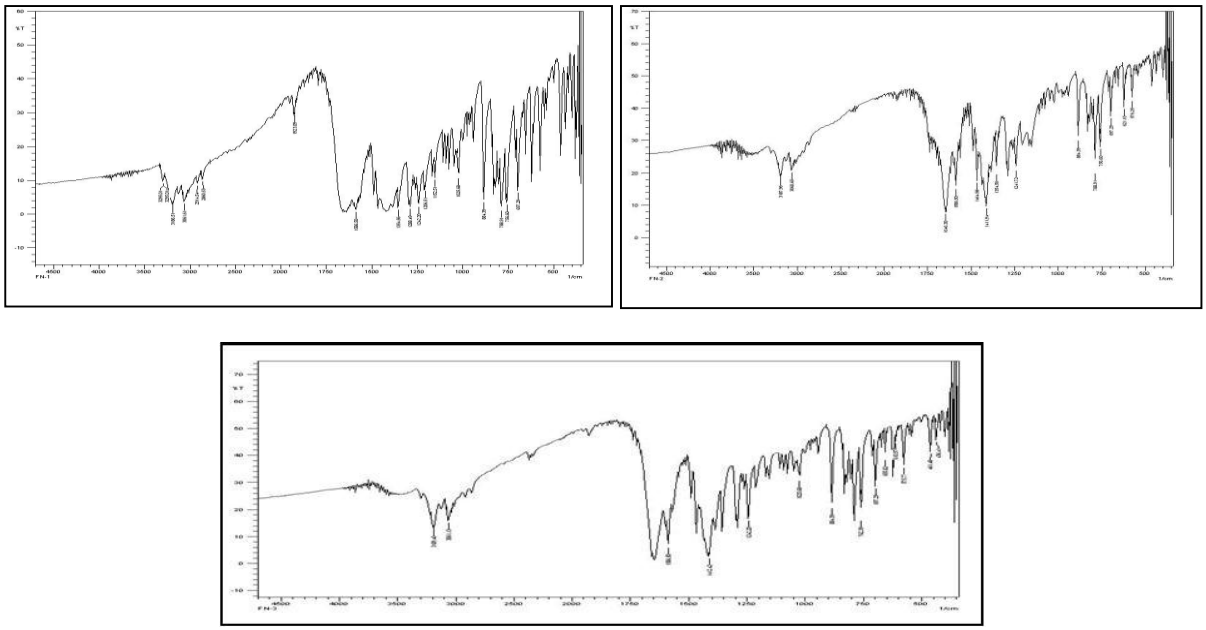

Fig 1: FT-IR spectra of (A) pure Nevirapine, (B) Nevirapine+ Chitosan
(C) Nevirapine+ Eudragit l 100

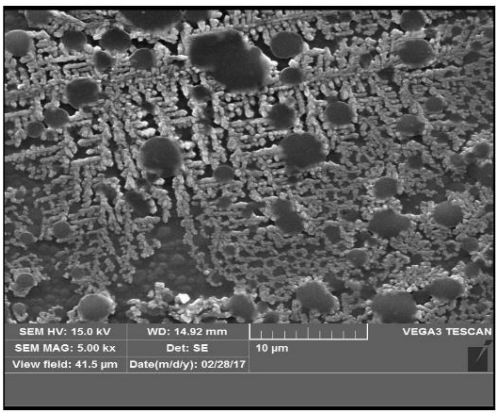

Fig 3: SEM of formulation FE-3

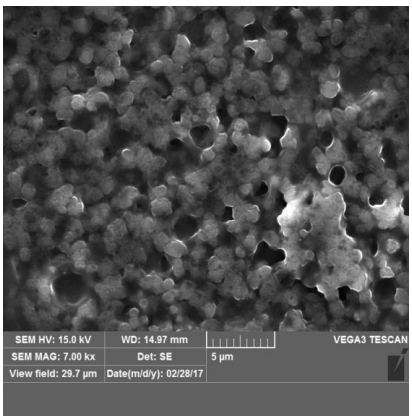

Fig 4: SEM of formulation FC-4

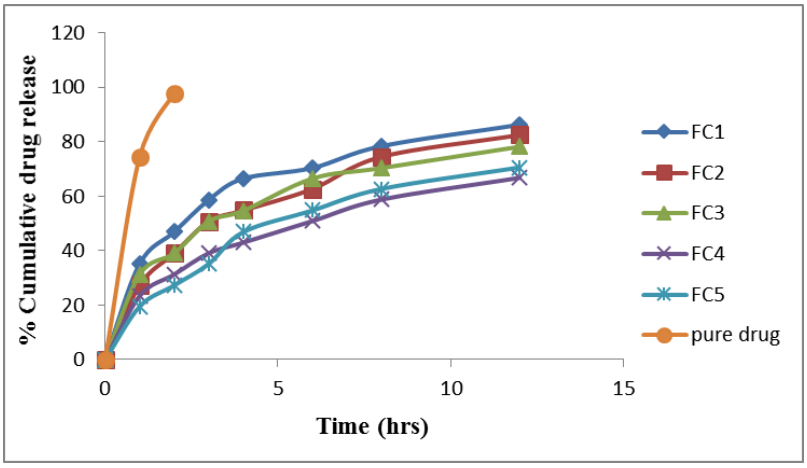

Fig 5: % cumulative drug release of nanoparticle by ionic gelation method

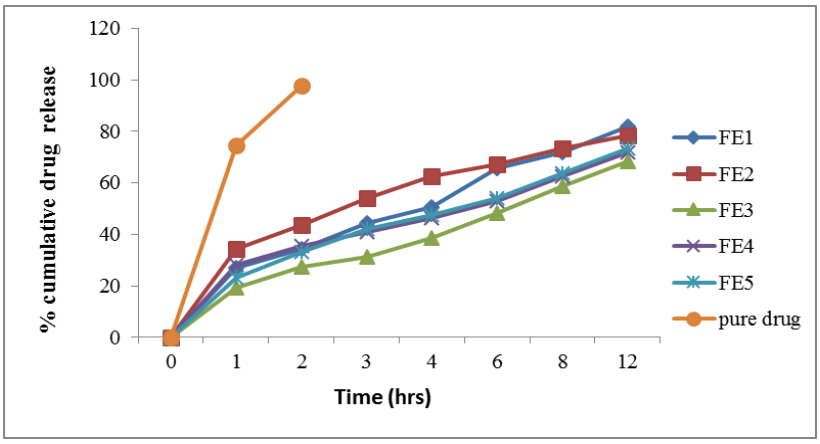

Fig 6: % cumulative drug release of nanoparticle by solvent evaporation method

Conclusion

Nevirapine nanoparticles were prepared by Ionic gelation technique were found to be suitable for controlled release than Solvent evaporation technique. The nanoparticles prepared by using Chitosan as a polymer show prolonged release rate when compared with other formulations than Eudragit l 100.

Referance

1. Sirisha Gubbala, Geethalakshmi A, Reshma Lamsa, Sambath L. co-solvent evaporation method for anti-hiv drug nevirapine to enhance solubility and dissolution rate. World journal of pharmacy and pharmaceutical sciences. 2014; 3(6):972-81.
2. Sellappan Velmurugan, Mohamed Ashraf Alia, Praveen kumar. Microparticulate drug carriers: a promising approach for the delivery of anti hiv drugs. Int J Pharm Pharm Sci. 2014; 6(2):31-39.
3. Ena J, Amador C, Benito C, Pasquau F. Pharmacological and clinical evidence of nevirapine immediate- and extended-release formulations. HIV AIDS (Auck). 2012; 4:169-79.
4. Kappelhoff BS, Huitema AD, Van Leth F, Robinson PA, Mac Gregor TR, Lange JM. Pharmacokinetics of nevirapine: Once-daily versus twice-daily dosing in the 2NN study. HIV Clin Trials. 2005; 6:254-61.
5. Anju Raju, Jagdeesh Reddy, Satheesh J, jithan V *et al*. preparation and characterisation of nevirapine oral nanosuspensions. Indian J Pharm Sci. 2014; 2:62-71.
6. Adlin Jino Nesalina J, Anton Smith *et al*. Preparation and evaluation of Chitosan nanoparticles containing zidovudine. Asian J Pharm Sci. 2012; 7(1):80-84.
7. Basu SK, Adhiyaman R. Preparation and characterization of Nitrendipine loaded Eudragit RL 100 microspheres.Trop J Pharm Res. 2008; 7(3):1033-41.
8. Adlin Jino Nesalin J, Gowthamarajan k, Somashekhara CN. Formulation and evaluation of nanoparticles Containing flutamide. Int J Chemtech Res. 2009; 1(4):1331-34.
9. Patil AS, Deshapande TD, Joshi PM, Gadad AP. Influence of organic solvent of nanoparticles loaded with Gatifloxacin. Universal journal oh pharmacy. 2013; 2(2):95-101.
10. Sanjukta Duarah, Kunal Pujari, Jyotirmoy Ghosh, Dhanalekshmi Unnikrishnan. Formulation and Evaluation of Metformin Engineered Polymeric Nanoparticles for Biomedical Purpose. Res J Pharm Bio Chem Sci. 2015; 6(3):1005-19.

11. Maysaa Chasib Al-Moahmmedawi *et al*. Nanoformulation and antibiotic releasing property of cefotaxime nanoparticles. Afr J Biotechnol. 2016; 15(14):539-48.
12. Nishanth Kumar, Parthiban S, Senthil Kumar GP, Tamizh Mani T. Ocular Drug Delivery of Levofloxacin Loaded Chitosan Nanoparticle by Emulsion Solvent Diffusion Method. Imperial Journal of Interdisciplinary Research. 2016; 2(5):1137-42.
13. Naveen HP, Adlin Jino Nesalin J, Tamizh Mani T. Preparation and characterization of microsphere encapsulating Ritonavir by solvent evaporation technique. Imperical journal of interdisciplinary research. 2016; 2(2):64-72.