FORMULATION AND EVALUATION OF ETRAVIRINE NANOSUSPENSION BY NANO PRECIPITATION METHOD

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Abstract

The aim of the present work is to develop oral Nanosuspension of Etravirine by Nano Precipitation method using various Stabilizers & Surfactant such as β-cyclodextrin, Soluplus, Poloxamer 407, Polyvinyl alcohol, Sodium lauryl sulfate, Polysorbate 80 Various formulation as well as process parameters were optimized in order to achieve desirable size and saturation solubility. Characterization of the prepared Nanosuspension was done with respect to Drug Content, Percentage yield, Entrapment efficiency, Viscosity, Sedimentation volume, Scanning electron microscopy, Particle's size, zeta potential, dissolution rate, in-vitro dissolution study. Zeta potential value for the optimized formulation (NS12) was found to -4.49 mv which was found to be within the acceptable limits. Average particle size of Nano suspension of optimized formulations (NS12) which is in ratio with Poloxamer 407 was found to be 500.4 nm. From the invitro studies we can say that formulation NS12 shows best drug release of 98.42±1.27% within 30 minutes whereas all the other formulations didn't release the drug. The drug release from the Nanosuspension was explained by the using mathematical model equations such as zero order, first order, and equation methods. Based on the regression values it was concluded that the optimized formulation NS12 follows first order kinetics with super case-II transport mechanism and the stability studies shows that the formulation was stability upto 3Months of time period.

Keywords: Etravirine, Poloxamer 407, Sodium lauryl sulfate, Nanoprecipitation technique, SEM, Zeta potential, PSD.

INTRODUCTION

As of 2020, 37.7 million individuals were HIV +, according to UNAIDS. People with acquired immunodeficiency syndrome (AIDS) have severely weakened immune systems, which increases their susceptibility to neoplasms and opportunistic infections [1,2]. HIV-1 is the principal cause of AIDS globally because it is the more infectious of the two types of the HIV virus.

This virus consists of two single-stranded RNA molecules. Two of the most important HIV enzymes are reverse transcriptase (RT), which transcribes RNA into DNA that may be integrated into the host's DNA, and protease, which cleaves protein precursors. These enzymes are often the focus of HIV therapy [3]. HIV medication now allows patients to maintain immunologic function by lowering viral replication, which lowers the risk of HIV transmission and death, with almost 90% less HIV being transmitted sexually. The usual treatment for HIV infection consists of two nucleoside-analog RT inhibitors (NRTIs) plus an oral protease inhibitor (PI), non-nucleoside RT inhibitor (NNRTIs), or integrase inhibitor [4].

The HIV virus primarily targets CD4+ T cells, which accumulate in lymphoid organs and serve as viral reservoirs. Memory CD4+ T cells become latently resting and may even become less in number when they become infected. Other cells that HIV dysregulates include CD8+ T cells, B cells, natural killer cells, and non lymphoid cells. The immunological response to HIV and other infections is compromised as a result, in addition to the loss and dysregulation of CD4+ T cells already mentioned [5].

1.1 Anti-Retroviral: For the past 25 years, treating an HIV-1 infection with antiretroviral therapy consisting of three active medications has been accepted as the standard of care. This therapeutic approach, which consists of a combination of two nucleos(t)ide reverse transcriptase inhibitors (NRTIs) as the main agent and a third agent—which could be an integrase strand transfer inhibitor (INSTI),[6] a boosted protease inhibitor (PI), or a non-nucleoside reverse transcriptase inhibitor (NNRTI)—has allowed for progressive immune system restoration, control of HIV-1 infection with efficacy rates exceeding 90% in recent clinical trials,[7] and, consequently, a marked decrease in acquired immunodeficiency syndrome (AIDS) events and other complications related to HIV-1 infection itself. A rise in the CD4+ cell count was considered a sign of immune system restoration enhanced CD4/CD8 ratio >0.9 and a T-cell count returning to normal levels [8].

Even while triple treatment has improved in terms of potency, effectiveness, convenience of dosing with one pill per day regimens, safety, and tolerability, there are still some tolerability and toxicity concerns with the NRTI backbones that are now in use.[9,10] Due to the ongoing toxicity of NRTIs combined with the growing potency of novel third-party medications (INTIs), research into and approval of dual therapy—a treatment approach combining two medications—has made it feasible to skip traditional NRTIs.[11] The use of these combinations in patients with and without experience has been approved.[12] In addition to being undetectable, they also provide improved tolerability and safety profiles, with a lower chance of any short- or long-term toxicities, particularly

in individuals with comorbidities. Furthermore, some patients may have an improvement in adherence, and the Expenses are decreased.[13]

1.2 Nanosuspension:

The effective formulation of medications depends on a number of factors, including solubility, stability at room temperature, compatibility with solvent, excipient, and photostability. Currently, approximately 40% of newly created chemical entities resulting from drug development initiatives are lipophilic or poorly soluble in water substances.[21,22] Drugs with limited solubility and low bioavailability can be solved using a variety of formulation techniques. Conventional methods such as micronization, fatty solution application, penetration enhancer or cosolvent application, surfactant dispersion method, salt creation, precipitation, etc., have limited effectiveness in improving the solubility of poorly soluble pharmaceuticals. Other strategies include vesicular systems like liposomes, solids dispersion, emulsion and microemulsion techniques, and inclusion complexes with cyclodextrins. These strategies demonstrate promise as drug delivery systems, but their main drawback is that they are not universally applicable to all medications.[23] Nanoparticle engineering has been researched and reported for use in pharmaceuticals throughout the past few decades.[24] The challenges posed by the previously discussed methods can be resolved via nanotechnology. The study of science and engineering at the nanoscale, or 10–9 m, is known as nanotechnology. Techniques like Bottom-Up Technology and Top-Down Technology are used to transfer the drug microparticles/micronized drug powder to drug nanoparticles.[25] Submicron colloidal dispersions of medication particles that are nanosized and stabilized by surfactants are called nanosuspensions.[26] The weakly water-soluble medication is suspended in a dispersion with no matrix material in nanosuspensions.[27] These can be applied to improve the solubility of medications that have low solubility in lipid and water environments. Increased solubility causes the active ingredient to flood at a quicker pace, reaching the maximum plasma level more quickly. This method works well for compounds that are difficult for formulators to work with because they have poor permeability, poor solubility, or both. Because of the smaller particle size, poorly soluble medications can be administered intravenously without obstructing blood vessels. The suspensions can also be formed into a solid matrix by lyophilization. It also has the benefits of liquid formulations over other formulations in addition to these advantages.[28] The benefits, drawbacks, and pharmaceutical use of these various preparation techniques as a drug delivery mechanism are the primary topics of this review.

1.2.1 Advantages of Nanosuspension [29]

Its simplicity and broad application to most medications.

- Suitable for medications that are not well soluble in water.able can be sent by any method.
- Decreased irritation of tissue whether administered subcutaneously or intramuscularly.
- The intravenous mode of delivery can facilitate rapid breakdown and tissue targeting.
- Taking Nanosuspensions orally results in a quicker start, a lower fed/fasted ratio, and enhanced bioavailability.

- Because of the smaller particle size, the medications' absorption from their absorption window can be boosted.
- Increased absorption and more reliable dosage when administered topically and inhaled
- It is possible to construct medications with high log P values as nanosuspensions in order to boost their bioavailability.
- Enhancement of biological performance as a result of the drug's high dissolution rate and saturation solubility; batch-to-batch variation is minimal and manufacturing is straight forward. Physical stability over the long period (since Ostwald ripening is absent).
- Adding nanosuspensions to tablets, pellets, hydrogel, and suppositories makes them ideal for a variety of delivery methods.
- Increasing the proportion of amorphous particles in the particles may result in a change in the crystalline structure and increased solubility.
- The ability to modify the surface of nanosuspensions to enable site-specific delivery.
- The potential for large-scale manufacturing, which is a need for releasing a delivery system onto the market.

1.2.2 Methods of Preparation of Nanosuspensions:

There are primarily two ways to prepare nanosuspensions. "Bottom Up technology" refers to the typical ways of precipitation (Hydrosols [34]). In Bottom Up Technology, the medication is dissolved in a solvent and introduced to a nonsolvent mixture to cause crystals to precipitate. The utilization of inexpensive, basic equipment is the precipitation technique's main benefit. The main difficulty with this method is that, in order to prevent the creation of microparticles, the growth of the drug crystals during the precipitation step must be regulated by adding surfactant. The medication must be soluble in at least one solvent and miscible with a nonsolvent in order for this precipitation procedure to work. Furthermore, medications cannot be treated using precipitation approach since they are weakly soluble in both aqueous and nonaqueous media [33]. The other is known as "Top Down Technologies," which are ways of disintegration and are favored above methods of precipitation. The "Top Down Technologies" consist of the following: precipitation and high-pressure homogenization combined (Nanoedege), high-pressure homogenization in non-aqueous medium (Nanopure), high-pressure homogenization in water (Dissocubes), and media milling (Nanocrystals) [30,31]. Emulsion as templates and microemulsion as templates are a few of other methods for creating nanosuspensions [32].

1.3 METHODOLOGY

1.3.1 Pre-formulation studies:

Prior to the development of nanosuspension dosage form, it is essential that certain fundamental physical and chemical properties of the drug molecule alone and when combined with excipients are determined. This first learning phase is known as pre-formulation. The overall objective of the pre-formulation is to generate

information useful to the formulator in developing stable and bioavailable dosage forms which can be mass produced.

The goals of pre-formulation studies are:

- To evaluate the drug substance analytically and determine its necessary characteristics, and
- To establish its compatibility with different excipients.

1.3.2 Buffer preparations

1.3.3 6.8 pH Phosphate buffer:

Dissolve 13.872 g of potassium dihydrogen phosphate and 35.084 g of disodium hydrogen phosphate in sufficient water to produce 1000 ml

1.3.4 0.1N HCL Buffer:

Take 8.33ml of concentrated HCL and transfer it to a 1000ml volumetric flask, then dilute to volume (1 L) with water.

1.3.5 7.4 pH Phosphate Buffer

Dissolve 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8.0 g of sodium chloride in 900ml of water and dilute upto 1000ml with water.

1.3.6 Solubility studies:[65]

Solubility of Etravirine was carried out in different solvents like- 0.1N HCL, 7.4 pH Phosphate buffer and 6.8 pH Phosphate Buffer and also in organic solvents like ethanol and methanol. Solubility studies were performed by taking excess amount of drug in different beakers containing the solvents. The mixtures were shaken for 24 hrs at regular intervals. The solutions were filtered by using whatmann's filter paper grade no.41. The filtered solutions were analyzed spectrophotometrically.

1.3.7 Identification of pure drug:

1.3.7.1 Description:

The Pure Drug was identified by using the Melting point and Spectroscopic studies like determination of λmax of drug and constructing the calibration curve to that pure drug.

1.3.7.2 Determination of Melting Point:[66,67]

Melting point of Etravirine was determined by capillary method. Fine powder of Etravirine was filled in glass capillary tube (previously sealed at one end). The capillary tube was tied to thermometer and the thermometer

was placed in the Thais tube and this tube was placed on fire. The powder at what temperature it melted was noticed.

1.3.7.3 Spectroscopic studies

1.3.7.4 Determination of absorption maximum (λmax):

The wavelength at which maximum absorption of radiation takes place is called as λmax. This λmax is characteristic or unique for every substance and useful in identifying the substance. For accurate analytical work, it is important to determine the absorption maxima of the substance under study. Most drugs absorb radiation in ultraviolet region, as they are aromatic or contain double bonds. Accurately weighed 10mg Etravirine separately was dissolved in 0.1N HCL Buffer in a clean 10ml volumetric flask. The volume was made up to 10ml with the same which will give stock solution-I with concentration 1000µg/ml. From the stock solution-I, 1ml was pipette out in 10ml volumetric flask. The volume was made up to 10ml 0.1N HCL Buffer to obtain stock solution-II with a concentration 100µg/ml. From stock solution-II, 2ml was pipette out in 10ml volumetric flask. The volume was made up to 10ml using pH buffer to get a concentration of 20µg/ml. This solution was then scanned at 200-400nm in UV-Visible double beam spectrophotometer to attain the absorption maximum (λ-max). The λ-max of Etravirine of 100% solution i.e 20ppm (µg/ml) by using Single Beam Spectrophotometer (YIS-294) was found to be at 280 nm by using 0.1N HCL Buffer.

1.3.7.5 Construction of calibration curve using 0.1N HCL Buffer:

Accurately weighed 10mg Etravirine was dissolved in 0.1N HCL Buffer taken in a clean 10ml volumetric flask. The volume was made up to 10ml with 0.1N HCL Buffer which gives a concentration of 1000µg/ml. From this standard solution, 1ml was pipette out in 10ml volumetric flask and volume was made up to 10ml using 0.1N HCL Buffer to obtain a concentration of 100µg/ml. From the above stock solution, aliquots of 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ml each was transferred to a separate 10ml volumetric flask and solution was made up to 10ml using 0.1N HCL Buffer to obtain a concentration of 4, 6, 8, 10, 12, & 14µg/ml respectively. The absorbance of each solution was measured at 280 nm.

1.3.7.6 Drug excipient compatibility study:

Excipients are integral components of almost all pharmaceutical dosage forms. The successful formulation of a stable and effective solid dosage form depends on the careful selection of the excipients, which are added to facilitate administration, promote the consistent release and bioavailability of the drug and protect it from degradation.

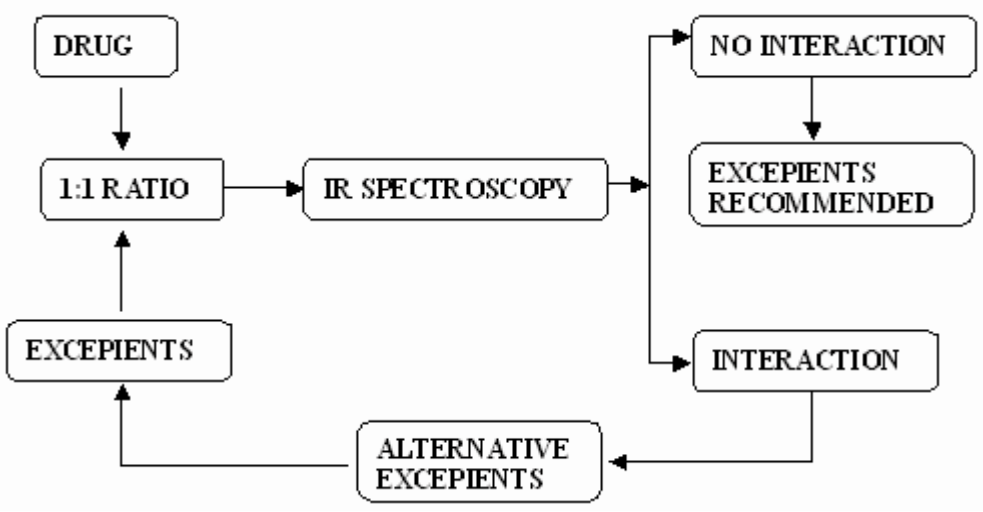


Figure No.13 Schematic representation of compatibility studies

1.3.7.7 Fourier-transform infrared (FTIR):

Infra Red spectroscopy is one of the most powerful analytical techniques to identify functional groups of a drug. The drug and excipient compatibility was observed using Fourier Transform – Infra Red spectroscopy (FT-IR). The FT-IR spectra obtained from Bruker FT-IR Germany (Alpha T) was utilized in determining any possible interaction between the pure drug and the excipients in the solid state. The potassium bromide pellets were prepared on KBr press by grounding the solid powder sample with 100 times the quantity of KBr in a mortar. The finely grounded powder was then introduced into a stainless steel die and was compressed between polished steel anvils at a pressure of about 8t/in2. The spectra were recorded over the wave number of 4000 to 400cm-1.

1.3.7.8 Differential scanning calorimetry (DSC)

DSC was performed utilizing DSC Q20 Universal V4.5A TA Instruments. Samples were allowed to equilibrate for 1 min and then heated in an atmosphere of nitrogen over a temperature range from 0 to 300°C. Thermograms were obtained using TA Instruments universal analysis software 2000.

1.3.7.9 X-Ray diffraction (XRD)

The samples were recorded on XRD (PW 1729, Philips, Amsterdam,Netherlands). XRD patterns were recorded using monochromatic Cu Kα radiation with Ni filter at a voltage of 40 kV and a current of 30 mA between 10° and 80° 2θ values. The data were processed with the software Diffrac Plus V1.01.

1.4 Preparation of Nanosuspensions:[68-71]

Table No.1 Formulation table of Etravirine loaded Nano suspensions using by Nano Precipitation method

Ingredients	N S1	N S2	N S3	N S4	N S5	N S6	N S7	N S8	N S9	N S10	N S11	N S12	N S13	N S14	N S15	N S16
Etravirine	100	100	100	100	100	100	100	100	100	100	100	**100**	100	100	100	100
β-cyclodextrin	50	100	150	200	-	-	-	-	-	-	-	**-**	-	-	-	-
Soluplus	-	-	-	-	150	225	300	450	-	-	-	**-**	-	-	-	-
Poloxamer 407	-	-	-	-	-	-	-	-	150	225	300	**450**	-	-	-	-
Polyvinyl alcohol	-	-	-	-	-	-	-	-	-	-	-	-	150	225	300	450
SLS(mg)	5	5	5	5	5	5	5	5	5	5	5	**5**	5	5	5	5
Polysorbate 80(ml)	3	3	3	3	3	3	3	3	3	3	3	**3**	3	3	3	3
Methanol	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Water	30	30	30	30	30	30	30	30	30	30	30	**30**	30	30	30	30

1.4.1 Method of Preparation of Nanosuspensions by Nano Precipitation method:

Nanosuspension of Etravirine was prepared by Nano precipitation method with various carriers and drug. At first the weighed amount of Etravirine was taken and dispersed into the beaker containing Ethanol which acts as organic solvent. This drug and ethanol solution is termed as organic phase. Now the carriers β-cyclodextrin, Polyvinyl alcohol (PVA) and Poloxamer 407 was dissolved in water and add surfactant (SLS) to the surfactant solution. we can label as aqueous phase. This solution was kept on magnetic stirrer for uniform mixing. Addition of organic solvents by means of a syringe positioned with the needle directly into stabilizer/surfactant containing water (aqueous phase). After 1 hour, the solution was kept in sonicator for about 60 mins. Then formed Nanosuspensions were collected by filtration through whatman filter paper and dried.

1.5 Evaluation parameters of Nanosuspensions:

The Nanosuspensions was evaluated for various parameters: -

- Drug Content
- Percentage yield
- Entrapment efficiency
- Viscosity
- Sedimentation volume

- Scanning electron microscopy
- Particle's size and shape
- Zeta potential
- In-vitro drug release studies
- Drug release kinetics studies
- Stability studies

1.5.1 Drug content

An accurately measured nanosuspension equivalent to 10mg of drug was taken in 100ml volumetric flask and diluted to 100ml with ethanol. (To prepare the stock solution of 100µg/ml). The amount of drug determined spectrophotometrically at 280 nm by using Single Beam Spectrophotometer (YIS-294).

1.5.2Percentage yield

Percentage practical yield of Etravirine Nanosuspensions is calculated to know about percentage yield, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of Etravirine Nanosuspensions recovered from each batch in relation to the sum of starting material.

The percentage yield of prepared nanosuspensions was determined by using the formula.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

1.5.3 Entrapment efficiency

The 100 mg of the Etravirine weight equivalent Nanosuspensions was analysed by dissolving the sample in 10ml of dichloromethane. After the drug was dissolved 10ml of clear layer of dissolved drug is taken. There after the amount of drug in the water phase was detected by a UV-Spectrophotometric method at 280 nm. The concentration of the drug is determined with the help of calibration curve. The amount of drug inside the particles was calculated by subtracting the amount of drug in the aqueous phase from the total amount of the drug in the Nanosuspensions. The entrapment efficiency (%) of drug was calculated by the following equation.[72]

$$\%\ \textbf{of Drug entrapment} = \frac{\textbf{Mass of drug in Nanosuspensions}}{\textbf{Mass of drug used in formulation}} * \textbf{100}$$

1.5.4Viscosity:

The rheologic parameters of the prepared suspensions, in terms of Viscosity, were determined by use of the steady shear method, Measuring the "non-Newtonian viscosity". Rheology of all Nanosuspensions was performed with a RVT Brookfield viscometer from Choksi Lab. (Indore, M. P.) All measurements were performed after Eliminating all thixotropy from the suspension.

1.5.5 Sedimentation volume:

The suspensions were stored individually in a 50ml measuring cylinder for 8hours at room temperature. Observations were made at every hour upto 8hours. The sedimentation volume (F) was then calculated using the following equation:

$$\mathbf{F = \frac{Vu}{Vo} * 100}$$

Where, **Vu** is the ultimate volume of the sediment and **Vo** is the original volume of the suspension.

1.5.6 Scanning electron microscopy:

The morphological features of Etravirine nanosuspension are observed by scanning electron microscopy at different magnifications.

1.5.7 Particle Size analysis:

The particle size of the formulated nanosuspension batches was determined by using the Malvern Instrument Ltd Particle Size Analyzer and the particle size of the batches were recorded in Malvern software v2.0. The formulations were diluted with an appropriate volume of phosphate buffered saline solution (0.1N HCL Buffer). The measurements were carried out three times where the mean value was used.

1.5.8 Zeta potential:[73]

Zeta potential (ZP) is a physical property that controls electrostatic interactions in particle dispersions and is essential in understanding the stability of colloidal dispersions. It is identified as the difference in potential between the particle and its ionic atmosphere surrounding the medium and is measured in the plane of shear.

1.5.9 In-vitro drug release studies:

The invitro release of various nanosuspension formulations were performed by dialysis bag diffusion technique. Dialysis tubing will act as dialysis sac. (Sigma dialysis membrane MW 12000 Da). Length of dialysis tube is 4 - 5 cm., The sac was then emptied and 1 ml of the formulated liquid nanosuspension was accurately transferred into the sac, which served as the donor compartment. The sac was once again examined for leak and then suspended in the stoppered vessel containing 100 ml 0.1N HCL Buffer, which behave as the receptor compartment. [74,75,76] The Media temperature should be 37º± 0.5ºC at 500 rpm speed. At predetermined time intervals, 3 ml of the sample was withdrawn from the receptor compartment and analyzed for the quantity of drug released. Fresh buffer was used to replenish the receptor compartment at each time point. The samples were withdrawn at 5, 10, 15, 20, 25 and 30 min. The diffusion studies and sample analysis were carried out for all the developed formulations. Collected samples were suitably diluted with 6.8 Phosphate Buffered Saline and analyzed at 280 nm using 0.1N HCL Buffer as blank by using a UV spectrophotometer. The cumulative percentage drug release was calculated and graphs were plotted against time Vs % cumulative drug release.[77,78,79]

1.5.10 Invitro drug release kinetic studies

Kinetic model had described drug dissolution from nano suspension where the dissolved amount of drug is a function of test time. In order to study the exact mechanism of drug release from the nanosuspension, drug release data was analyzed according to zero order, first order, Higuchi square root, Korsmeyer- Pappas model. The criteria for selecting the most appropriate model were chosen on the basis of goodness of fit test.[80]

1.5.10.1 Zero-order model:

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation

$$Qt = Q0 + K0t$$

Where Qt is the amount of drug dissolved in time t, Q0 is the initial amount of drug in the solution (most times, Q0 = 0) and K0 is the zero order release constant expressed in units of concentration/time. To study the release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug released versus time.

Application: It is used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as tablets with low soluble drugs in coated forms, osmotic systems, etc.

1.5.10.2 First Order Model:

The first order equation describes the release from systems where the dissolution rate is dependent upon the concentration of the dissolving species.

Release behaviour generally follows the following first order equation:

$$\text{Log } C = \text{Log } C_o - kt/2.303$$

Where C is the amount of drug dissolved at time t,

C_o is the amount of drug dissolved at t=0 and

k is the first order rate constant.

A graph of log cumulative of % drug remaining vs time yields a straight line.

The pharmaceutical dosage forms following this dissolution profile, such as those containing water-soluble drugs in porous matrices, release the drugs in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminishes.

1.5.10.3 Higuchi model: The first example of a mathematical model aimed to describe drug release from a system was proposed by Higuchi in 1961. Initially conceived for planar systems, it was then sustained to different geometrics and porous systems. This model is based on the hypothesis that

- initial drug concentration in is much higher than drug solubility;

- drug diffusion takes place only in one dimension (edge effect must be negligible);
- drug particles are much smaller than system thickness;
- swelling and dissolution are negligible;
- drug diffusivity is constant; and
- Perfect sink conditions are always attained in the release environment.

In a general way the Higuchi model is simply expressed by following equation

$$Q = KH - t1/2$$

Where, KH is the Higuchi dissolution constant.

The data obtained were plotted as cumulative percentage drug release versus square root of time.

1.5.10.4 Korsmeyer-Peppas model: Korsmeyer et al.(1983) derived a simple relationship which described drug release from a polymeric system equation. To find out the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer-Peppas model,

$$Mt / M\infty = Ktn$$

where Mt / M∞ is a fraction of drug released at time t, k is the release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices.

In this model, the value of n characterizes the release mechanism of drug as described in the following table.

Table No.4 Drug transport mechanisms suggested based on 'n' value.

S. No	Release exponent	Drug transport mechanism	Rate as a function of time
1	0.5	Fickian diffusion	$t^{-0.5}$
2	0.45 < n = 0.89	Non-Fickian transport	t^{n-1}
3	0.89	Case II transport	Zero order release
4	Higher than 0.89	Super case II transport	t^{n-1}

1.5 Stability studies

The stability study of the optimized formulation of nanosuspenion were carried out under different environmental conditions. The film was packed in the aluminum foil and stored in a stability chamber for stability studies at 2-8°C (45% RH), 40^0C/75%RH, after a period of 1 Month and at 40^0C/75%RH after a period of 3 Months. The patches were characterized for the Percentage yield, Drug Content, Entrapment efficiency and In-vitro dissolution study parameters during the stability study period.

1.6 RESULTS AND DISCUSSIONS

1.6.1 Determination of melting point:

The melting point of found to be in range of 242.20 °C, which was determined by capillary method.

1.6.2 Saturation Solubility:

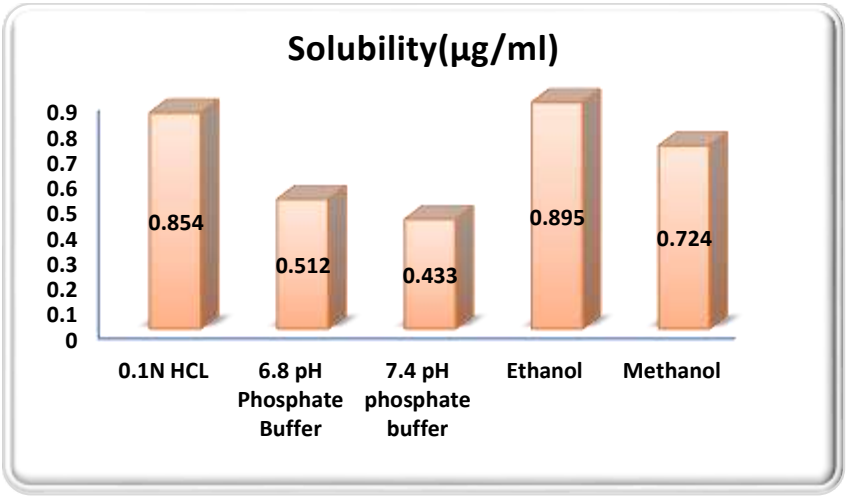


Figure No.1 Solubility studies of Etravirine

1.6.3 Calibration curve of pure Drug:

Table No.2 Standard graph of Etravirine in 0.1 N HCL (λmax 280 nm)

Concentration (µg/ml)	Absorbance
0	0
4	0.279
6	0.398
8	0.537
10	0.671
12	0.785
14	0.929

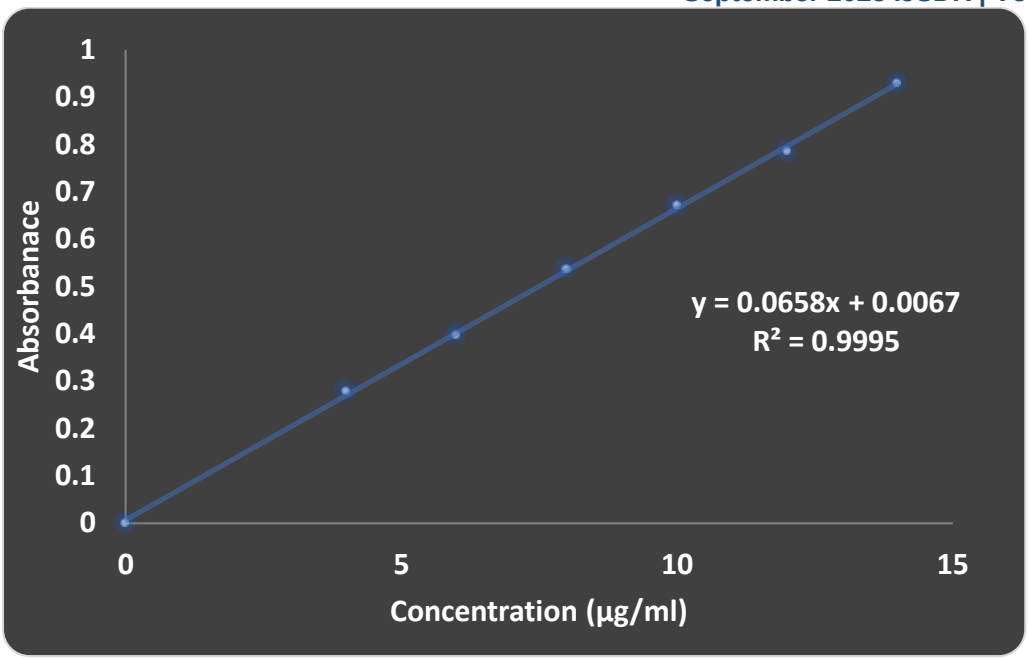
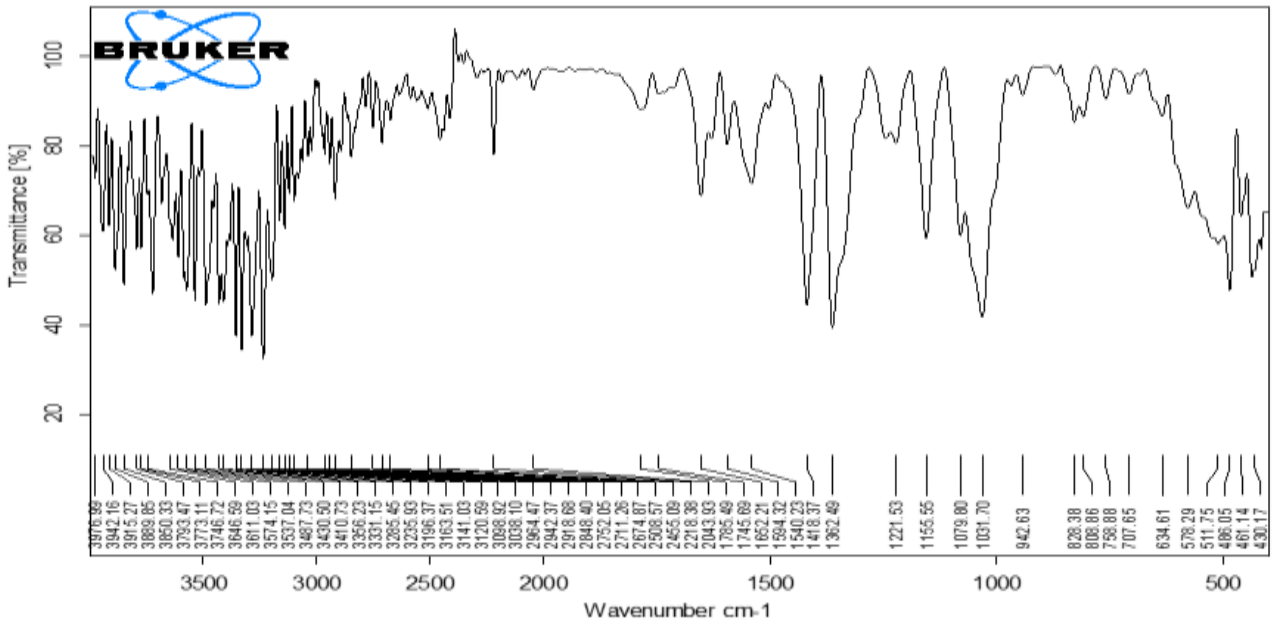


Figure No.2 Standard calibration curve of Etravirine in 0.1 N HCL

1.6.4 Drug excipient compatibility(FTIR)

Drug and excipient compatibility was confirmed by comparing spectra of FT-IR analysis of pure drug with that of various excipients used in the formulation.

1.6.5 Optimized Formulation IR Spectrum



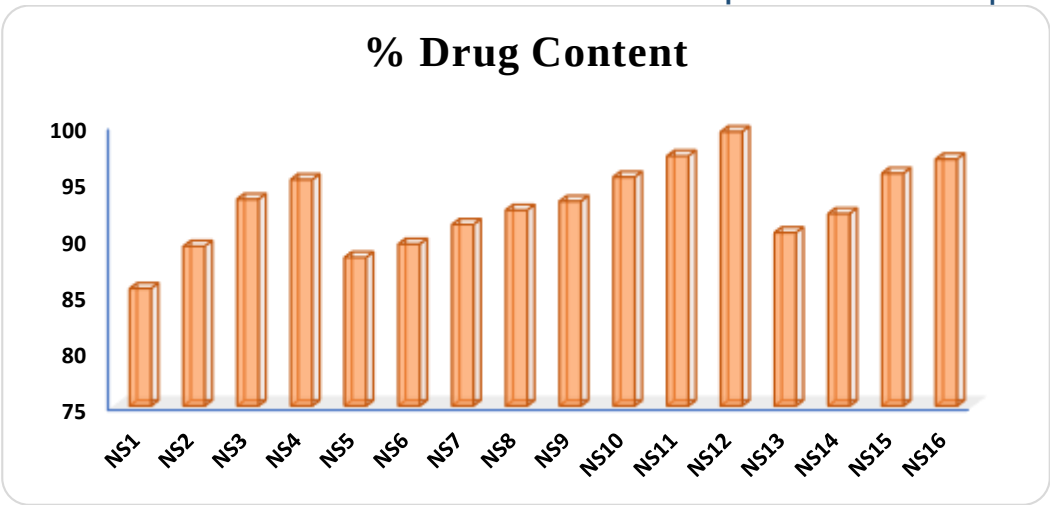


Figure No.3 % Drug content graphs for formulations

1.6.6 Entrapment efficiency:

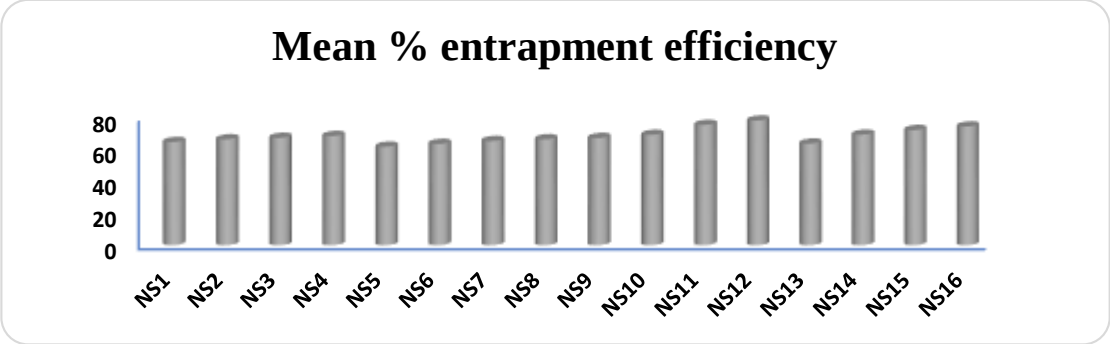


Figure No.4 % Entrapment efficiency graphs for formulations

1.6.7 Viscosity:

The viscosity results are mentioned in Table No. The Viscosity of the formulation and NS12 were found to cps 0.54 cps,which was lower compared than other batches. A lower viscosity means that the fluid flows more easily, while a higher viscosity suggests that the fluid is thicker and flows less easily. In the context of nanosuspensions, viscosity plays a crucial role in their behavior and performance.

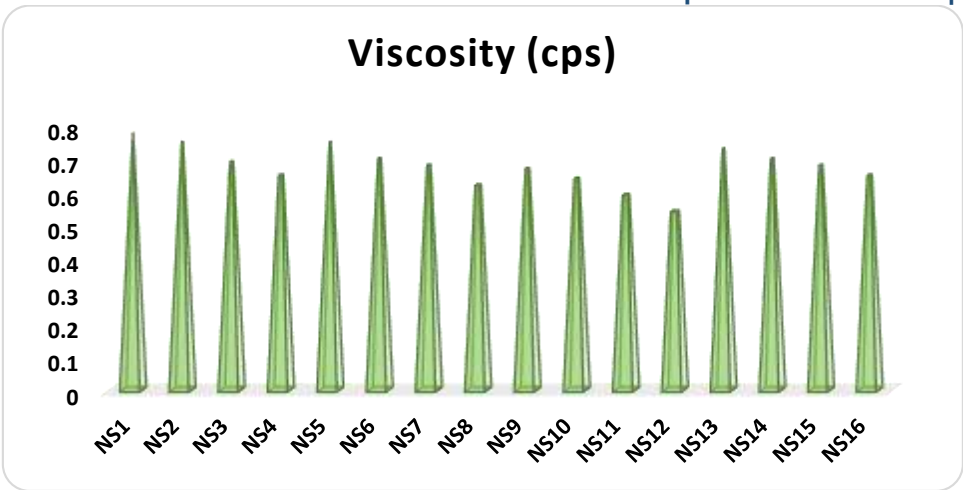


Figure No.5 Viscosity graphs for formulation

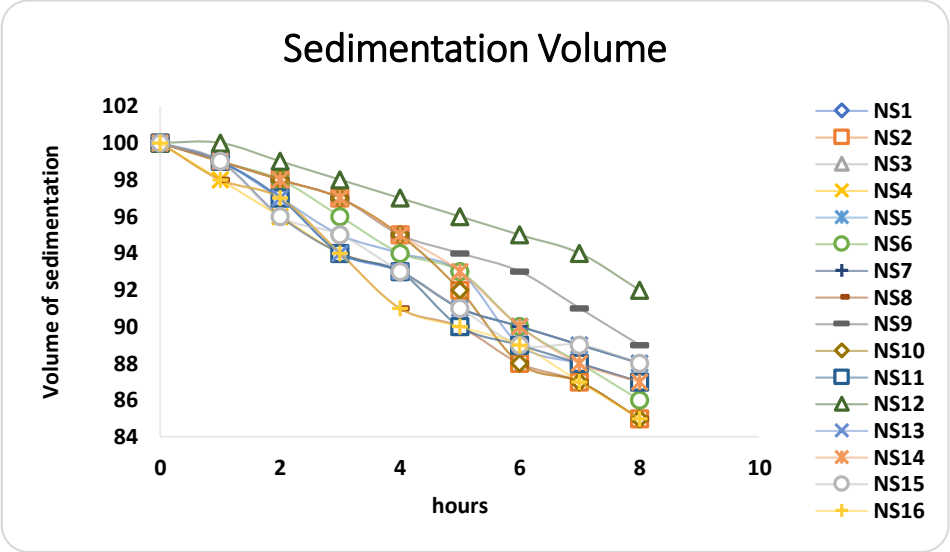


Figure No.6 Sedimentation volume of the Nanosuspensions (NS1-NS16)

1.7 Particle size analysis:

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	500.4	Peak 1:	545.2	100.0	493.2
PdI:	0.251	Peak 2:	0.000	0.0	0.000
Intercept:	0.920	Peak 3:	0.000	0.0	0.000
Result quality :	Good				

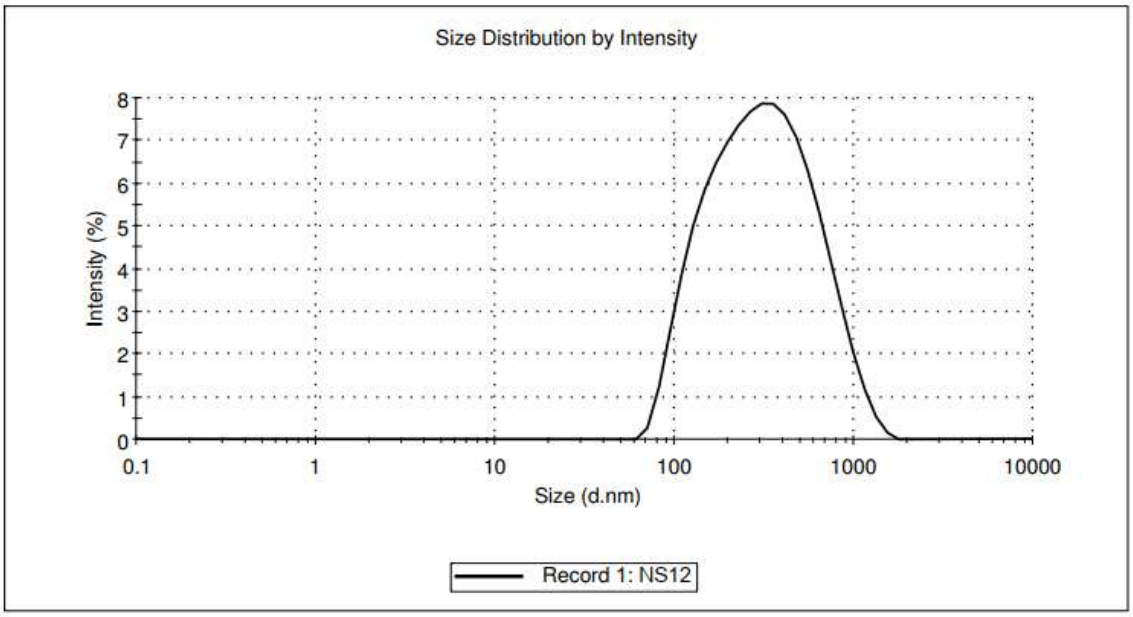


Figure No.7 Particle Size Analysis of Optimized Formulation

1.8 *In-vitro* drug release:

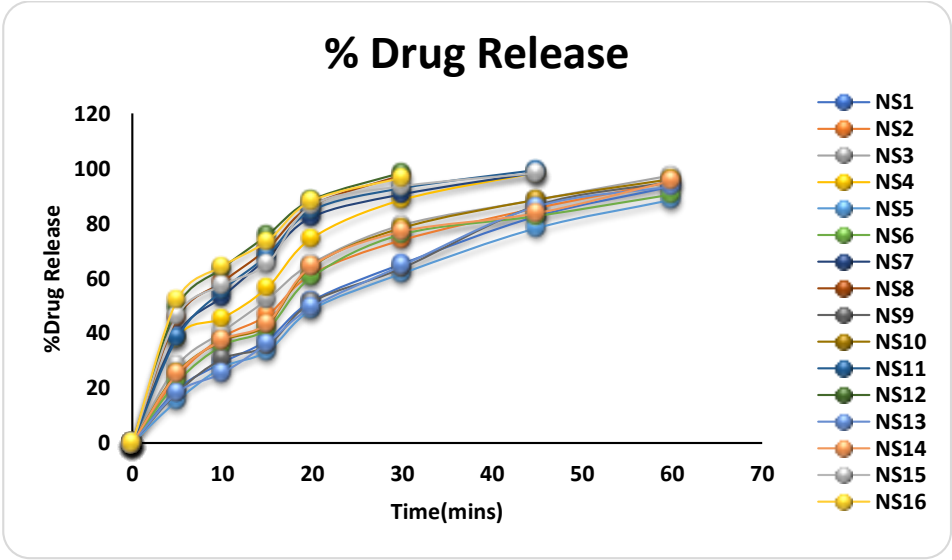


Figure No.8 Dissolution parameters for the formulations NS1-NS16

Discussion: From the above in vitro studies we can say that increase in the polymer concentration of polymers decrease in the dissolution time of all the formulations. Increase in the stabilizer concentration of Poloxamer 407 shows 98.42±1.27% of drug release, so the formulations prepared by using Poloxamer 407 releases more drug release at the end of 30 mins than the other Stabilizers. So NS12 was considered as optimized formulation as it shows drug release with in 30mins.

1.9 Drug release kinetics studies: Optimized formulation NS12

Zero Order Release Kinetics:

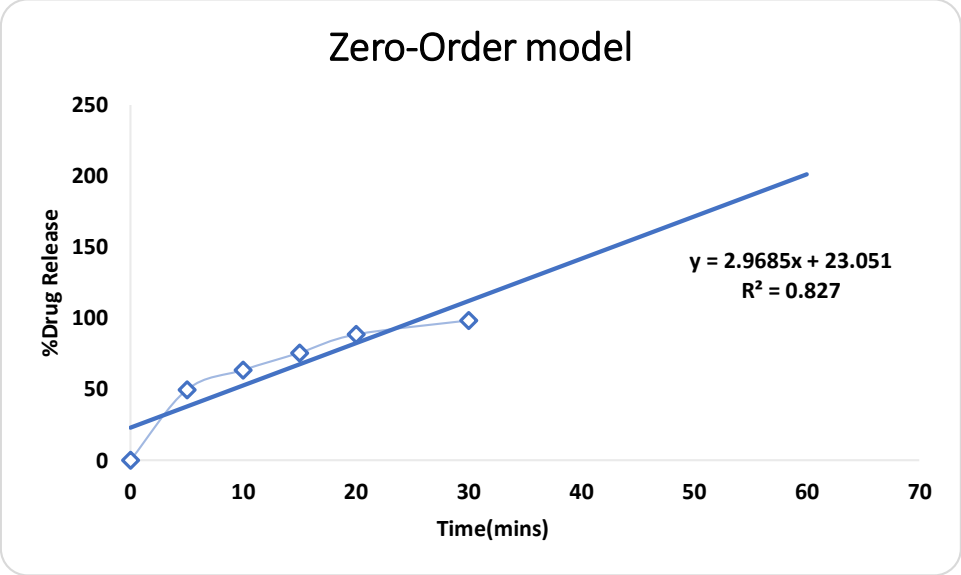


Figure No.9 Zero order release profile of formulation NS12

First Order Release Kinetics:

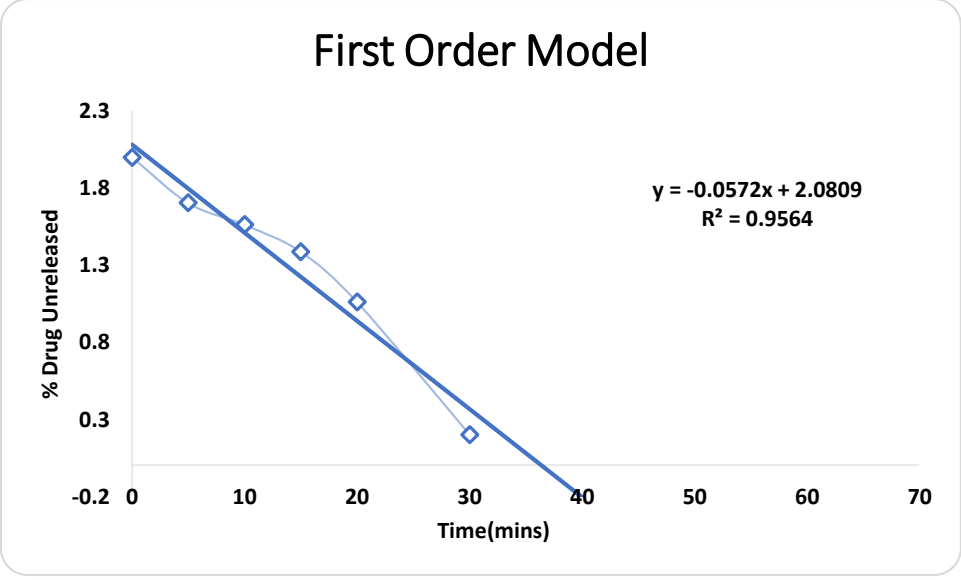


Figure No.10 First order release profile of formulation NS12

Higuchi model:

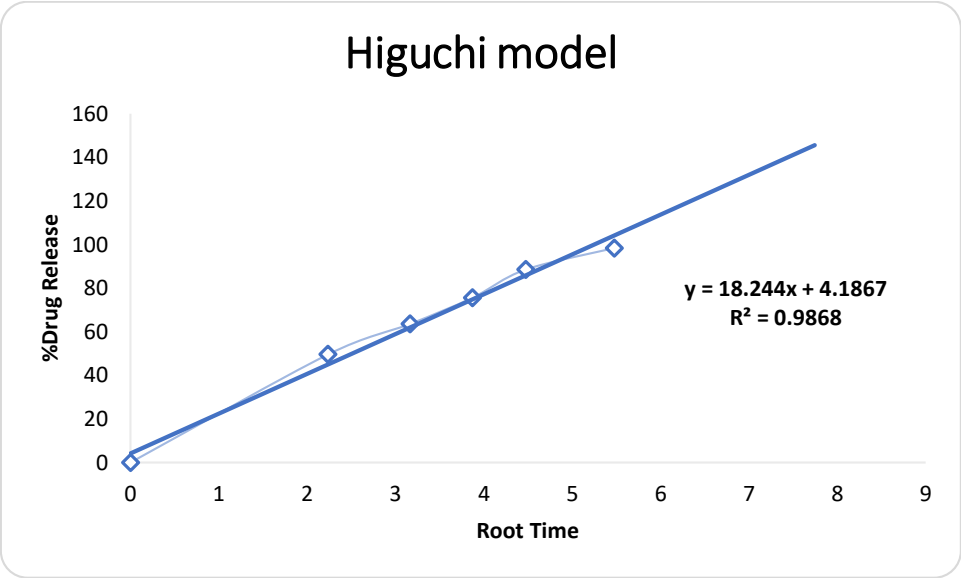


Figure No.11 Higuchi model of formulation NS12

Korsmeyer-Peppas model

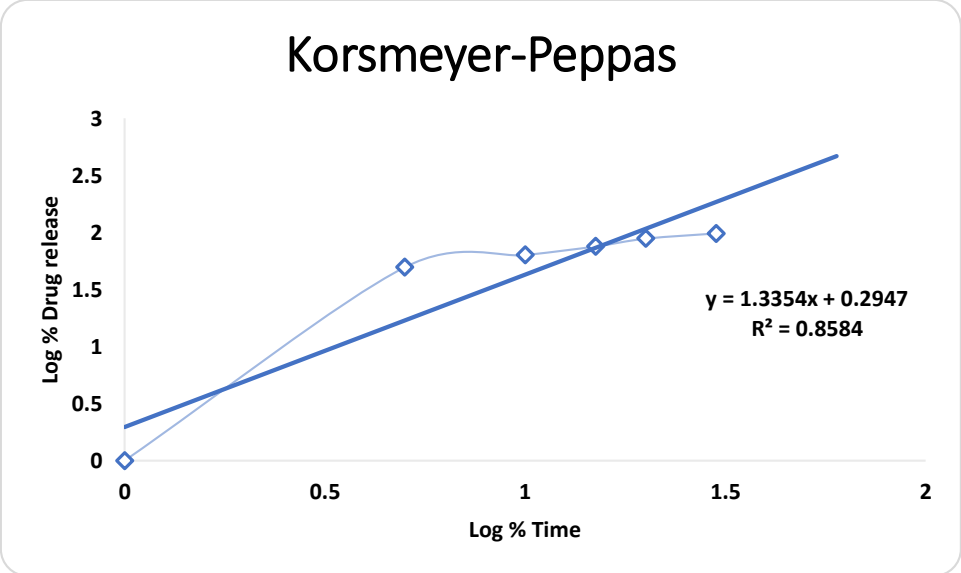


Figure No.12 Korsmeyer-Peppas model of formulation NS12

1.10 SUMMARY AND CONCLUSION

In present investigation Nanosuspensions of Etravirine was prepared by Nano Precipitation method.

The Nano suspensions are novel promising target and controlled released dosage form which is gaining importance because of ease of manufacturing and diversified applications. The present trend of pharmaceutical research lies in the usage of biodegradable polymer because of its availability and low toxicity.

Nanosuspension containing drug was prepared by Nano precipitation method by using combinations of β-cyclodextrin, Soluplus, Poloxamer 407, Polyvinyl alcohol, Sodium lauryl sulfate, Polysorbate 80 and quantity sufficient water and ethanol.

Estimation of Etravirine was carried out spectrophotometrically at 280.0nm. The Nanosuspension were evaluated for parameters such as drug excipient interactions i.e FTIR, DSC, XRD and Drug Content, Percentage yield, Entrapment efficiency, Viscosity, Sedimentation volume, Scanning electron microscopy, Particle's size and shape, Zeta potential, In-vitro drug release studies, Drug release kinetics studies finally stability studies. The stability data was also subjected to statistical analysis.

The melting point of Etravirine was Found to be in range of 242.20 ˚C which was determined by capillary method.

Saturation solubility was carried out at 25^0C using 0.1N HCL, 6.8 phosphate buffer, 7.4 pH buffer and ethanol, methanol.

From the drug excipient compatibility studies we observe that there are no interactions between the pure drug (Etravirine) and optimized Formulation (Etravirine + excipients) which indicates there are no physical changes.

The Evaluation parameters like Percentage yield, Drug content , entrapment Efficiency, viscosity and sedimentation volume for the Nanosuspension formulations are being conducted and in all parameters, the formulation NS12 yields the best results.

The morphological studies was conducted by using scanning electron microscopy and it was observed that in slightly spherical in shape and particle size was reduced upto to 1 µm. Zeta potential value for the optimized Formulation (NS12) was found to be be -4.49 mv that indicates good stability of the formulation indicates that it was in the acceptable limits. The Average particle size of nanosuspension of optimized Formulations (NS12) was found to be 500.4 nm with the PDI of 0.251 which was in acceptable limit.

From the invitro studies we can say that formulation NS12 shows best drug release of 98.42±1.27% within 30 minutes.

Then the optimized drug release of the Nanosuspension was explained by the using mathematical model equations such as zero order, first order, Higuchi model and Korsmeyer-Peppas model methods. Based on the regression values it was concluded that the optimized Formulation NS12 follows first order kinetics with super case-II transport mechanism.

The stability investigations were conducted on the optimized trails (NS12). The trials were sealed in an impermeable container and stored in a stability laboratory at 40 ± 2°C and 75 ± 5% RH for the first and third months. The samples were subsequently withdrawn at intervals of 30days and 90days was evaluated for percentage yield, drug content uniformity, entrapment efficiency, and in vitro dissolution. The stability experiments concluded that the optimized nanosuspension was stable for up to 3 months.

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