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Research Article

Development and Characterization of Emtricitabine Loaded Nanoparticles

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Abstract

The present research work is based on the formulation, optimization and characterization of the delivery system of nanoparticulate drugs. The main aim of the work is to formulate a targeted drug delivery system with higher drug entrapment efficiency. Drug used in the work is Emtricitabine, an antiretroviral drug, while the polymer used is Eudragit, a biodegradable polymer. The development of the analytical method is carried out using acetonitrile and phosphate buffered saline. Optimizing the formulation also involves optimizing its various process and formulation parameters. Different organic solvents were tested and various surfactants were used to optimize the formulation of the nanoparticles. Size range and zeta potential were measured using the Malvern zetameter. Lyophilization was carried out using two different cryoprotectants. The stability test carried out revealed that the formulation was good. The best formulation was considered for zeta potential determination. Formulation (F9) showed a maximum deviation of 27 mV which showed the particles to be separate and highly repellent. This repellent property has been found to be more useful in reducing opsonization and promotes target specificity. The prepared formulation was sterilized by a membrane filtration technique.

Keywords: Antiretroviral drug, Lyophilization, membrane filtration technique, repellent property

Introduction

Targeted drug delivery is sometimes referred to as smart drug delivery. It is a method of administering drugs to a patient in such a way as to increase the concentration of drugs in some parts of the body compared to others. The objective of the targeted drug delivery system is to prolong, localize, target and precede the drug interaction with diseased tissue. The advanced targeted delivery system is the reduction in the frequency of the dose taken by the patient, with a more uniform effect of the drug, a reduction in side effects and a reduction in fluctuations in the circulation of the drug level. The targeted drug delivery system has been developed to optimize regeneration techniques [1]. Several carriers were used as pilot molecules to selectively deliver the drug to the intended cells have been reported.

The colloidal carriers based on biodegradable and biocompatible systems have largely influenced the controlled and targeted drug delivery

concepts. Nanoparticles are sub-nanosized colloidal structures composed of synthetic or semi synthetic polymers. Nanotechnology is the study of nanoparticles, nanosuspention, nanoemulsion etc . Nanoparticle is a collective name for nanospheres and nanocapsule. Nanospheres have a matrix type structure. Drugs may be adsorbed at their surface, entrapped in the particle or dissolved in it.

These are colloidal particles ranging in the size from 10-1000 nm. They consist of macromolecular materials and can be used therapeutically, e.g. as adjuvant in vaccines, drug carriers, in which the active principle (Drug or biological active material) is dissolved, entrapped or encapsulated and the active principle is adsorbed or attached[5].

Polymeric nanocarriers are generally composed of polymers like gelatin, chitosan, poly (D, L-lactide- coglycolide), poly (a-caprolactone) etc. These are again classified into 3 types: polymeric micelles, nanoparticles & dendrimer type. Among polymeric nanocarriers, dendrimers and nanoparticles are important classes. Most of the nanoparticle based targeted delivery of antiretroviral drugs has been studied to target cells of the mononuclear phagocytic system (MPS), such as the monocytes/macrophages (Mo/Mac) that act as a reservoir for the HIV virus.

There are several advantages of polymeric nanoparticles like Reduction of toxicity and occurrence of adverse reactions, better drug utilization, controlled rate of drug release, specific site of drug release, greater patient convenience and better patient compliance, enhancement of the therapeutic effectiveness of the drug etc.

Human Immunodeficiency Virus type 1 (HIV-1) infection is the major cause of impaired immune system function that leads to progression of disease and death in patients with acquired immunodeficiency syndrome (AIDS). Antiretroviral therapy has been limited by several factors, such as its inherent toxicity, insufficient efficacy, and drug resistance. In 2007, it was estimated that the total number of people infected by HIV accounted for around 33 million, while another 25 million more have already died since the first reported cases in 1980. In 1980s first antiretroviral drugs were introduced and after the development of resistance when treated with single drug regimens, Highly Active Antiretroviral Therapy (HAART) was introduced in the late1990s, comprising the intense use of combination drug regimens. Several classes of ARVs including i) nucleoside reverse transcriptase inhibitors (NRTIs) ii) non-nucleoside reverse transcriptase inhibitors (NNRTIs) iii) protease inhibitors (PIs) iv) intergraded inhibitors, v) entry inhibitors. The primary rationale for using multiple agents is to disrupt HIV replication at multiple points in the lifecycle. Each of these combination regimens often comprises two nucleoside analogues and a PI to achieve synergistic effect. These therapeutic combinations are referred as the highly active ARV therapy (HAART) in general, less effective for the treatment of CNS complications than other AIDS-related illnesses. By using targeted nanoparticle drug delivery systems, anti-HIV drugs can accumulate in HIV-infected tissues or cells selectively and quantitatively, while their concentration in non-infected tissues or cells should be much lower. Therefore, side effects are reduced, lower doses are needed and drug administration regimens are simplified. Alongside, inadequate physical, chemical properties of most of these antiretroviral drugs (e.g. poor solubility, permeability, and stability) impair optimal absorption, bio-distribution, and sustained antiretroviral effect, thus contributing to poor clinical outcome. In order to solve these problems, several new and improved delivery systems and dosage form have been proposed in the literature.

The present study was aimed to development and characterization of Emtricitabine loaded nanoparticles drug delivery system using bio-degradable polymer such as Eudragit RS100 &

Eudragit RL 100. The polymer enhances the binding of Emtricitabine nanoparticles in specific (or) targeted site with sustained release of drug increasing therapeutic efficacy.

Materials

Emtricitabine was procured from Bafna pharmaceuticals, Mumbai. Eudragit RS 100, Eudragit RL 100 were obtained from Micro Labs Hosur. Poly Vinyl Alcohol (PVA), Potassium dihydrogen phosphate, Sodium chloride and Disodium hydrogen phosphate were obtained from S.D Fine Chemicals Ltd, Boisar. Methanol of Merck, India was used.

Methodology

Standard Curve for Emtricitabine[6, 7]

Preparation of pH 7.4 Phosphate Buffer Saline[8]

Disodium hydrogen phosphate 2.38 gm, potassium di-hydrogen phosphate 0.19 gm, sodium chloride 8 gm accurately weighed and it is transformed in to 1000 ml volumetric flask and volume is made up with distilled water. The pH was adjusted if necessary.

Determination of Absorbance maximum (λmax)

Emtricitabine was dissolved in phosphate buffer saline pH 7.4. Solution with 20 µg/ml concentration was prepared by suitable dilution. The solution was scanned in UV spectrophotometer at 200 to 400 nm using phosphate buffer saline pH 7.4 as blank. Absorbance maximum was determined as 270 nm. The standard curve is shown in **Figure 1** of results and discussion section.

Preparation of Stock solution

Stock solution was prepared by dissolving 100 mg of Emtricitabine drug in 100 ml of solvent medium, so as to get a solution of 1000 µg/ml concentration (Primary stock solution) From primary stock solution 2 ml was taken in 100 ml standard flask and it diluted to100 ml with solvent medium PBS pH 7.4 (secondary stock solution) to get the concentration of 2-20 mcg/ml.

Preparation of Standard solution

From the secondary stock solution aliquots ranging from 1to10 ml with PBS to get the final concentration ranges from 2 to 20 µg per ml. Absorbance of the solution was measured at 270 nm UV spectrophotometrically against drug free PBS pH 7.4 media as blank.

Fourier Transform Infrared Spectroscopy (Ftir)

In the present work, Emtricitabine pure drug, pure Eudragit RS 100 & Eudragit RL 100 was submitted to FTIR and spectra are obtained. They are compressed under 10 tonnes pressure in a hydraulic press to form a transparent pellet. The pellet was scanned from 4000 to 400 cm-1 in a spectrophotometer.

Method of Preparation of Emtricitabine Nanoparticles:

Emulsion Solvent Evaporation Method[9-11]

All batches of nanoparticles were prepared by Emulsion Solvent Evaporation method. The required quantity of polymer was dissolved in 4 ml methanol and 2 ml chloroform in (2:1) ratio as organic phase. The organic phase was then mixed with an aqueous phase containing drug and 0.2% polyvinyl alcohol (4 ml). The polymer concentration differs in various batches formulation as given in **Table 1**.

Table 1: Various Composition of Nanoparticles Formulation

S.NO	Formulation Code	Drug (Entricitabine) mg	Polymer Eudragit RS 100	Polymer Eudragit RL100	Drug:Polymer ratio
1.	F1	10	30	-	1:3
2.	F2	10	20	-	1:2
3.	F3	10	10	-	1:1
4.	F4	10	-	30	1:3
5.	F5	10	-	20	1:2
6.	F6	10	-	10	1:1
7.	F7	10	15	15	1:1
8.	F8	10	20	10	2:1
9.	F9	10	25	5	2.5:0.5
10.	F10	10	27.5	2.5	2.75:0.25

This mixture was homogenized by vortex mixture for 1min and then sonicated using a probe sonicator set at 55W of energy output for 1min to form an oil- in-water emulsion. The emulsion thus formed was further evaporated by flash rotatory evaporator for 20 min. The nanoparticle was collected by ultra- centrifugation (15,000 rpm). The prepared nanoparticles are washed with water. The washed liquid was eliminated by centrifugation and purified nanoparticle was collected.

Evaluation and Characterization of Nanoparticles[12]

Ph and Physical Appearance:

The pH of the formulation was measured using pH meter. It plays a vital role in process of stability and formulation activity. The physical appearance of the formulation such as colour and suspended foreign particulate matter were to be examined.

Entrapment Efficiency Study

The entrapment efficiency study was determined by free drug content in the supernatant which is obtained after centrifuging the solid lipid suspension at (15,000rpm for 20 min at zero degree using ultra centrifuge) The absorbance was measured at 270 nm by UV spectrophotometrically.

***Invitro* Drug Release Studies**[13-15]

The in vitro drug release study was carried out by using the diffusion membrane technique. The nanoparticles preparation was placed in a dialysis membrane and it is dropped in to a beaker containing 200 ml of diffusion medium (phosphate buffer saline pH 7.4) the medium was maintained at 37^0 C under magnetic stirring at constant speed. At fixed time interval 1ml of sample was taken from the diffusion medium for every 1 hour and it was replaced by 1ml fresh medium. This process was carried out for 24 hours. The sample was measured UV spectrophotometrically at 270 nm. The percentage of drug released at various time intervals was calculated from calibration graph.

Morphology of Nanoparticles by Simple Microscopy

The optimized formulation was morphologically characterized by microscopy. The small amount sample was placed in a glass slide and investigated in microscopy.

Scanning Electron Microscopy[16]

The optimized formulation was morphologically characterized by scanning electron microscopy (SEM). The sample for SEM analysis was mounted in the specimen using an adhesive small sample was mounted directly in scotsch double adhesive tape. The sample was analyzed in hitachi scanning electron microscope operated at 15 kv and photograph was taken.

Surface Charge (Zeta Potential) Determination[17]

Zeta potential is an important parameter to evaluate and establish an optimum condition for stability of colloidal or dispersed systems. The prepared nanoparticle suspensions were characterized with respect to zeta potential by using zeta potential analyser (Malvern Zeta seizer). Zeta potential is electrical charges on particles surface it create electrical barrier it is very important for drug stability. The effect of Eudragit RS100 & Eudragit RL100 (polymer) on the surface characteristics of the nanoparticle was studied.

Sterilization of Nanoparticles

The prepared nanoparticles were administered as injection, so it needs to sterilize. The sterilization process was carried out by membrane filter placed with 0.4micron membrane. The prepared nanoparticle suspension was allowed to flow through the membrane filter. This whole process can carry out in aseptic condition maintained by laminar air Flow Bench. The sterilized nanoparticle suspension was stored in suitable container.

Sterility Testing Of Nanoparticles[18]

Procedure:

The sterility test is a important for nanoparticle because it's a parental formulation. The sterility test for nanoparticles is done by various medium as per IP.

Preparation of Soyabean Casein Medium (Scdm);

25gms of dehydrated media was weighed and mix with small amount of freshly prepared hot water and made up to 1000 ml with hot water in a 1000 ml beaker. The medium was cooled to room temperature and pH adjusted to 7.3 ± 0.2. The medium was dispensed in suitable container and sterilized at 121oC for 15 min.

Preparation Of Fluid Thioglycollate Medium (Ftm):

26gm of dehydrated media was weighed and mix with small amount of freshly prepared hot water and made up to 1000 ml with hot water in a 1000 ml beaker. The medium was adjusted to pH7.1 ± 0.2. The sterilized media should not have more than upper one third of the medium in pink color.

Preparation of rinsing fluid (Fluid A):

1 gm of peptic digest of animal tissue was weighed and taken and mixed up with small amount of freshly prepared hot water and made up to one 1000 ml in a beaker. The solution was filtered and pH adjusted to pH 7.1 ± 0.2. The solution was then dispensed in suitable container and autoclaved at 1210 C 15 min for sterilization.

Procedure:

The vials containing Emtricitabine loaded nanoparticles were broken open under aseptic condition provided by laminar air flow works. All precautions and preventive measures were taken to avoid contamination by the process or by the analyst. The drug solution was then passed through sterile membrane lodged on a membrane holder assembly. After passing through the solution the membrane was rinsed with thrice with 100 ml of sterile peptone (Fluid A). The membrane was then cut into two halves using sterile scissors. One half of the filter paper was introduced into the container with SCDM and the

other half in to the container with FTM. This prepared medium is placed in different temperature. Soybean casein digest medium (SCDM) is incubated at 22.5 C^0 ± 2.5 C^0 and Fluid thioglycolate medium (FTM) is incubated at 32.5 C^0 ± 2.5 C^0. The containers were observed for turbidity or appearance growth of microorganism for 14 days. Positive control and negative control test were done to validate the sterility testing procedure.

Stability Studies of Nanoparticles [18, 19]

The Stability studies of nanoparticles involves observing the formulation at 45°C/70% RH which constitutes Accelerated condition and 4°C on refrigerator and room temperature. The formulations were kept in both the temperature for 3 months and sufficient amount of samples were taken at periodic intervals for performing the following tests.

1. Physical appearance
2. pH of the solution
3. In vitro drug release (Dissolution)
4. Percentage of drug entrapment

Results and Discussion:

Standard Curve for Emtricitabine

From the standard curve obtained in Figure 1 it was determined that slope was found to be 0.037 and $r^2$was 0.9995.

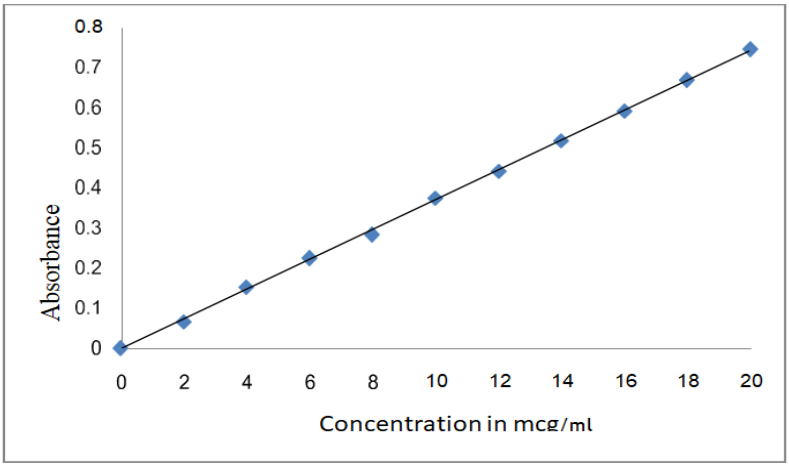

Figure 1: Standard curve of Emtricitabine at 270nm

Drug & Polymer Compatibility Studies by Ftir [20]

IR spectrums for pure drug alone and physical mixture of drug and polymers are taken. The spectrum of physical mixture was compared with spectrum of pure drug. Bands seen in pure drug also recognized in physical mixture. Hence there was no significant interaction between drug and excipients. IR Spectra data of drug, polymers and mixture is given in **Tables 2-5.**

Table 2: IR spectra data for drug Emtricitabine

Frequency cm^{-1}	Groups assigned
1637.71	C=O Stretching
3328.32	NH Stretching
1286.72	C-O Stretching
3214.01	O-H Stretching
2924.56	C-H Stretching

Table 3: IR spectra data for Eudragir RS 100

Frequency cm^{-1}	Groups assigned
2928	CH Stretching
1740	C=O Stretching
1466	CH Stretching
1021	C-O Stretching

Table 4: IR spectra data for Eudragir RL 100

Frequency cm^{-1}	Groups assigned
2932	CH Stretching
1740	C=O Stretching
1466	CH Stretching
1021	C-O Stretching

Table 5: IR spectra data for physical mixture

Frequency cm^{-1}	Groups assigned
1637.71	C=O Stretching
3328.32	NH Stretching
1286.72	C-O Stretching
3214.01	O-H Stretching
2924.56	C-H Stretching

Entrapment Efficiency of Nanoparticles[19, 20]

The Entrapment efficiency of all prepared nanoparticles were calculated as prescribed method and given in Table 6. This is due to repulsive force between drug and the polymer.

Table 6: Entrapment efficiency formulations with Drug and polymer

S.NO	Formulation Code	Drug (mg)	Eudragit RS 100	Eudragit RL 100	Entrapment Efficiency
1.	F1	10	30	-	61±0.12
2.	F2	10	20	-	68±0.09
3.	F3	10	10	-	72±0.17
4.	F4	10	-	30	49±0.141
5.	F5	10	-	20	56±0.11
6.	F6	10	-	10	60±0.08
7.	F7	10	15	15	73±0.12
8.	F8	10	20	10	83±0.17
9.	F9	10	25	5	94±0.05
10.	F10	10	27.5	2.5	85±0.08

In formulation (F2) polymer concentration was decreased (Emtricitabine 10 mg Eudragit 20 mg) the entrapment efficiency was increased to 68%. Further decrease in polymer concentration in formulation F3 (Emtricitabine 10 mg Eudragit 10 mg) entrapment efficiency was 72%. Formulation F4, F5, F6, carried out by same process as like first three formulation but changes in polymer

concentration 30 mg, 20 mg, 10 mg of (Eudragit RL 100) is taken. The entrapment efficiency was Formulation F7 was carried out by combination of both polymers initially with same concentration (Emtricitabine 10 mg Eudragit RS100 15 mg RL 100 15 mg) were taken. Entrapment efficiency was increased in great number F7 73% and in F8 83%. In order to study the polymer concentration, the formulation F9 was carried out in different concentration (Emtricitabine 10 mg with 25 mg of Eudragit RS 100 and 5 mg of Eudragit RL 100) it gives high percentage of entrapment efficiency 94%, which indicate the steady increased in entrapment efficiency. The drug entrapment efficiency is based on chemicals structure of drug and polymer used. Eudragit RS100 contains more number of non polar group but Eudragit RL 100 contain more number of polar group, at the same time Emtricitabine have 49 ± 0.03 for F4, 56 % for F5, 60% for F6. Based on above result.

more number of non polar. So Eudragit RS 100 has less repulsive force with Emtricitabine drug molecules. So the entrapment efficiency is high in Formulation F9.

***Invitro* Drug Release Profile of Nanoparticles** [18-20]

In *vitro* drug release studies were performed for all formulations. The results obtained in *in vitro* release studies were plotted in percent cumulative drug release Vs time and shown in **Figure 2**.

From the above formulations (F1 to F10) it confirms that the percentage of drug release was satisfactory in formulation F9 and it shows higher percentage of drug release of 92.89 %. So it was decided to be the optimum formulation and is chosen for further study.

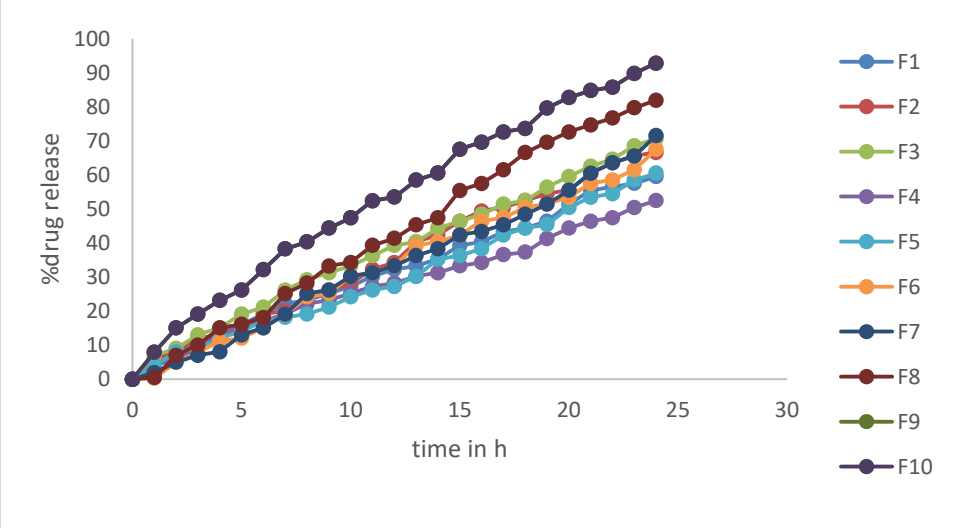

Figure 2: Summarized *in vitro* drug releases of nanoparticles

Morphology of Nanoparticles:

The characteristics of optimized nanoparticles formulation (F9) particle size were studied by simple microscopy. Small amount of sample was placed in glass slide and placed in simple microscope. Image of prepared nanoparticle formulation shows the encapsulation of polymer mixture on drug particles and is shown in **Figure 3**.

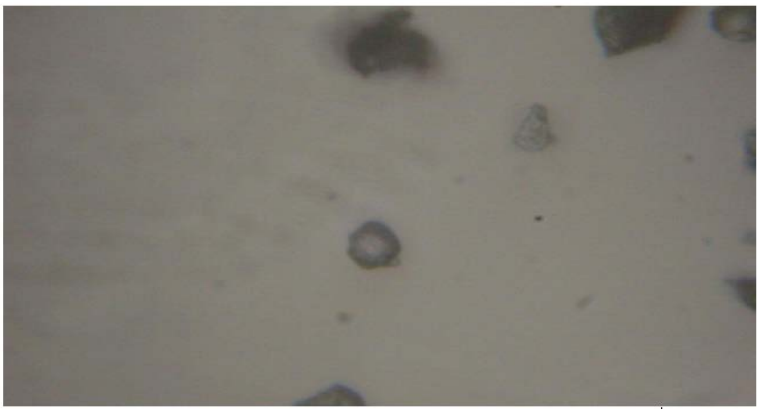

Figure 3: Microscopic image of F9 formulation

Scanning Electron Microcopy (Sem) [18-20]

The surface characteristics of optimized formulation (F9) particle size were studied by scanning electron microscopy. SEM image of prepared nanoparticle formulation shows the coating of polymer mixture on drug particles. The size distribution of nanoparticles in SEM is 400 nm, which indicates a thin and uniform coating over the drug and is shown in **Figure 4**.

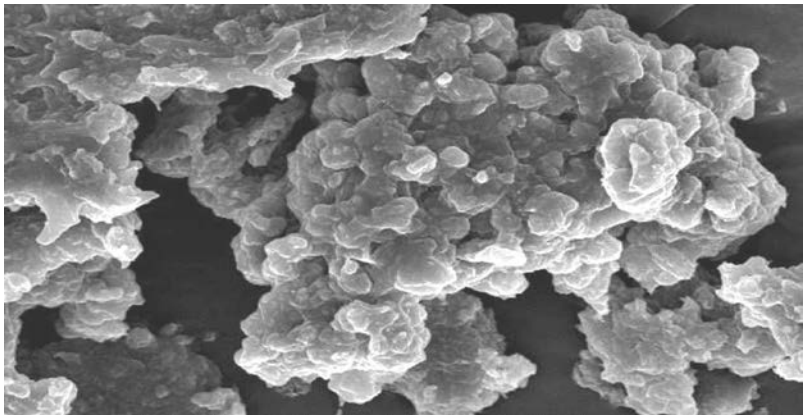

Figure 4: SEM image of F9 Formulation

Surface Charge (Zeta Potential) [20]

The zeta potential of a nanoparticle is commonly used to characterize the surface charges property of nanoparticles. It reflects the electrical potential of particles is influenced by the composition of the particles and the medium in which it is dispersed. When nanoparticle formulations are administered through intravenous route they are easily identified and detected by the phagocytes. The particle size and the hydrophobicity surface of the nanoparticle determine the adsorption of blood components (proteins) called as opsonins. This opsonin in turn decides the fate of the nanoparticles. Binding of these opsonins on to the surface is known as Opsonization. Non modified nanoparticles were rapidly opsonized and gets easily eliminated from the body. Hence, to increase the likelihood of the success in drug targeting by nanoparticles, it is necessary to minimize the opsonization and to prolong the circulation of nanoparticles in vivo.

The zeta potential of the nanoparticle formulation with Eudragit (RS 100 &RL100) (formulation F9) particles which present in the formulation are de- aggregated and remain same and more stable in the suspension and zeta potential (mV) is 59.0 and Zeta Deviation (mV) is 5.29 and conductivity (mS/cm) is 0.0866. So this polymer is more

suitable for nanoparticle preparation and the result shows smooth surface character and efficient repelled action and it decreases the opsonization.

Sterility Test [21]

Negative control:

Negative control confirms the sterility of the sterilized media. It was the uninnoculated sterile media and observed for 14 days. Negative control was maintained for both (FTM) and (SCDM) Medias and incubated in the appropriate temperature.

Positive control:

Positive control confirms the suitability of the media for the growth of microorganism. The positive control for SCDM and FTM was inoculated with Bacillus subtilis suspension with less than 100 CFUs and incubated recommended temperature respectively for 14 days. The growth of microorganism witnessed by the turbidity of the medium confirms the presence of nourishments favoring Microorganism.

Figure 5: Observation of Sterility test done in Soya bean Casein Digest medium (SCDM)

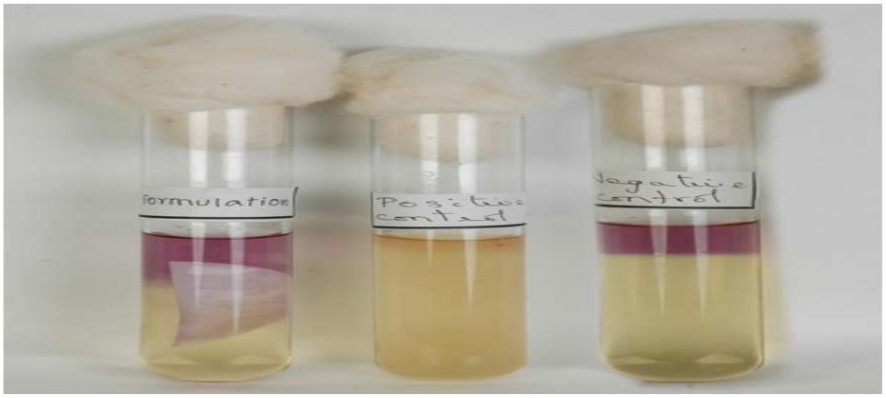

Figure 6: Observations of sterility test done in Fluid Thioglycolate medium (FTM)

Stability Studies of Emtricitabine Nanoparticles [21, 22]

The stability studies of the optimized nanoparticle formulation F9 was carried out for 3 months. The test was performed in three condition 4^0C, room temperature and 45^0C/70%RH. At the time interval of one month the nanoparticle formulation were evaluated for entrapment efficiency. The stability of nanoparticle formulation was more stable in refrigerator (4°C) when compared to room temperature and at (45^0C/70%RH).

Table 7: Stability studies of nanoparticles

S.No	Storage Conditions	Test Parameters	1st Month	2nd Month	3rd Month
1	4^0C	pH	7.5	7.5	7.5
		Colour	Clear& Colourless	Clear& Colourless	Clear& Colourless
		Sterility	Passes	Passes	Passes
2	Room Temperature	pH	7.4	7.4	7.3
		Colour	Clear& Colourless	Clear& Colourless	Clear& Colourless
		Sterility	Passes	Passes	Passes
3	Accelaration Conditions at 45^0C/70% RH	pH	7.3	7.3	7.3
		Colour	Clear& Colourless	Clear& Colourless	Clear& Colourless
		Sterility	Passes	Passes	Passes

Table 8: In vitro release for optimized formulation F9 stability study at 4^0C

Time (Hrs)	Cumulative% drug release		
	1st month(%)	2nd month(%)	3rd month(%)
1	8.0	12.0	7.0
2	15.08	19.12	9.07
3	19.18	21.19	13.09
4	23.19	23.21	19.13
5	26.23	24.23	24.19
6	32.26	29.24	25.24
7	38.32	31.29	32.24
8	40.38	39.31	39.32
9	40.40	41.39	48.39
10	47.44	44.41	50.48
11	52.47	48.44	54.50
12	53.52	50.48	56.54
13	58.53	52.50	60.56
14	60.58	54.52	63.60
15	67.60	57.54	65.63
16	69.67	58.57	69.65
17	72.69	64.58	72.69
18	73.72	68.64	75.72
19	79.73	69.68	77.75
20	82.79	75.69	81.77
21	84.82	79.75	82.81
22	85.84	84.79	83.82
23	89.85	86.84	87.83
24	92.89	92.86	91.87

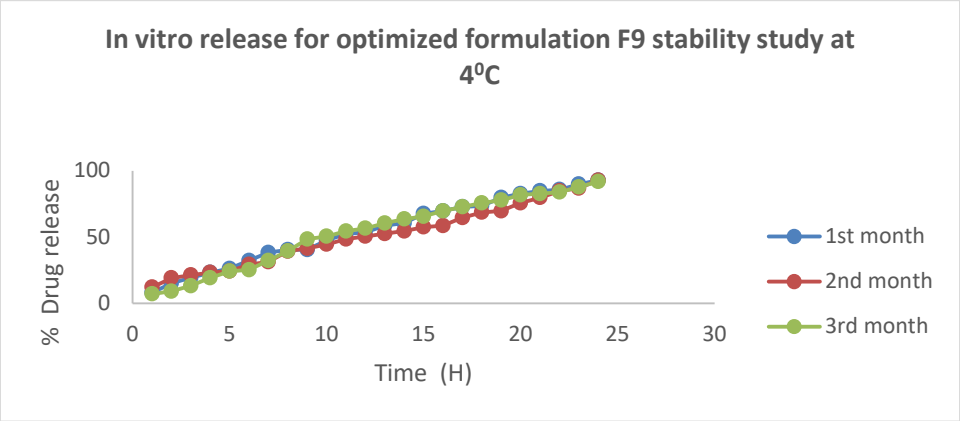

Figure 7: Stability study release data for formulation F9 after 3 months at 4°c

Table 9: In vitro data for optimized formulation F9 stability study at room temperature

Time (Hrs)	Cumulative% drug release		
	1^{st}month	2^{nd}month	3^{rd}month
1	8.0	5.0	0.5
2	10.08	7.05	07.05
3	15.10	13.07	10.07
4	16.15	17.13	15.10
5	18.16	24.17	16.15
6	23.18	29.24	18.16
7	26.23	31.29	25.18
8	28.26	34.31	28.25
9	33.28	39.34	33.28
10	35.33	45.39	34.33
11	39.35	47.45	39.34
12	40.39	49.47	41.39
13	44.40	51.49	45.41
14	46.44	53.51	47.45
15	51.46	56.53	55.47
16	55.51	61.56	57.55
17	60.55	63.61	61.57
18	66.60	68.63	66.61
19	67.66	69.68	69.66
20	69.67	71.69	72.69
21	71.69	72.71	74.72
22	72.71	77.72	76.74
23	78.72	80.77	79.76
24	83.78	82.80	81.97

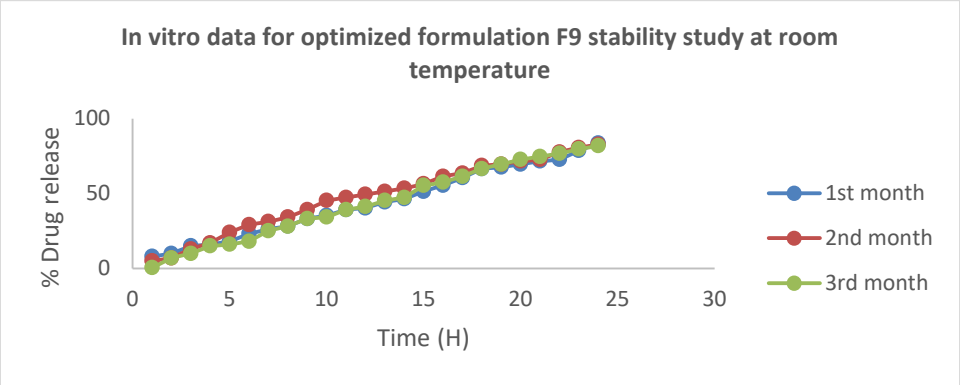

Figure 8: Stability study release data for formulation F9 after 3 months at room temperature

Table 10: In vitro data for optimized formulation F9 stability study at 45°c/75%RH

Time (Hrs)	Cumulative% drug release		
	1^{st}month	2^{nd}month	3^{rd}month
1	6.0	2.0	4.0
2	11.06	5.02	8.04
3	13.11	7.05	10.08
4	17.13	8.07	13.10
5	21.17	13.08	14.13
6	22.21	15.13	19.14
7	26.22	19.15	25.19
8	28.26	25.19	28.25
9	30.28	26.25	30.28
10	31.30	30.26	32.30
11	34.31	31.30	35.32
12	40.34	33.31	36.35
13	42.40	36.33	40.36
14	48.42	38.36	41.40
15	51.48	42.38	43.41
16	53.51	43.42	46.43
17	57.53	45.43	48.45
18	59.57	48.45	49.48
19	61.59	51.48	54.49
20	63.61	55.51	55.54
21	67.63	60.55	61.55
22	68.67	63.60	64.61
23	73.68	65.63	66.64
24	75.73	71.65	70.66

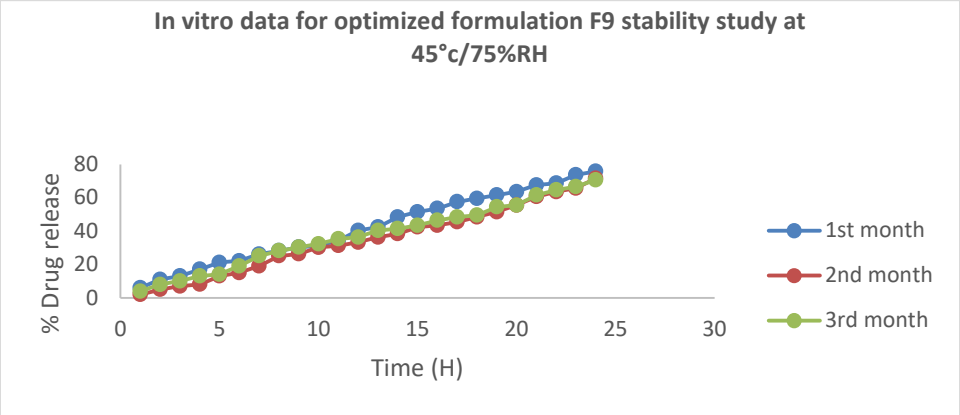

Figure 9: Stability study release data for formulation F9 after 3 months at 45°c /75% RH

Kinetics of drug release for optimized formulation F9 [37]

The optimized formulation F9was introduced in to graphical treatment for kinetics of drug release.

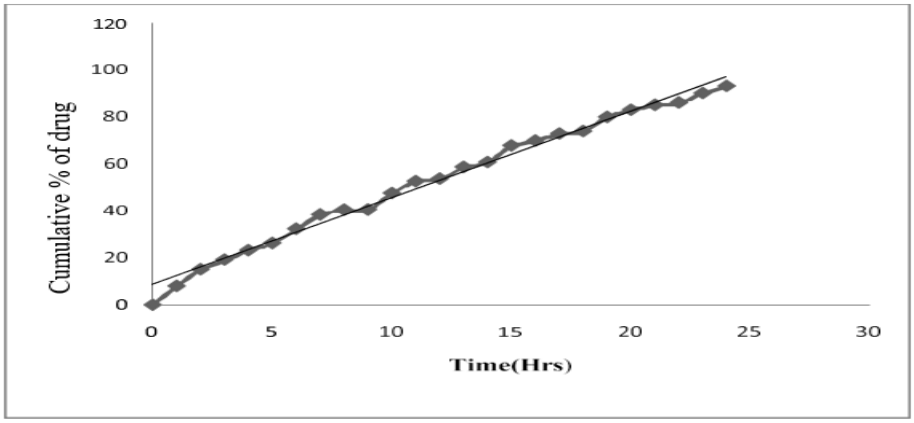

Figure 10: Zero order Plot for formulation F9

Slope=3.704 Regression=0.988

The optimized formulation F9 of nanoparticle.is more suitable for parenteral administration it shows good in the in vitro release kinetic study. The zero order plots were obtained by plotting cumulative percentage drug release versus time. The regression value is 0.988.

Higuchi's Plot:

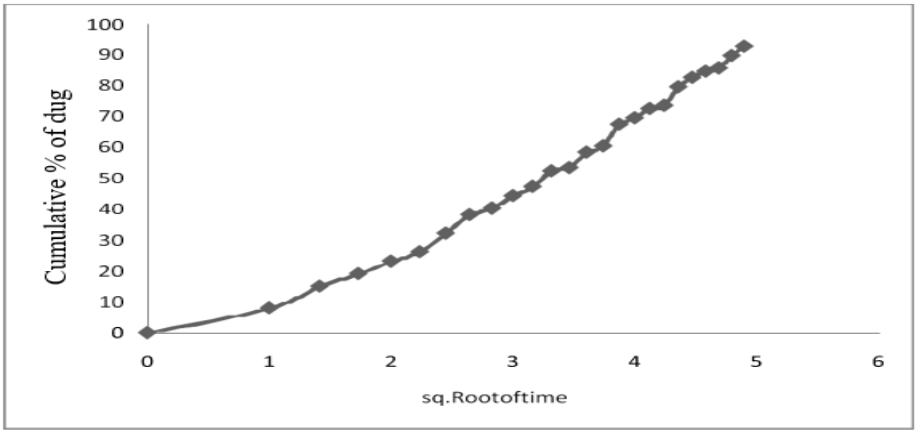

Figure 11: Higuchi's plot for formulation F9

Slope = 20.93 Regression = 0.973

Higuchi plot was made by plotting cumulative % drug release against square root of time. The regression value was found to be 0.973. This indicates that diffusion is one of the mechanisms of drug release.

Korsemeyer Plot

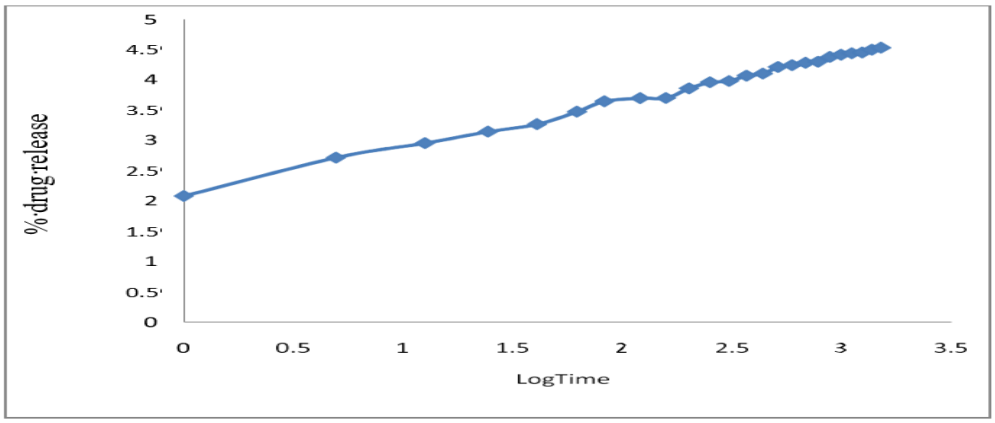

Figure 12: Korsemeyer's plot for formulation F9

n = 0.764

The graph was plotted between log cumulative % of drug release and log time. The value was found to be 0.45 < n < 0.89 anomalous (non – fickian) diffusion. This indicates that the diffusion and erosion could be the reason for the mechanism of drug release.

Summary and Conclusion

The present study Emtricitabine nanoparticles aimed to develop a nanoparticulate drug delivery system of antiviral drug Emtricitabine using biodegradable polymer Eudragit RS 100 & RL 100. The polymer enhances the binding of Emtricitabine nanoparticles in specific or targeted site with sustained release of drug increasing therapeutic efficacy. These nanoparticles may also reduce the dose & dose frequency with desired therapeutic response.

The pre-formulation studies were performed by using FTIR. The spectra of pure drug, pure polymer and nanoparticle formulation were examined. The study revealed the absence of significant interactions between drug and polymer.

All batch of nanoparticles (F1-F10) were prepared by emulsion solvent evaporation method, formulation was subjected to various evaluation tests. The entrapment efficiency of the optimized formulation was 94 ± 0.05% and invitro drug release was 92.89% after 24 hours. It also obeys the zero order follows diffusion and erosion mechanism of release. Particle size determination by Scanning Electron Microscope shows the best formulation containing size of about 100 nm.The formulation passed the sterility test performed as per specifications of Indian pharmacopoeia. The stability test performed revealed that the formulation was good. The best formulation was examined for zeta potential determinations. The formulation (F9) showed maximum deviation of -27mV which demonstrated that the particles are separate and highly repelling. This repelling property found to be more useful in decreasing opsonization and favors target specificity. The prepared formulation was sterilized by Membrane filtration technique. It was an aseptic technique involving the use of laminar air flow workstation.

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