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RESEARCH ARTICLE

Design and Development of Chitosan Based Etravirine Nanosuspension

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ABSTRACT

Objective(s): The objective of this study was to design, develop, optimize, and evaluate Chitosan based Etravirine Nanosuspension to enhance their solubility and extent of release.

Methods: The Chitosan based Etravirine Nanosuspension was formulated by the modified solvent evaporation combined ionic cross-linking method and it was optimized through design of experiments (Box-Behnken Design). The impact of Drug, Chitosan and Sodium Tripolyphosphate concentration on the drug release, particle size and drug entrapment efficiency were evaluated. The optimized Nano formulation were characterized using, Zetasizer, Transmission electron microscopy, X-ray diffractogram and saturation solubility study.

Results: The three level, three factor response surface design was applied in design of experiments which yields regression equations for individual responses against composition variables to arrive a design space to produce nanosuspension constantly with desired attributes. Among the trials conducted, the Batch # F6 exhibits optimal results in various parameters like particles size (226 nm), poly dispersity index (0.2), zeta potential (+30 mV), 92 % of drug release and 80 % entrapment efficiency. The saturation solubility of Batch # F6 was enhanced to about 22 times (89.19±2.56 µg/ml) of pure crystalline drug moiety (4.02±0.03 µg/ ml).

Conclusions: Etravirine, an antiretroviral drug with poor bioavailability has several constraints like lipophilicity, low permeability, crystallinity and first pass metabolism. Hence, Etravirine loaded chitosan based nanosuspension was formulated and considerably improved the solubility and drug release profile. This Nanosuspension could ease the oral administration and facilitate the absorption of Etravirine to attain enhanced bioavailability.

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INTRODUCTION

A revolutionary therapeutic tool for HIV could be Nano based antiretroviral (ARV) formulations, because Nano formulations can influence and align the kinetics of the poorly soluble drug candidate to the soluble, permeable drug products efficiently. Etravirine (ETR), a widely used ARV, is a new non-nucleoside reverse transcriptase inhibitor (NNRTI) having typical low soluble inherence with the chemistry of 4-[6-Amino-5- bromo-2-[(4-cyanophenyl) amino] pyrimidin-4-yl] oxy- 3, 5-dimethylbenzonitrile. ETR blocks HIV's reverse transcriptase enzyme which is required to convert RNA to DNA and prevent the virus from infecting the CD4 cell [1]. ETR demonstrates superior efficacy than other NNRTIs, it is being considered indispensable in standard drug composition regimen (HAARTs), due to their sustained virological efficacy and lack of lipodystrophy

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problems [2]. However, ETR has a high lipophilicity, a high log P value, poor solubility across the entire pH range of the gut, first-pass metabolism, low to moderate permeability and high faecal excretion (93%), which are all account for poor bioavailability. [3-4].

Only a few researchers made efforts to improve the solubility and bioavailability through various principles like solid dispersion, co-solvency and co-crystal engineering. Ramesh et al. have enhanced the solubility of ETR by nine-fold and bioavailability by two-fold using Kolliphor® P407(Poloxamer) with the solvent evaporation technique. [5]. With Povidone-copovidone polymers, Pavan Kommavarapu et al. demonstrated a 4.52-fold of solubility enhancement. [6].

FDA approved commercial product is Intelence® marketed by Tibotec was formulated by Spray drying (SD) process to improve apparent solubility and hence bioavailability. This SD process would yield low density granules and it is compressed to be bigger size tablets as the 100 mg strength has dimension of 19 X 9.5 X 6.6 mm and 200 mg is with 22 X 11 X 8.2 mm. The bulky and larger size of these pills often causes problems with oral administration which leads to poor compliance. Also, the dissolution is one of the critical quality attributes listed in the Intelence® drug product development [7]. Furthermore, their absolute bioavailability was reported to be 0.13% [8]. This suggests that Nano formulations could be a viable solution to enhance bioavailability as well as making into ease of administration.

But, very few research works have been carried out in the nano-formulations of ETR with the aim of enhancement of their bioavailability. Of which, Kim A. Woodrow et al., developed nano formulation of combination of Maraviroc (MVC), ETR and Raltegravir (RAL). ARV drug-nanoparticles were prepared using emulsion-solvent evaporation techniques with poly (lactic-co- glycolic acid) (PLGA) nanoparticles and compared with individual nano formulations. ETR nano formulation was developed with the size of 371+/- 71.6 nm and higher encapsulation efficiency (91 %) with least IC 50 value (1.4 nM) [9].

Ryanet al., developed etravirine nanosuspension loaded Dissolving Micro Needle (DMNs) by sonoprecipitation method with an objective of prolonged action for HIV therapy and achieved the release of the etravirine up to 240 hrs. This long-acting intradermal depot system shows 29.07-fold increment in the AUC compared to the commercial Intelence® 200 mg/kg tablet. [10].

Nano dosage forms are not only warranted for solubility enhancement, but it is also meant for permeation and absorption enhancement of oral administration [11]. Considering the need to improve the bioavailability of ETR, it is decided to design the Nanosuspension using suitable polymer and adjuvants with the objective of enhancing solubility and release rate.

Chitosan is a naturally available mucopolysaccharide polymer, it has been used against viral infections and their syndromes in therapeutic and prophylactic nano formulations. Since, Chitosan can open tight junctions between contiguous cells and deliver the actives into the tissue to achieve abundant concentrations through paracellular pathways compare to the conventional dosage forms [12]. The cationic polymer adheres to the anionic mucus layer and enhance the transcellular absorption which in turn delays the elimination of formulation from gastrointestinal tract to reduce the high elimination rate of ETR. Also, chitosan-based nano formulations would prolong the drug release which may increase the contact time at absorption site [13]. Such prolong adhesion may reduce unchanged portion of faecal excretion.

D-α-tocopheryl polyethylene glycol succinate (TPGS) is a functional excipient, made up of hydrophobic vitamin E and a hydrophilic PEG, both of which provide an amphipathic feature. Since, it has multi-functional properties, it can perform as solubilizer, emulsifier, permeation enhancer, absorption enhancer and stabilizer. Low concentration of TPGS can form stable amphiphilic micelles which plays essential role in drug delivery system. [14].

The uniqueness of nanotechnology inspired to fabricate chitosan based ETR loaded Nano suspension (ETR-CS-NS) to curtail the constraints and limitations associated with inherent nature of the active pharmaceutical ingredient and make it into ease of administration. The Nano formulation is designed to develop with naturally available, affordable Chitosan and TPGS by modified solvent evaporation combined ionic cross-linking method. The formulation was optimized through Box-Behnken method of design of experiments (DoE) using Minitab (version 20) software. Also, the optimized formulation was characterized by XRD, TEM, PSD and Zetasizer. Additionally, the in-vitro release study was performed and their kinetics were

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characterized using model dependent approaches.

EXPERIMENTS

Materials

ETR of purity 99.27% was gifted by Hetero Labs Pvt Ltd, Hyderabad, India. Chitosan (extra pure) was purchased from Sisco Research Laboratories, Mumbai. Sample of D-alpha tocopherol polyethylene glycol 1000 succinate mono ester (TPGS) was supplied and supported by Isochem, France. Based on the analyte molecular weight, MWCO 1 kDa Dialysis membrane (Spectra/ Por7®) was selected and purchased from Spectrum Laboratories Inc., Rancho Dominguez, CA, U.S.A. Sodium Tripoly phosphate was gifted by Anmol Chemicals, Mumbai. The chemicals and reagents used in the analysis and for testing purposes are all of suitable analytical grade.

Methods

Differential Scanning Calorimetry (DSC)

The thermal characteristics of active, inactive ingredients and the proposed drug mixtures were studied. Each sample was sealed in aluminum cases and placed them in the Mettler TA 4000 calorimeter and proceeded in an N2 environment at a rate of 100 degrees Celsius per minute throughout a range of 30 to 3000 degrees Celsius.

Fourier transform infrared study (FT-IR)

The compatibility of the drug with excipients was confirmed by FT-IR studies. The FT-IR spectra of ETR and ETR-CS-NS were studied by using Perkin Elmer RX1 model. An admixture of 1 mg sample and 200 mg potassium bromide were grounded well together and underwent high compaction pressure to form pellets. The pellets thus prepared were scanned and recorded over the range 4,000–450 cm^{-1} with a spectral resolution of 4 cm^{-1}[15].

Calibration curve

A series of ETR working standard solutions were prepared by appropriate dilutions of the stock ETR standard solution with a methanol and absorption measured at 236 nm^{-1}, plotted against known concentration and linearity curve was established [16].

Preparation of Nanosuspension

The chitosan based nanosuspension was prepared by solvent evaporation with the combination of the ionic cross-linking method. Chitosan solution was prepared in 0.2% (v/v) acetic acid (2mL), and the pH was adjusted to 5.5 using NaOH solution (dropwise). Then required quantity of the drug was dissolved in the chloroform (1mL). TPGS solution (20mg/mL) was prepared in distilled water. Afterwards, TPGS solution (1mL) was mixed with chitosan solution followed by the addition of drug containing chloroform solution and sonicated to form uniform nano emulsion using probe sonicator (Hielscher UP200H, Germany) and kept on stirring for overnight to get evaporate the chloroform. Afterwards, 2.5 mL of NaTPP solution was added dropwise under stirring for cross linking. The final volume of the nanoparticles (10 mL) was adjusted with distilled water. Nanoparticles were centrifuged at 10000 rpm, and the sediment of nanosuspension was redispersed in distilled water. The nanoparticle formulation was placed in the petri plates (5mL in each) and kept for deep fridge for 24 hrs at -20°C. Then the frozen nanoparticles were lyophilized (Labocon freeze dryer) for 10 hrs (two cycles) [17-18].

Optimization of Nanosuspension by Box- Behnken Design

Optimization of ETR-CS-NS was carried out based on the data generated from various pre-optimized batches. Impact of different formulation variables of drug, polymer, and cross-linking agent concentration on Drug release, Particle size distribution and Entrapment efficiency of ETR-CS-NS were studied. The 3-factor, 3-level randomized response surface Box-Behnken design (BBD) was chosen with sixteen trial runs to understand the impact of independent variables on the key response variables. BBD is simplest three level designs for three continuous variables. The three levels are usually referred as minimum, medium, and maximum. These levels are numerically expressed by the digits -1, 0, and +1. One variable was (A) drug concentrations of 1, 2 and 3mg. As for the other variables, the second was Chitosan at 10,20,30 mg, and the third was Na TPP at 1.66,3.33, and 5.0 mg in 2.5 mL. Total sixteen (16) no of batches of nanosuspension were prepared as per the random order of composition derived. The results were filled in Minitab software (Version 20) and tested statistically using analysis of variance (ANOVA). [19]. The results of the same are discussed in the respective section.

One-way ANOVA was used to examine the statistical significance of the differences in dissolution, particle size, and percentage of entrapment efficiency.

$$Y = b0 + b1X1 + b2X2 + b3X3 + b1b2X1X2 + b1b3X1X3 + b2b3X2X3 + b1b2b3X1X2X3$$

where $Y$is the measured response, $b0$ is the arithmetic mean response, $b1$ is the main effect of Drug concentration($X1$), $b2$ is the main effect of Chitosan concentration ($X2$), and $b3$ is the main effect of Na TPP concentration ($X3$); $b1b2$, $b1b3$, $b2b3$ and $b1b2b3$ are the interactions of the main factors. The data were also subjected to 3-D response surface methodology to determine the influence of concentration of Drug, Chitosan and NaTPP on dependent variable responses and design space were arrived by plotting overlaid plot. Additionally, all the batches were evaluated for Poly dispersity Index and saturation solubility study.

Physical Characterization of Nanosuspension

a. Particle size and poly Dispersity

Dynamic light scattering study principle is used for the determination of Particle Size distribution (PSD), Average particle size and poly Dispersity index (PDI) (Malvern Zetasizer Nano S-90). As such ETR-CS-NS suspensions (without dilution) were used for the PSD and PDI measurements.

b. Zeta potential measurement

The influence of surface charge on the stability of ETR-CS-NS was measured by Zeta potential. Zetasizer (Nano ZS, Malvern Instruments, Malvern, UK) instrument was used for the determination of zeta potential of ETR-CS-NS.

X- Ray Diffraction Studies

X- Ray Diffraction (XRD) studies were carried out for the pure drug and nano formulations by using Smart Lab (Rigaku, Japan) diffractometer at 15 mA amperage, 40 kV tube voltage, 0.02◦ step width, in 20–80◦ 2θ scanning range. The XRD pattern meant for interpretation of solid-state stability, crystallinity of the pure drug and their changes in nano formulations were studied.

Electron microscopy studies

External morphology of ETR-CS-NS was characterized by using Transmission electron microscopy (TEM) (Morgagni 268D).

The optimized ETR-CS-NS was diluted with 50 parts of distilled water and kept it in sonication for 15 minutes. After addition of staining agent of 2% phospho tungstic acid and a tiny drop was placed on 300 mesh carbon-coated copper grid, proceeded for images of representative areas at appropriate magnifications.

Entrapment efficiency

An UV spectrophotometer (Shimadzu 1800, Tokyo, Japan) was used to measure the entrapment efficiency of the prepared Nano formulation. The supernatant liquid was discarded after centrifugation of nanosuspension at 10,000rpm, sediment was redispersed in 1ml of distilled water. Representative portion was withdrawn and dried in rotary vacuum drier under reduced pressure at 35˚C (Buchi R-210 Advanced, Switzerland). The residue at bottom was solubilized in 10 ml methanol and kept in slow shaking for 24hr at 37˚C, centrifuged and the clear supernatant liquid was filtered (0.45 µm) and measured the absorption at 236 nm. The entrapment efficiency is the ratio of the quantity of ETR loaded in the nanoparticles, and that quantity taken for formulation process.

Saturation Solubility

The volume equivalent to 100 mg / ml of nanosuspensions was dispersed to 20 ml of 0.01N HCl and stirred by a magnetic stirrer at 700 rpm for 24 hrs. Then samples were withdrawn in test tubes and centrifuged at 25,000 rpm for 30 minutes. Then, the filtrate of the supernatant solution was diluted appropriately and analysed by UV spectrophotometer at 236 nm. The experiment was repeated in triplicate and the average value has been considered.

In Vitro Release Profile

The release profile of nano formulation was characterized by using highly versatile dialysis membrane method. The pre-determined quantity (10ml) of ETR-NS-CS was taken in a water-jacketed beaker containing 300ml of 0.01N HCl (pH 1.2) with 1.0 % sodium lauryl sulphate at 37± 1^{O}C and agitated by magnetic stirrer with speed of 50 rpm. Periodically, 5 ml of aliquot was drawn from the media for 0, 0.5, 1, 2, 4, 6, 8, 10 and 12 hrs and the same volume was replaced with fresh media. Appropriately diluted aliquots were filtered (0.45 µ) and ETR content was determined by UV spectrophotometer at 236 nm. The results

of in-vitro drug release were analysed using model dependent approach for various kinetic models of zero order, first order, Higuchi, Hixson Crowell and Korsmeyer-Peppas, and Weibull by Kinet DS software [19].

RESULTS AND DISCUSSION

DSC

Pre-formulation study initiates systematic development of Nano formulation with the compatibility study of the composition where the thermogram (Fig. 1) of Chitosan, Etravirine, TPGS, NaTPP and ETR-CS-NS (F6) were studied by DSC. The pure ETR displayed an endothermic peak at 262^{O}C, corresponding to its melting point. There was no degradant peak or endotherm shift observed with thermogram of excipients and their mixtures, indicating compatibility of the drug with all the excipients.

FT-IR

The FT-IR spectra of pure ETR, excipient, and formulation were showed in Fig. 2. Pure ETR presents characteristic infrared spectra in the region of 3445 cm^{-1} explains primary and secondary amine (-NH_2 and -NH-) functional group, while 2925 cm^{-1} suggests CH_3 group. It also exhibits characteristic infrared spectra in the C=O stretching region of the functional carbonyl group band at 1635 cm^{-1}, showing its crystalline nature. In the case of FT-IR spectrum of physical mixture characteristic amine peak at 3417 cm^{-1} and C=O peak at 1617 of pure ETR appeared unchanged in physical mixture. From the spectra (Fig.2) there are no any extra peaks were observed for drug and drug excipient mixture which proves that there is no interaction occurred and ensures the solid-state stability of the formulation. Further, to determine the solubility and to test release characteristics of development batches by UV method, standard concentration curve to be constructed which should demonstrate the linearity. A series of concentrations of ETR working standard solutions were prepared by appropriate dilutions of the stock ETR standard solution (1 mg/mL) with a methanol to obtain the following ETR concentrations: 2, 4, 6, 8, 10 and 12 mcg/mL and measured absorption at 236 nm $^{-1}$. The linearity (Fig.3) was arrived with straight-line of y= 0.0658x-0.0045 and R^2 value of 0.9994.

Optimization of Nanosuspension by Box- Behnken Design

The ETR-CS-NS formulation design were optimized and demonstrated their robustness using design of experiments (DoE). A 3-factor,3-level BBD was used to suitably reveal out the main interaction and quadratic terms and arrive the second order polynomial equation using software Minitab (version 20) [20]. A design matrix of sixteen batches were constructed to generate a non-linear quadratic model equation for the responses. All three independent variables and their interaction were studied at their three levels concentration. The result of dependent variable responses of % drug release at 12 hours (Y1), particle size (Y2) and % entrapment efficiency (Y3) as per the order of experiments (Table. 1)As result generated quadratic equation for all responses could be arrived as follows.

This section describes full-model polynomial with significant value (P< 0.05) of Chitosan and NaTPP concentrations that quantify the effect of formulation variables on the responses YI, Y2 &Y3.

Y1 = 86.6+13.8A-2.05B+9.96C- 5.59AA+0.0413BB-2.93CC-
0.092AB+1.66AC+0.191BC

Y2 = 1158-282A-47.9B- 122C+12.6AA+1.230BB+
25.0CC+8.14AB+17.7AC-3.78BC

Y3 = 69.6+14.5A-2.64B+15.07C- 5.78AA+0.0620BB-3.54CC-
0.019AB+1.39AC+0.178BC

Also, the model suitability was confirmed with their regression coefficients which ensured the fitness as the lack of fit was insignificant. As shown in the Fig .4 the % drug release increases when Chitosan and NaTPP decreases irrespective of drug concentration. Also, it, decreases when Chitosan and NaTPP increases with respect to drug concentration. Curvature effect of NaTPP shows that significant quadratic effects on the % release. But, Particle size increases when chitosan, NaTPP increases at presence of drug. But, Particle size increases irrespective of Chitosan concentration at absence of drug. So that, it infers that Chitosan is significant variable for the particle size of Nanosuspension. The Entrapment

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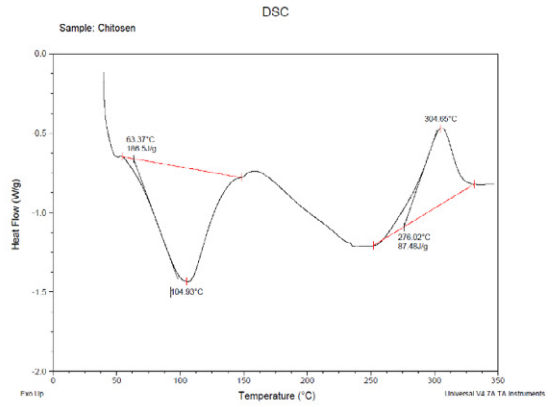

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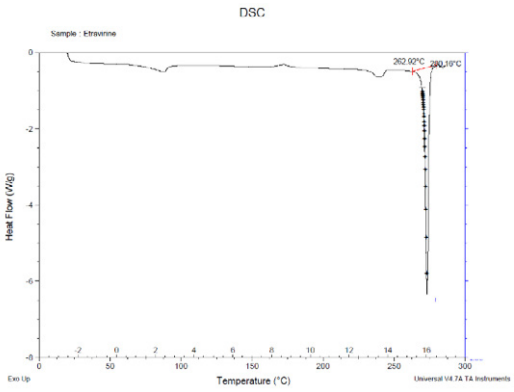

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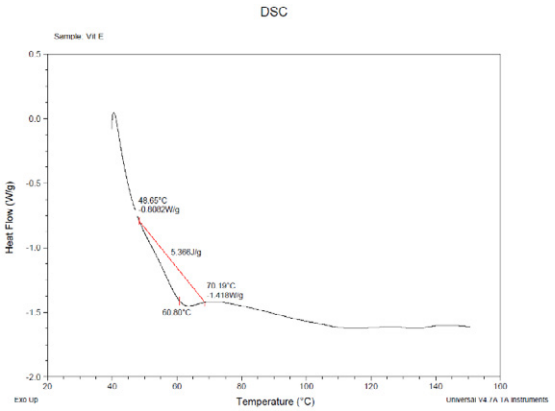

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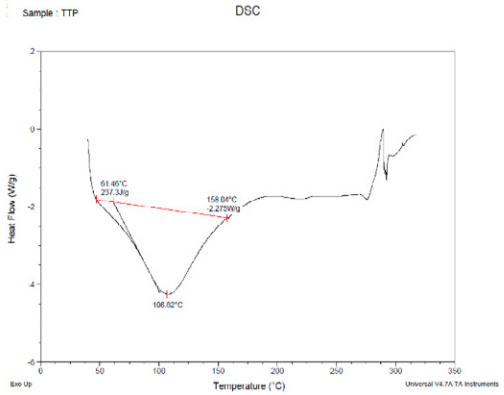

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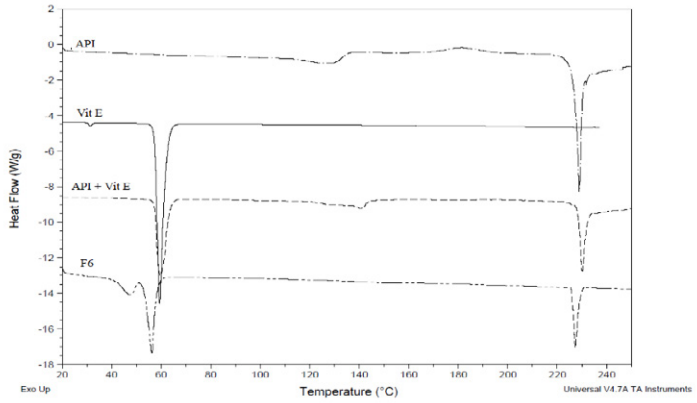

E

Fig. 1.

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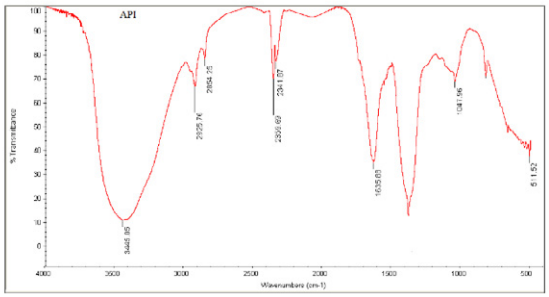

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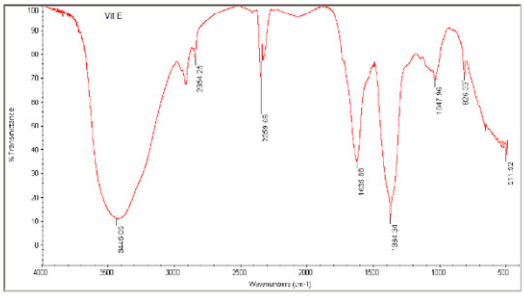

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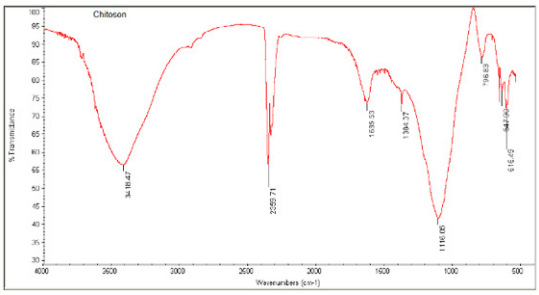

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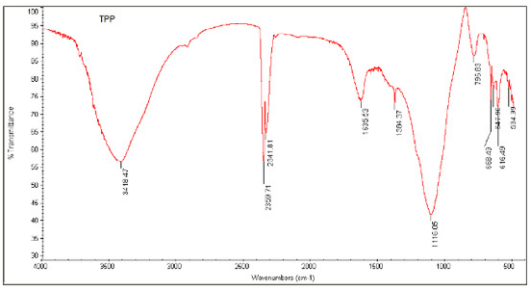

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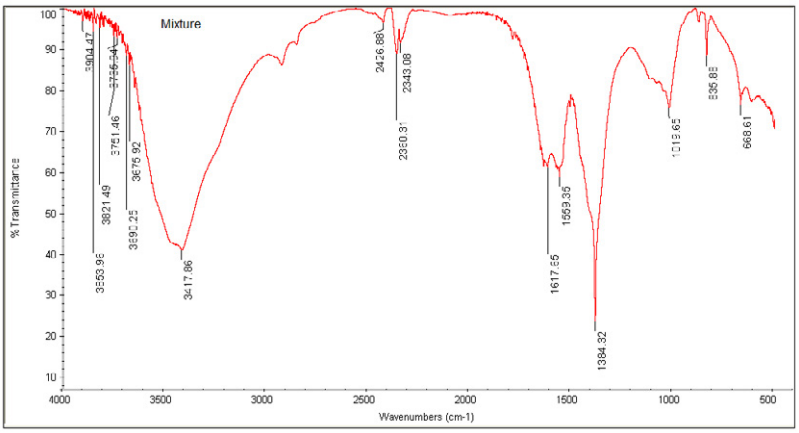

E

Fig. 2.

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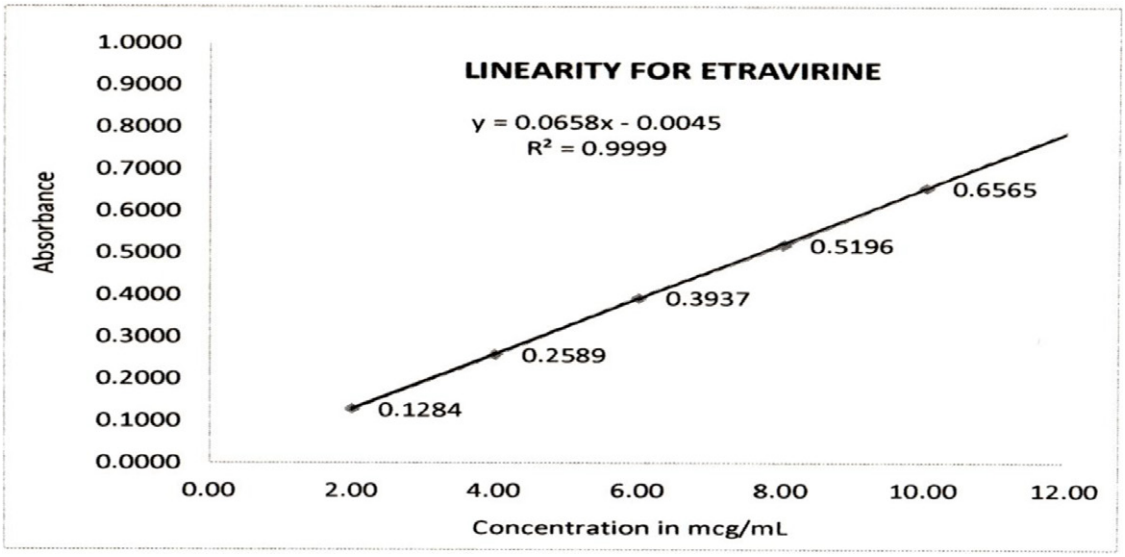
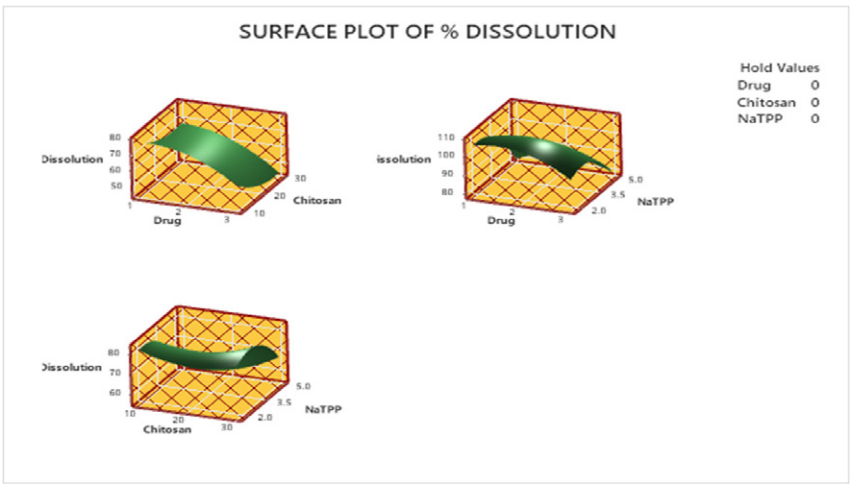


Fig. 3.

Table 1. Effect of various formulation variables on response variables.

EXP #	Proportion (mg)			Dissolution % (Y1)	Size (nm) (Y2)	EE %(Y3)
	Drug(A) in mg	Chitosan(B) in mg	NaTPP(C) in mg /2.5 mL			
F1	2	10	1.66	88.5	348	75.2
F2	1	20	5.00	67.0	301	56.0
F3	3	20	1.66	70.2	236	60.0
F4	2	10	5.00	77.1	480	66.0
F5	1	20	1.66	87.0	359	75.0
F6	2	20	3.33	92.0	226	80.0
F7	2	30	5.00	80.9	378	76.2
F8	2	30	1.66	86.5	550	80.2
F9	3	30	3.33	81.2	552	78.7
F10	2	20	3.33	85.2	236	74.4
F11	1	10	3.33	91.2	365	80.4
F12	2	20	3.33	89.2	265	84.2
F13	3	20	5.00	68.2	348	57.0
F14	1	30	3.33	87.2	301	82.2
F15	1	20	3.33	89.0	336	79.0
F16	1	30	5.00	88.0	394	81.6

efficiency showed maximal impact by Chitosan and minimal with NaTPP. Also, the design space was constructed by drawing the overlay plot between two formulation variables and constant of third one, the response variables were plotted within the acceptable criteria to search for the best compromise visually (Fig. 5). From, the design space the best formulation composition was arrived as Drug (2 mg), Chitosan (20 mg) Na TPP (3.33 mg) specified by the selected point. Hence, ETR- CS-NS nanosuspension has been developed and optimized successfully with particle size of 226



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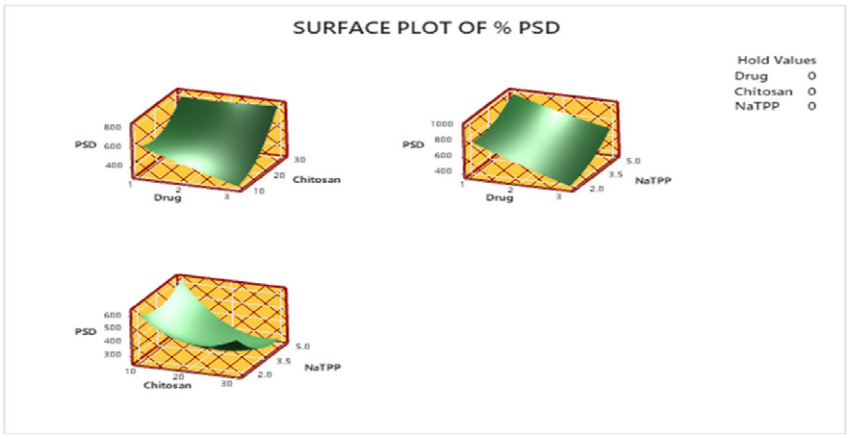

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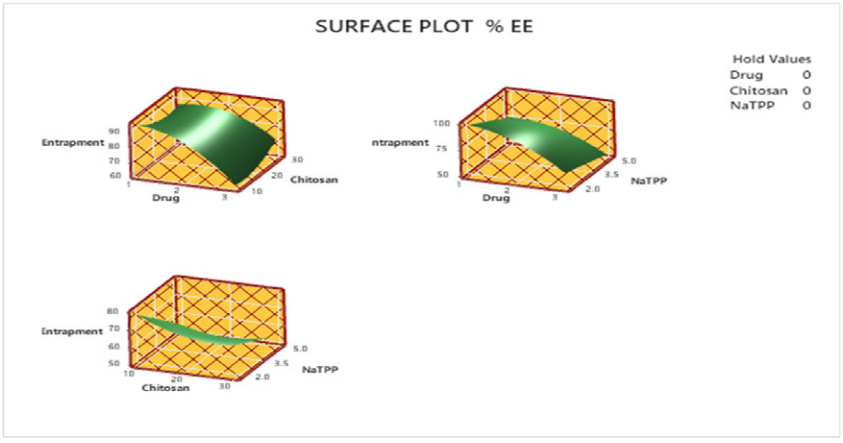

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Fig. 4.

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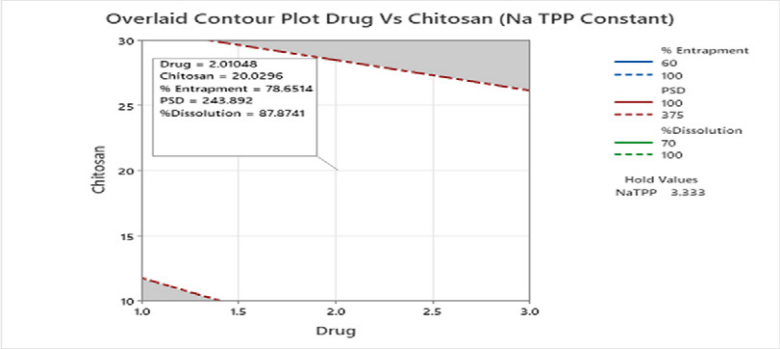

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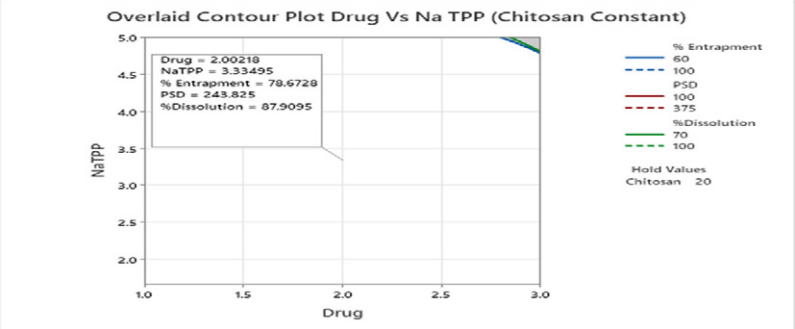

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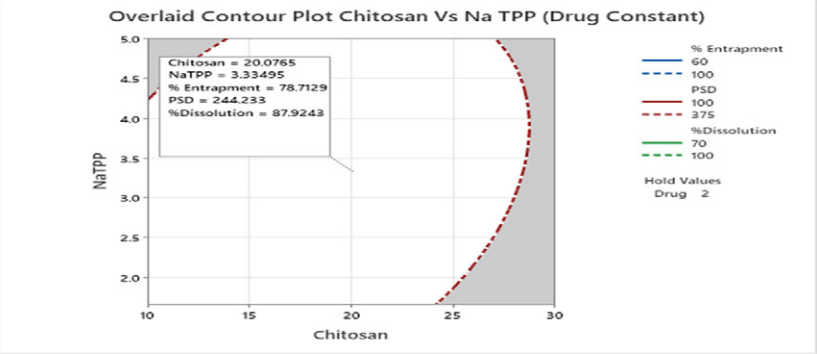

C.

Fig. 5.

-265 nm, 85.2 - 92 % drug release and 74.4 – 84.4% of entrapment efficiency which has PDI of 0.2-0.5 and zeta potential of + 30 mV.

Physical Characterization

The physical characters PDI, Average Particle size and Entrapment efficiency for the batches F1-F16 were tabulated in Table 2. The batches F1, F3, F6, F10, F11, F12, F13, F15 and F16 are all having PDI less than 0.4, it shows the uniform, narrow distribution of particles. The batch F8 only exceed the criteria with 0.846. As per the International Organization for Standardization ISO 13321:1996 E and ISO 22412:2008, the PDI value < 0.05 describes that highly monodisperse system and PDI> 0.7 are all polydisperse system with bigger particle size

Table 2. Particle size, PDI and % EE of ETR-CS-NS batches

Code	PDI	Size (nm)	EE (%)
F1	0.201	348.7	75.2
F2	0.560	301.4	56.0
F3	0.234	236.3	60.0
F4	0.520	480.0	66.0
F5	0.518	359.5	75.0
F6	0.209	226.2	80.0
F7	0.499	378.0	76.2
F8	0.846	550.0	80.2
F9	0.499	551.8	78.7
F10	0.490	468.0	74.4
F11	0.360	365.0	80.4
F12	0.321	260.0	84.2
F13	0.400	348.0	57.0
F14	0.420	301.0	82.2
F15	0.302	236.0	79.0
F16	0.395	394.0	81.6

distribution.

It can be seen from the results that the formulation batch # F6 has the minimum particle size and it was reported to be 226 nm, whereas batch # F9 has 552 nm, which is the highest size amongst batches manufactured. The particle size increases with either aggregation of particles under impact of anionic charge of abundant cross connecting NaTPP or higher concentration of Chitosan. Among all batches F6, F10 and F12 are all in the size between 226 to 265 nm which are all having intermediate concentration of Chitosan and NaTPP depicted in A, B & C of Fig.6. Increasing chitosan concentration focuses increment on viscosity, and exorbitant NaTPP builds cross linkage firmness; the two of them are reason behind the expansion in particle size. Optimized formulation (batch # F6) has a zeta potential value of +30mv (Fig. 7) resulting in good formulation stability. As per DVLO theory, the stability of the colloidal system based on the sum of attractive and repulsive forces with the dispersed particles. Also, the zeta potential of +30 mV explains the stability of the formulation as nanosuspensions with positive charges which repulse each other particles and prevents the aggregation. However, the low zeta potential values, prone to lesser stability of the system. [15&21].

Entrapment efficiency (%EE)

Many of the prepared batches with entrapment efficiency of more than 70%. But the batches F2, F3, F4 and F13 were shown entrapment efficiency of less than 70 %, because of either maximum concentration of drug active or low concentration of polymer. These batches of F7, F8, F9, F14 and F16, which are all contributed by maximum level chitosan (30 mg). and batches F7 and F16 is contributed by both maximum level of Chitosan and Na TPP (30 mg & 5 mg). The results reflect with the conclusion that higher concentration of Chitosan has greater capacity to form an ionic gel, thus preventing rifampicin from escaping the encapsulating phase, and therefore increasing encapsulation efficiency. [22]. The extent of drug entrapment in polymer matrix determined by hydrophobic interactions, electrostatic interactions and hydrogen bonding occurred between drug and polymer chemistry. Chitosan is composed of mixture of molecules with varying degree of deacetylation, which plays vital role in emulsification process; accordingly, high degree of deacetylated molecules promotes oil-in water emulsion and low degree facilitate water -in-oil system [23].

XRD study

The XRD study (Fig. 8) shows the potential changes in the inner structure of ETR crystalline lattice as ETR-CS-NS has broad humps at low to moderate intensities which conforms the existence of amorphous nature. But, few sharp peaks were found which may be due to electrostatic interaction of chitosan and Na-TPP.

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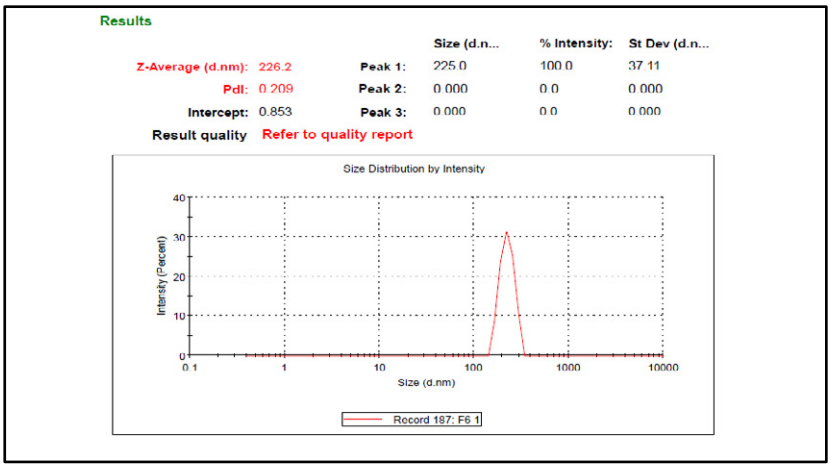

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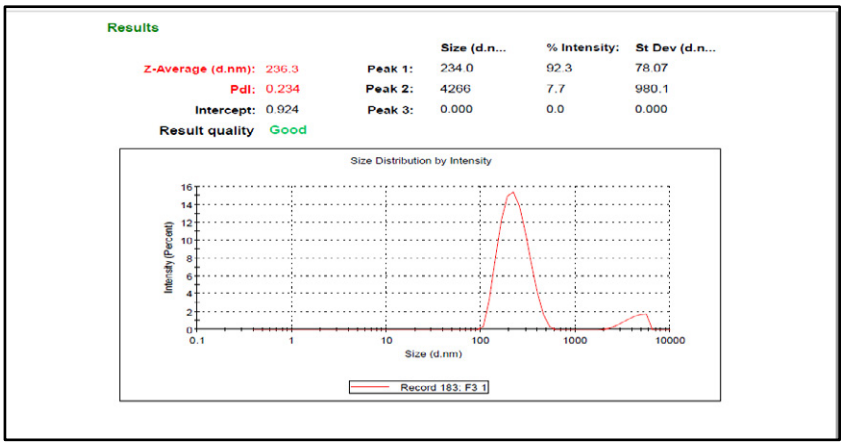

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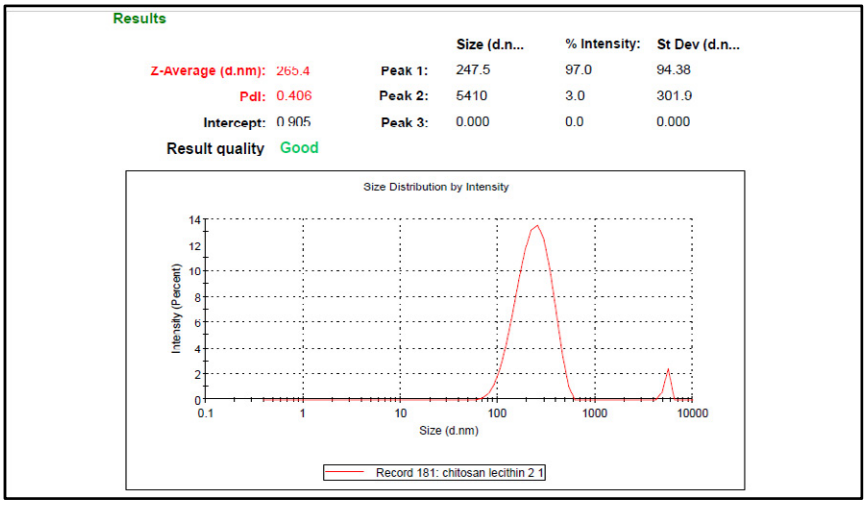

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Fig. 6.

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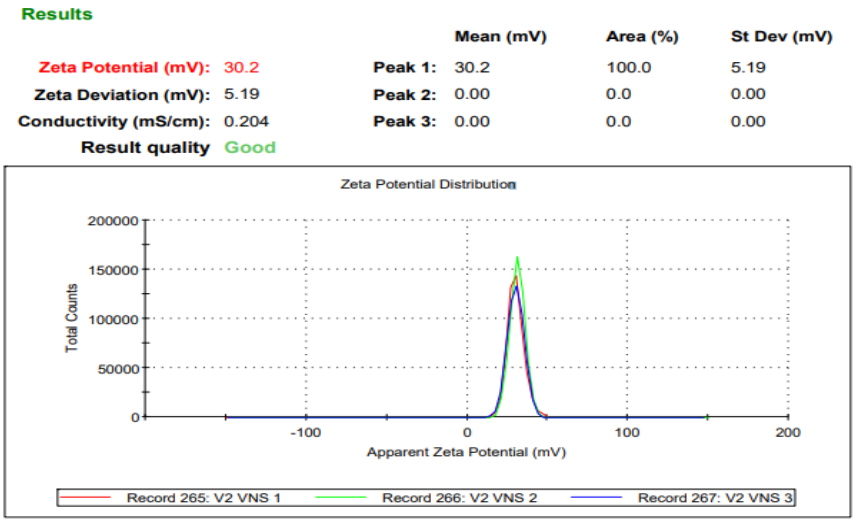

Fig. 7.

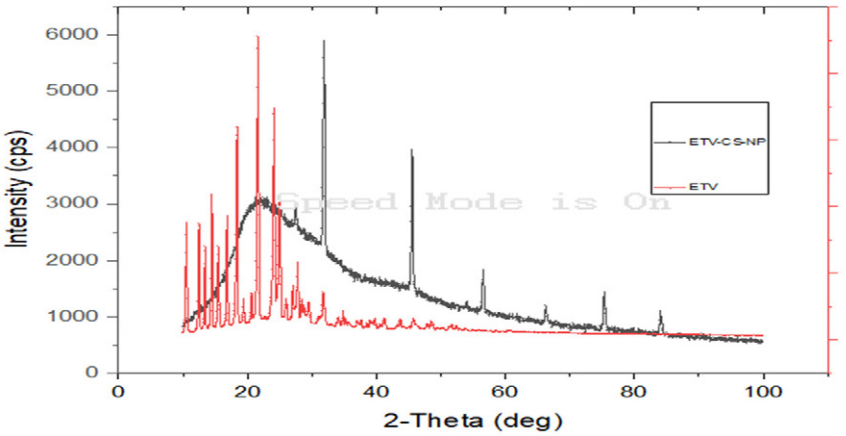

Fig. 8.

Electron microscopy studies

The morphological characterization of formulation F6 was investigated by TEM. The TEM image (Fig. 9) of optimized nanosuspension showed that particles are spherical in shape, smooth morphology and discreted particles. Since, there is no precipitation of the particles observed, ensures the stability of nanosuspension.

Saturation solubility

The result of saturation solubility of all batches and pure drug were shown in Table. 3. Of which batch F6 exhibited the highest saturation solubility (89.19±3.22µg/ml) which is 22-fold higher than that of pure drug. Thus, remarkable increase in solubility of ETR was obtained through formulation of nano suspension. ETR shows poor aqueous solubility because, of strong intermolecular hydrogen bonding involved in their molecular structure [24]. The nano sized drug particles and low crystallinity nature could be the reason behind for the significant increase in saturation solubility.

In Vitro Release Study

The % drug release profile of all batches of ETR-CS-NS have been displayed in Table. 4 and their graphical pattern in Fig.10. The batch F6 exhibited maximum % cumulative drug release (92±3.1%)

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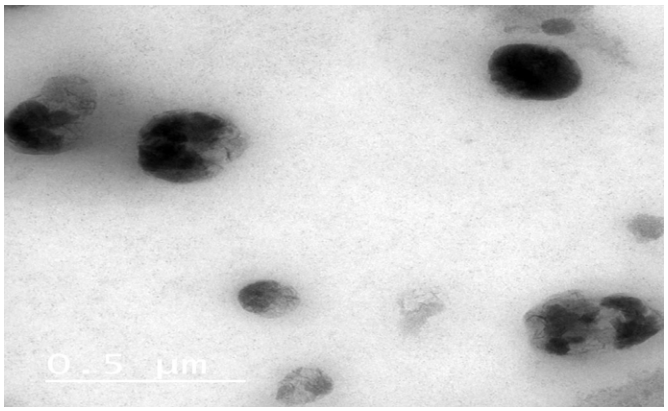

Fig. 9.

Table 3. Saturation solubility of ETR-CS-NS batches.

Sl no	Formulation code	Solubility µg/ml
1	API	4 .02±0.03
2	F1	80.21±3.22
3	F2	64.25±2.45
4	F3	64.52±3.61
5	F4	72.61±4.16
6	F5	81.20±2.56
7	F6	89.19±3.22
8	F7	73.61±3.98
9	F8	80.20±2.61
10	F9	76.52± 2.84
11	F10	79.50±3.85
12	F11	82.31±2.51
13	F12	80.62±1.84
14	F13	61.25±2.64
15	F14	80.23±3.57
16	F15	86.91±3.12
17	F16	87.91±3.55
18	F17	80.26±2.92

Table 4. In vitro % drug release profile of ETR-CS-NS batches.

	Cumulative drug release (%)									
Time (Hrs) / Formulation	0	0.5	1	2	3	4	6	8	10	12
API	0	10.2±3.9	18.2±3.7	24.1±3.2	29.1±2.4	36.2±2.4	41.2±2.1	44.3±1.9	47.5±2.1	51.0±1.8
F1	0	24.2±4.3	29.2±2.4	34.9±2.2	52.5±2.1	66.3±1.8	77.8±2.1	81.2±1.5	86.3±2.1	88.5±3.4
F2	0	18.2±3.7	27.0±3.6	32.6±2.7	40.2±2.1	48.2±1.8	54.5±2.1	59.8±1.5	65.2±2.4	67.0±3.8
F3	0	19.5±3.9	28.5±2.4	30.6±1.8	39.2±2.3	47.3±1.6	53.2±2.7	60.2±3.2	67.5±2.5	70.2±1.6
F4	0	20.3±2.7	25.5±3.1	31.4±4.1	39.2±5.1	48.5±3.2	58.7±4.1	69.7±2.5	75.3±2.3	77.1±3.4
F5	0	22.3±2.8	27.6±3.9	48.5±3.2	62.8±3.2	70.8±4.1	75.5±3.3	80.5±3.1	85.2±2.1	87.0±3.2
F6	0	21.8±3.4	34.2±2.1	42.2±1.8	56.2±2.3	67.4±1.5	76.0±0.9	82.5±0.6	85.6±3.2	92.0±3.1
F7	0	21.5±4.3	26.5±3.6	38.2±2.7	44.5±2.1	58.2±1.8	67.5±2.1	72.2±1.5	78.5±1.8	80.9±2.5
F8	0	25.9±3.3	36.6±2.6	44.8±3.1	50.2±1.1	60.2±2.8	68.9±3.1	75.6±4.4	80.2±3.4	86.5±3.4
F9	0	24.2±4.5	27.5±3.2	36.5±2.4	45.8±2.6	57.5±2.1	67.0±1.8	73.1±2.5	79.1±4.1	81.2±3.1
F10	0	23.2±3.2	34.2±2.7	40.2±2.1	47.6±1.3	60.2±1.8	70.5±1.5	74.5±0.6	82.5±3.4	85.2±2.7
F11	0	29.3±4.5	38.1±3.2	48.5±2.4	58.6±2.6	69.4±2.1	78.1±1.8	84.8±0.8	87.2±3.3	91.2±3.0
F12	0	22.7±2.1	27.4±1.9	37.2±4.2	49.5±2.6	61.2±4.5	71.5±2.3	80.9±3.8	86.2±1.8	89.2±2.0
F13	0	20.5±2.6	25.2±5.1	34.2±4.1	41.2±1.8	48.9±2.4	54.5±1.0	60.5±2.5	66.2±3.2	68.2±4.3
F14	0	24.2±2.5	31.5±1.6	39.5±4.1	48.8±4.2	55.5±2.3	65.8±2.6	76.5±3.7	84.6±1.5	87.2±1.5
F15	0	21.2±2.1	33.3±2.4	45.2±4.3	58.2±3.1	65.8±3.4	75.0±19	82.2±2.8	86.2±2.8	89.0±3.2
F16	0	20.5±2.4	31.3±3.5	42.6±2.8	51.8±3.1	60.5±3.6	70.2±3.4	80.2±2.1	85.5±2.8	88.0±3.1

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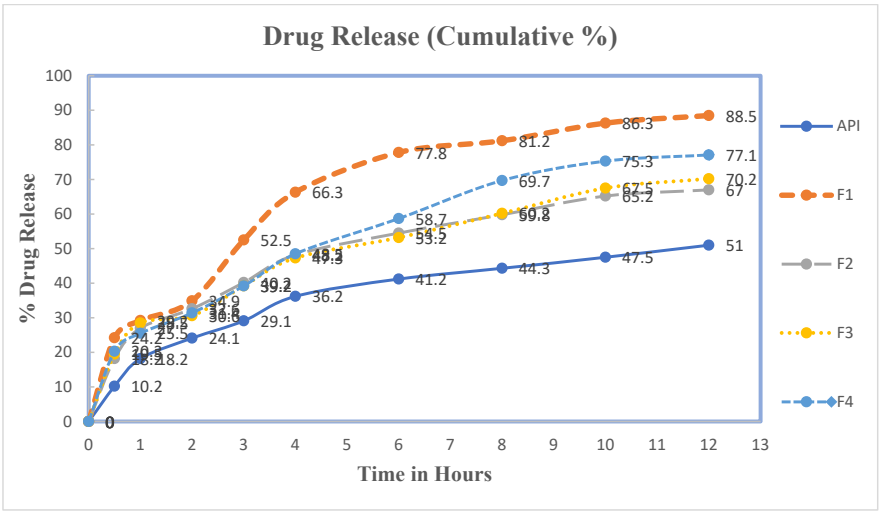

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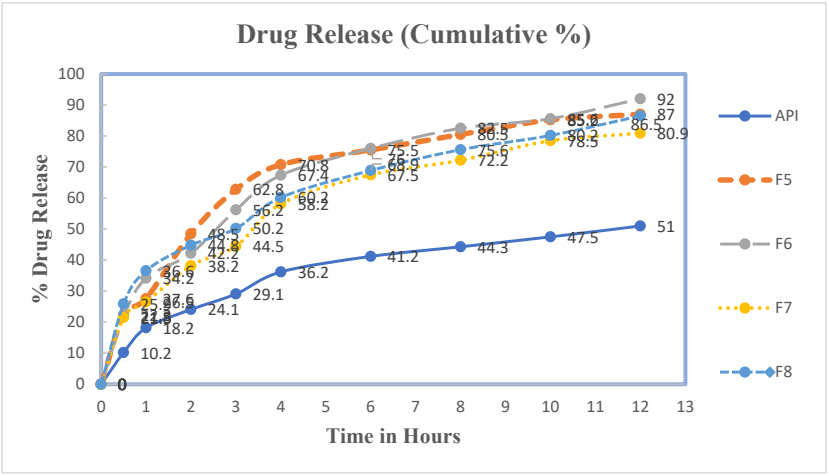

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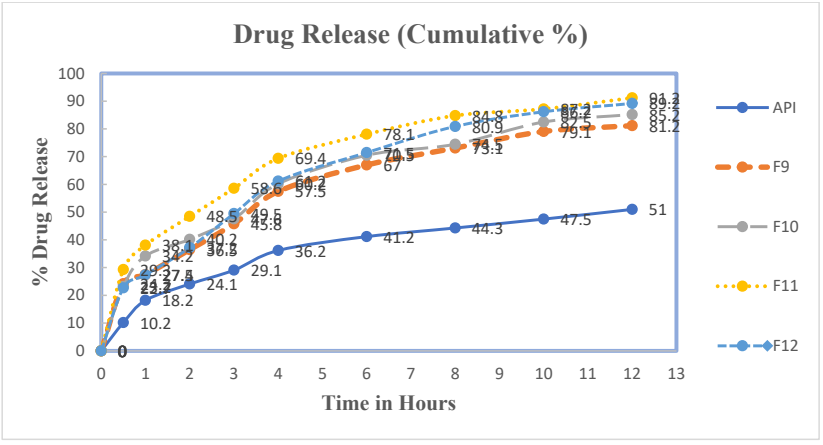

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Fig. 10.

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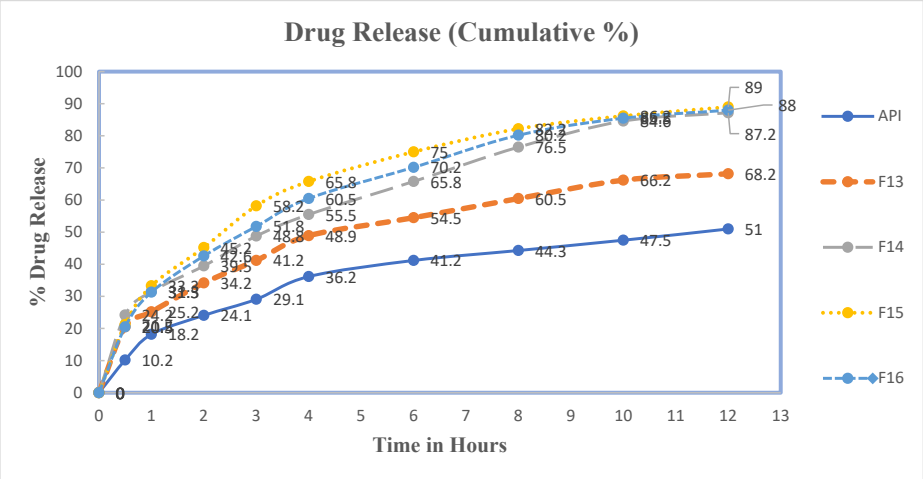

Continued Fig. 10.

Table 5. Regression co-efficient of Kinetic Models

Regression Coefficient (R^2) of Kinetic Models							
Batches	**Zero Order**	**First Order**	**Korsmeyer -peppas**	**Weibull**	**Hixson-crowell**	**Higuchi**	**Michaelis -Menton**
F6	0.8795	0.7549	0.9796	0.9905	0.8021	0.8021	0.9793
F10	0.9183	0.8167	0.9863	0.9809	0.8566	0.9094	0.9510
F12	0.9199	0.8247	0.9850	0.9797	0.8610	0.9739	0.9023

at 12 hours. Most of the batches shows the drug release more than 80 % other than that of F2, F3, F4 and F13. It may be likely due to higher drug concentration or higher crosslinking agent. Since, higher drug concentration may not be encapsulated properly by low or intermediate concentration of polymer and due to the retardation by high cross-linking agent, whereas the pure drug can release up to 51 % due to their inherent nature. The orally administered ETR-CS-NS would be delivered to the acidic environment of stomach and facilitate the swelling immediately. Chitosan based saquinavir Nano formulation shows faster and complete drug release at 12 h in acidic medium due to occurrence of enhanced swelling of chitosan [25]. Therefore, the drug release of all batches was studied in acidic medium. Also, the regression coefficients of the best batches (F6, F10& F12) were complied with both Korse-Meyer Peppas and Weibull kinetic models as shown in Table 5. This explains that the system is based on polymeric matrix system, and it follows swelling and non-fickian diffusion mechanism.

Thus, the analysis of the data obtained in this study reveals that the ETR-CS-NS possess enhanced release profile and patient friendly delivery system which could ensure high degree of patient compliance towards ETR based ARV therapy.

CONCLUSION

The unique aspect of ETR is the fact that it is a highly effective antiretroviral agent as it is resistant to seventeen clinically possible mutations associated with reverse transcriptional enzyme. However, their poor bioavailability and solubility restricts them to be convenient and easily palatable formulations. Therefore, the optimized formulation of ETR-CS-NS was successfully prepared with particle size of 226 – 265 nm with appropriate zeta potential. The result infers that both Chitosan and NaTPP have played vital role in the design and development of ETR-CS-NS as they have significant impact in drug release, particle size and drug encapsulation efficiency. Also, it exhibits remarkable enhancement of solubility and dissolution rate compared to ETR and thereby, it could have high vascular rich pharmacokinetic profile. i.e. C_{max} and AUC.

ETR-CS-NS could be an effective and highly feasible dosage form for paediatrics, geriatrics as

well as terminally ill HIV patients that ensures maximum compliance and effectiveness. Also, ETR-CS-NS can be administered once daily as it delivers the ETR over the period of time. So that it will be shown a highly favorable pharmaco kinetic attributes against HIV and their syndromes.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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