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Formulation and Evaluation of Anti-HIV Efavirenz Loaded Nanomicelles for Pediatrics

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ABSTRACT

In recent years, Micellar system have become a key tool toward the improvement of the aqueous solubility and the chemical stability of hydrophobic drugs. This work demonstrates that the encapsulation of EFV (poorly water soluble) into polymeric micelles of different co-polymers significantly improves the oral bioavailability and reduces the inter individual variability. This strategy appears a very promising one towards the development of a liquid aqueous EFV formulation for the improved pediatric HIV pharmacotheraphy. Nanomicelles are potentially useful vehicle for oral drug delivery system.Twelve batches of efavirenz loaded nanomicelles was prepared by sonication method following homogenization method by varying the concentration of polymers. The particle size of formulations were found to be from 146 to 3000 nm with the least particle size of 146.9 ± 167.0 for formulation F3. The surface charge of the formulation F4 to F12 possessed nearly negative and neutral charge. Formulation F1, F2, F3 possessed high negative potential. The entrapment efficiency was found to be higher for the formulation F1, F2, F3 (89.4%, 86.8%, 87.2%). In vitro release studies of F1, F2, F3 showed (63.01%, 81.46%, 83.04%) release respectively. Considering parameters like size, entrapment efficiency and in vitro release study, formulation F3 with a particle size of 146.9 ± 167.0 nm and 87.2 % of the drug in nanoparticles was chosen as the best nanomicelle formulation for liquid oral preparation. Thus it can be concluded that efavirenz loaded nanomicelles possesses promising future in delivery of oral formulation of drugs for pediatrics.

INTRODUCTION:

The acquired immunodeficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV) which causes a significant immunosuppression that is primarily brought on by the selective elimination of helper/inducer T cells that express the virus' receptor (the CD4 molecule). the characteristic feature of HIV infection is the virus's selective tropism for specific immune system and central nervous system (CNS) cells, which causes immunosuppression and neuropsychiatric disorders. (Anthony S. Fauci et al., 1988) The HIV-1 pandemic is unquestionably the most significant public health concern of our time. It is a complex mashup of other epidemics. Though preventative measures have increased and our knowledge of pathogenesis and transmission dynamics has deepened, a protective vaccination or treatment is still unattainable. In some settings, antiretroviral therapy has changed AIDS from a sickness that would inevitably result in death to a chronic, treatable illness.(Viviana Simon et al.,2006)The majority of the 1500 daily new HIV infections in children under the age of 15 are brought on by mother-to-child transmission MTCT due to a lack of preventative measures or issues with implementing prevention of mother-to-child transmission (P-MTCT).(Carlo Giaquinto et al.,2008)

In terms of anti-HIV/AIDS medication, children are the most difficult demographic to treat. (Diego A Chiappetta et al.,2010) Only 10% of infected children have appropriate access to ARVs and Most pediatric patients will probably succumb to the disease within the first two years of life. The lack of effective medications for pediatric HIV is one of the major challenges.(Diego A Chiappetta et al.,2011)Adult-approved ARV medications have no equivalent pediatric formulations and are solely offered in solid form. Pediatric pharmacotherapy requires liquid formulations since children less than 7 are frequently unable to take solid drugs. Pediatric pharmacotherapy requires liquid formulations since children less than 7 are frequently unable to take solid drugs. (Alejandro Sosnik., 2009) In the pediatric treatment cocktail, efavirenz is a first-line drug and it is a poorly aqueous soluble non-nucleoside reverse transcriptase inhibitor used in the pharmacotherapy of children over the age of 3 years old.(Diego A Chiappetta et al.,2010). Modern medicine is being revolutionized by the multidisciplinary science of nanotechnology. It has the potential to significantly advance HIV/AIDS therapy and prevention.

Amphiphilic copolymers can generate polymeric micelles, which are potential drug delivery systems. Poorly soluble medications can be made more soluble inside the hydrophobic inner

core of polymeric micelles, which have a core-shell structure. Micelles can thereby significantly increase the solubility and bioavailability of a variety of hydrophobic medicines. Numerous pharmaceutical applications, including medication and gene delivery systems, as well as diagnostic imaging as carriers for different contrasting agents, have explored the use of polymeric micelles. (Kyung T. Oh.,2004 & Lingbing Li et al.,2008) Amphiphilic protein casein self-organizes into core-shell micelles and act s as effective nanocarriers for oral drug delivery. About 36% of the caseins in cow milk are the calcium-sensitive phosphoprotein known as bovine -casein. At neutral pH and below the isoelectric pH (5.3) casein exhibits an amphiphilic property. The N-terminal region is strongly polar and negatively charged, whereas the C-terminal domain is extremely hydrophobic at the natural milk pH (6.6). A milk protein called casein is considered to be risk-free and will naturally release the drugs at the site of action. (Dganit Danino et al.,2012)

METHODOLOGY:

COMPACTIBILITY STUDIES:

Determination of lambda max (λ max) of Efavirenz using UV spectrophotometer: With appropriate dilution of the standard stock solution (100µg/ml) with 1% SLS with PBS pH 7.4, the solution was scanned using the double beam UV spectroscopy in the spectrum mode between the wavelength ranges of 400 nm to 200 nm. The λ max of Efavirenz was found to be 246nm, which was selected as the analytical wavelength for further analysis.

Construction of calibration curve of Efavirenz using UV spectrophotometer at 246 nm: 10 mg of pure drug diluted to 100 ml by using 1% SLS with PBS pH 7.4 to produce a concentration of 100 µg/ml which is a standard stock solution and it is sonicated for 15 min. From this 20 ml was taken and made up to 100 ml of buffer to give the concentration of 20 µg/ ml. The stock solution was further diluted to get the different concentrations (2,4,6,8,10,12,14,16,18, and 20µg/ml) to determine the linearity range. The standard sample were analyzed at 246 nm using UV spectrophotometer.

Identification/ purity of the drugs by Fourier Transform Infrared Spectroscopy (FR-IR): To investigate the possible interactions between drug and excipient were performed for the formulation. FT-IR spectrum of pure drug (Efavirenz), excipient (TPGS, pluronic F68, pluronicF127, CASEIN) were obtained. The FT-IR spectrum of Efavirenz were analyzed using FT-IR 8400S (CE) shimadzu spectrophotometer. The samples were prepared as KBr

pellets by compressing at 6 ton/nm2 pressure. The wavelength ranges were selected between 400-4000 cm-1 in Perkin Elmer FT-IR spectrophotometer. The IR spectrum of pure drug sample was compared with reference spectrum. Similarly an IR peak was obtained for physical mixture of Efavirenz and TPGS, pluronic F68, pluronic F127, and casein.

SOLUBILITY STUDIES: Efavirenz (5mg) is added to 1.5ml of various mediums like water, 0.5-2% SLS in water, pH 7.4, 0.5-2% SLS in pH7.4, 0.1N Hcl, 0.5-2%SLS in 0.1N Hcl and kept in thermo mixer maintained at 370C for 1hr. Samples are then analyzed spectrophotometrically at 246nm.

FORMULATION OF EFAVIRENZ LOADED NANOMICELLES

Isolation of casein: Take 50 mL milk in a 100 mL beaker and Heat the solution on a hot plate to bring the temperature to 55°C, control the temperature with thermometer. Add drop-wise the 10% acetic acid solution while stirring. Keep the beaker on the hot plate until the liquid is transparent and the casein stops precipitating. Collect the casein with suction filtration and then add 1:1 ethyl ether - ethanol solution this mixture to the casein precipitate. Stir the solution and collect the casein by suction filtration and allow it to dry for three days.

Fig.1 Image of prepared casein

PREPARATION OF NANOMICELLES

Sonication Technique: In this method, drug and polymers like TPGS, pluronic F68, Pluronic F127, casein are dissolved in phosphate buffer ph 7.4 containing 1% SLS. Then it is kept in bath Sonicator for half an hour. The size of the micelle formulation is decreased to desire extent using sonication. (sonication for 30 min).

Homogenization Technique: After sonication the formulations were carried out in high speed homogenizer in 15,000 rpm for 10 mts for each formulations to get a desire size range of less than 400 nm. On whole 12 formulations of Efavirenz loaded nanomicelles were prepared with various polymers. Table 1 displays the components of several formulations.

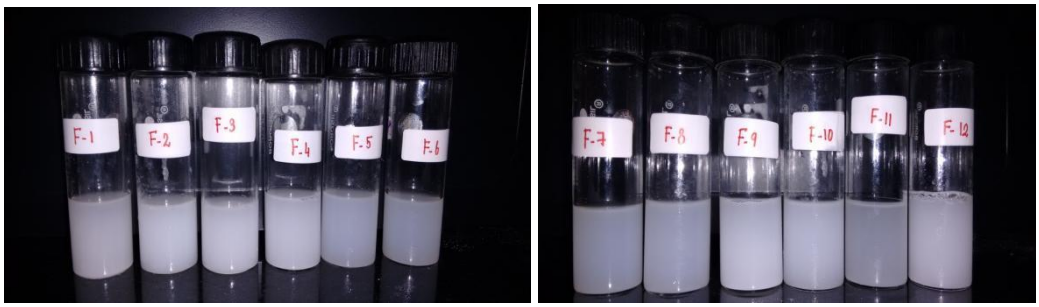

Fig.2. Images of Nanomicelles formulations

Table.1 Composition of Nanomicelles formulations

Code	Drug (mg)	Polymer (mg)				PBS solution (pH 7.4)	Sonication time (min)	Homogenization (min)
		Casein	TPGS	F-68	F-127			
F1	50	50	-	-	-	10	30	10
F2	50	75	-	-	-	10	30	10
F3	50	100	-	-	-	10	30	10
F4	50	-	50	-	-	10	30	10
F5	50	-	75	-	-	10	30	10
F6	50	-	100	-	-	10	30	10
F7	50	-	-	50	-	10	30	10
F8	50	-	-	75	-	10	30	10
F9	50	-	-	100	-	10	30	10
F10	50	-	-	-	50	10	30	10
F11	50	-	-	-	75	10	30	10
F12	50	-	-	-	100	10	30	10

CHARACTERISATION OF NANOMICELLES:

Determination of particle size by photon correlation spectroscopy: The average mean diameters and size distribution of nanomicelles was found out by photon correlation

spectroscopy using Zetasizer (nano ZS90, Malvern instruments) at 25°C. The samples were kept in polystyrene cuvette and the readings were noted at a fixed angle.

Determination of zeta potential: The electrophoretic mobility (zeta potential) measurements of nanomicelles were made using Zetasizer (nano ZS90, Malvern instruments). The sample were placed in a polystyrene cuvette (at 25°c) and zeta dip cell was used to find out the potential.

Freeze drying: Freeze drying was performed to convert aqueous nanosuspension into powder. Best formulation was kept in freezer for 4 hrs. Then the formulation were freeze dried using LYODOL freeze drier at the temperature of -40oC and -1.12 mbar pressure for 48 hrs. The lyophilized formulations were used for the further characterization studies. (Nakrani et al.,2010)

SEM analysis: Scanning electron microscopy analysis of lyophilized Nanosuspension was carried out to confirm the Nano-size of formulation. The sample-containing stub was put into a scanning electron microscopy chamber to study the surface morphology.

EVALUATION OF EFAVIRENZ NANOMICELLES

Entrapment efficiency of nanomicelles: The amount of Efavirenz entrapped within various nano micelle formulations was determined by centrifugation at 2000 rpm for 45 min to separate the untrapped aliquot. The supernatant was measured for drug amount by UV spectroscopy.

***In vitro* drug release studies:** The nanomicelle formulations (1ml) were placed in dialysis membrane where both ends of the membrane are sealed. The membrane with formulation is suspended in a beaker containing 100 ml phosphate buffer solution; pH 7.4 at 37°C containing 1% of SLS. The buffer solution was stirred with glass rod at 45mins interval. The samples were collected at 30min,1, 2, 4, 8, and 12 hrs interval and replaced with equal quantity of fresh buffer solution. The collected samples were analysed for drug release using UV spectroscopy.

Stability studies: Stability studies were carried out for formulation and the storage conditions was selected as per ICH guidelines. The formulations were stored at refrigerated condition (5°C±3ºC) and room temperature (25ºC ± 2ºC/60 %RH). At predetermined sampling time points, the samples were analyzed for changes in physicochemical properties

that are likely to change during long term storage and are likely to influence the quality , safety and efficacy of the product. The physicochemical properties are studied which include physical appearance, particle size, zeta potential.

Drug content: 50 mg of nanoparticles was dissolved in 50 ml of phosphate buffer using bath Sonicator. Then, the solution was filtered through the filter medium. The filtered solution was analysed for the drug content by UV spectroscopic method.

RESULTS AND DISCUSSION:

DETERMINATION OF LAMBDA MAX (λ max) 0F EFAVIRENZ USING UV SPECTROPHOTOMETER: The maximum absorption of Efavirenz was found to be 246 nm after scanning the stock solution of Efavirenz (100µg/ml) using UV- spectrophotometer. The r2 value was found to be 0.9808.

FT-IR SPECTROSCOPY: Drug excipients study was checked by comparing the IR spectra of pure drug, excipients and physical mixture of drug and excipients. The obtained result shows that the functional group of efavirenz was found to be remain intact. Therefore, there is no interaction between drug and polymers.

DETERMINATION OF PARTICLE SIZE BY PHOTON CORRELATION SPECTROSCOPY The particle size of the obtained nanomicelles using homogenization method ranged from to 146.9±167.0 to 2926±468.2 nm. The particle size of formulations F1, F2 and F3 was less than 300 nm when compared to that of formulations F4, F5, F6, F7, F8, F9, F10, F11, F12 which was ranging between 700 to 3000 nm. The particle size of the prepared formulations is given in table 2.

Table. 2. Particle size, Polydispersity index and zeta potential of formulated Nanomicelles

FORMULATION CODE	AVERAGE PARTICLE SIZE (nm)	POLYDISPERSITY INDEX (PDI)	ZETA POTENTIAL(mV)
F1	286.5±127.5	0.259	-31.4
F2	243.8±349.6	0.493	-29.9
F3	146.9±167.0	0.467	-37.7
F4	1187±183.4	0.319	-1.46
F5	2926±468.2	0.178	0.989
F6	1558±350.7	0.175	1.25
F7	915.0±133.5	0.601	-13.2
F8	382.3±67.57	0.188	0.543
F9	908.9±108.8	1.000	-0.0428
F10	816.9±124.3	0.717	0.862
F11	935.1±114.6	1	0.291
F12	756.7±146.2	0.123	1.42

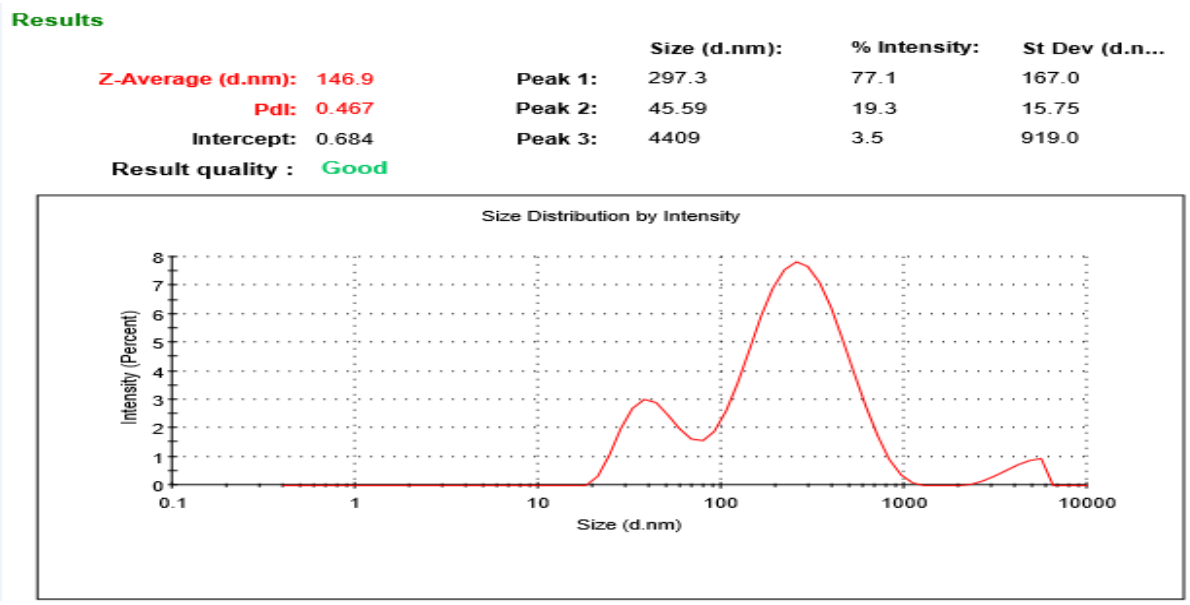

Fig.3: Particle size of optimized formulation F3

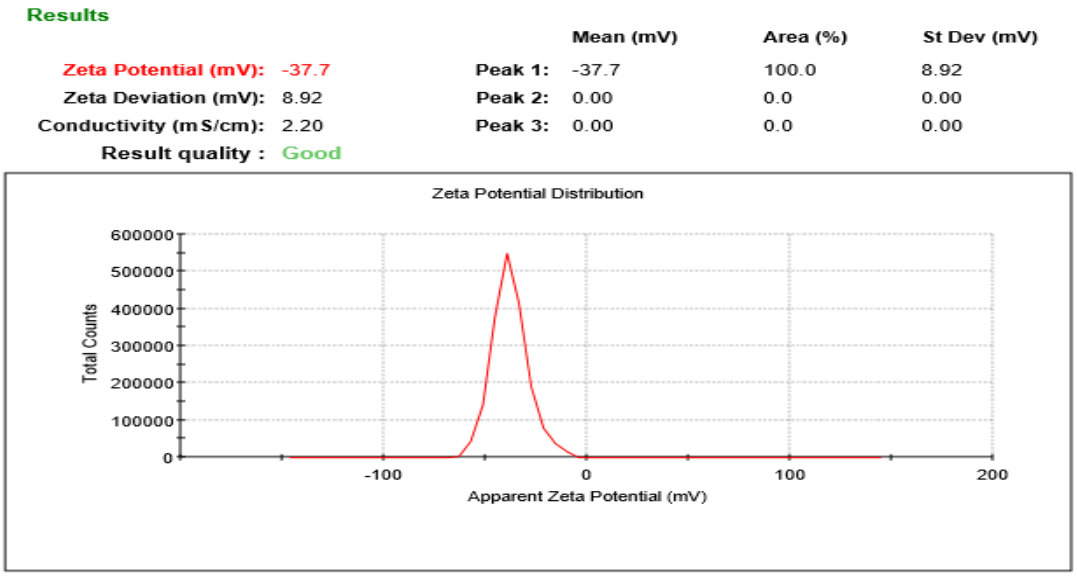

Fig.4: Zeta potential of optimized formulation F3

DETERMINATION OF ENCAPSULATION EFFICIENCY

The entrapment efficiency of the prepared nanomicelles reveals that the drug has been entrapped resulting in moderate entrapment efficiency. The result shows that the EE was in between 30% (lowest) for formulation F12 to 89.4% (highest) for formulation F1.

DETERMINATION OF DRUG CONTENT:

Drug content per ml of all the 12 formulations were determined. 10mg of formulation was dissolved in phosphate buffer and the solution is measured spectrophotometrically at 246 nm. Formulation F1, F2, F3 showed least particle size (286.5, 243.8, 146.9 nm), good zeta potential (-31.4, -29.9, -37.7 mV) and highest entrapment efficiency (89.4%, 86.8%, 87.2%). Therefore, these formulations were selected for further study.

Table.3. Entrapment efficiency and Drug content of prepared nanomicelles

FORMULATION CODE	ENTRAPMENT EFFICIENCY (%)	DRUG CONTENT (mg/ml)
F1	89.4%	4.45
F2	86.8%	3.52
F3	87.2%	3.03
F4	70%	4
F5	64%	3
F6	54%	2.13
F7	80%	4.3
F8	83%	3.56
F9	85.2%	2.98
F10	66%	3.97
F11	42%	2.19
F12	30%	1.3

IN VITRO DRUG RELEASE STUDIES

The in vitro drug release studies were performed using dialysis bag method in PH 7.4 phosphate buffer. Formulation (1ml) was taken in the dialysis membrane which is placed in 100 ml of 7.4 phosphate buffer containing 1% SLS and samples were withdrawn at regular intervals of time for measuring drug concentration. All the 3 formulations (F1, F2, F3) have shown that more than 30 % of drug release in 30 min. This may be due to the burst release of drug in the formulation. Further, the effect of casein concentration influences the release of drug from the nanomicelle formulation. As the concentration of casein was increased, the release rate of the drug get increased. After 12 hrs F1, F2, F3 showed 63.01, 81.46, 83.04 % respectively. The drug release pattern showed sustained release manner from 60 min to 24 hrs. The maximum drug release was 83.04 % from F3 and the minimum release of 63.01% was obtained from F1. Thus, the obtained in vitro drug release data will be correlated with in vivo drug release of suitable animal model.

Table.4. Cumulative percentage drug release of Nanomicelles formulation

TIME (min)	F1	F2	F3
0	0	0	0
15	25.02	28.02	40.05
30	32.34	33.18	54.53
60	39.05	39.04	60.17
120	42.33	46.03	63.03
240	46.12	62.01	67.03
360	52.34	66.03	69.16
480	55.52	69.03	70.07
1440	63.01	81.46	83.04

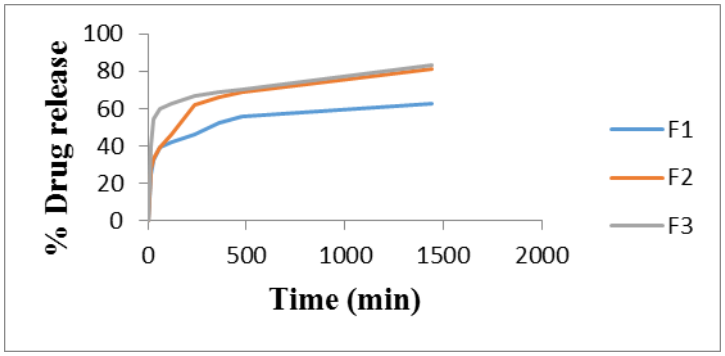

Fig.5: Percentage drug release of nanomicelles

Solubility study:

The solubility of the efavirenz was found to be higher in SLS solution while compared to water and 0.1N HCl.

Table .5. Solubility profile of efavirenz

Drug	Solvent	Concentration
EFAVIRENZ	1% SLS in phosphate buffer 7.4 pH	4.125

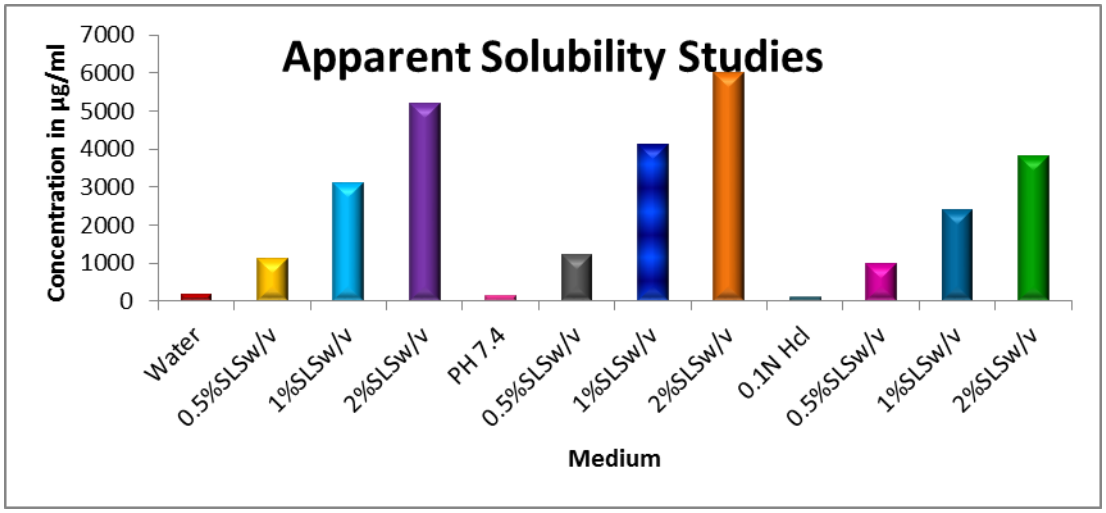

Fig.6: Graphical representation of solubility of drug in different solutions

STABILITY STUDIES OF NANOMICELLES:

Stability studies were carried out for formulation and the storage conditions was selected as per ICH guidelines. The formulations were stored at refrigerated condition (5ºC±3ºC) and room temperature (25ºC±2ºC/60 %RH). At predetermined sampling time points, the samples were analyzed for changes in physicochemical properties that are likely to change during long term storage and are likely to influence the quality, safety, and efficacy of the product. The physicochemical properties are studied which include particle size, zeta potential, and entrapment efficiency.

Particle size

Increase in particle size was noticed for the formulation stored in room temperature from nm to nm in the period of one month when compared to the refrigerated condition (5ºC±3ºC). There isn't much increase in the formulation stored in refrigerated temperature which confirms that they are stable. The zeta potential i.e., surface charge is not affected much in both the storage conditions and remained almost same as shown in the table 15 below.

Table.6. Stability study of nanomicelles

Initial day of formulation		**After 1 month (room temperature) (25ºC±2ºC/60 %RH)**		**After 1 month (refrigerated temperature) (5ºC±3ºC)**	
Particle size (nm)	Zeta potential (mV)	Particle size (nm)	Zeta potential (mV)	Particle size (nm)	Zeta potential (mV)
172.9	-34.3	243.9	-30.3	193.4	-32.4

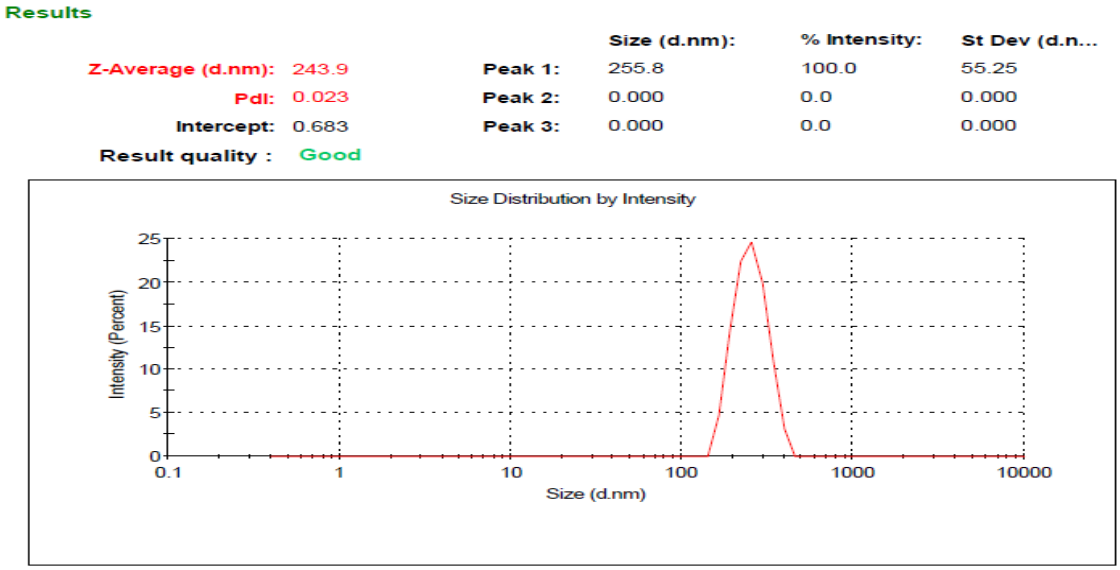

Fig.7: Particle size of nanomicelles in room temperature

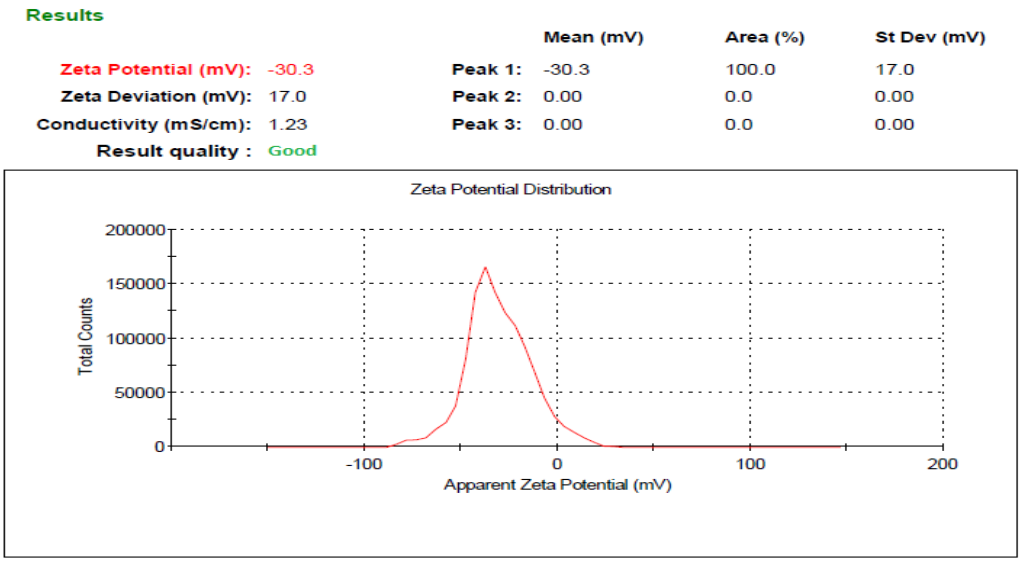

Fig.8. Zeta potential of nanomicelles in room temperature

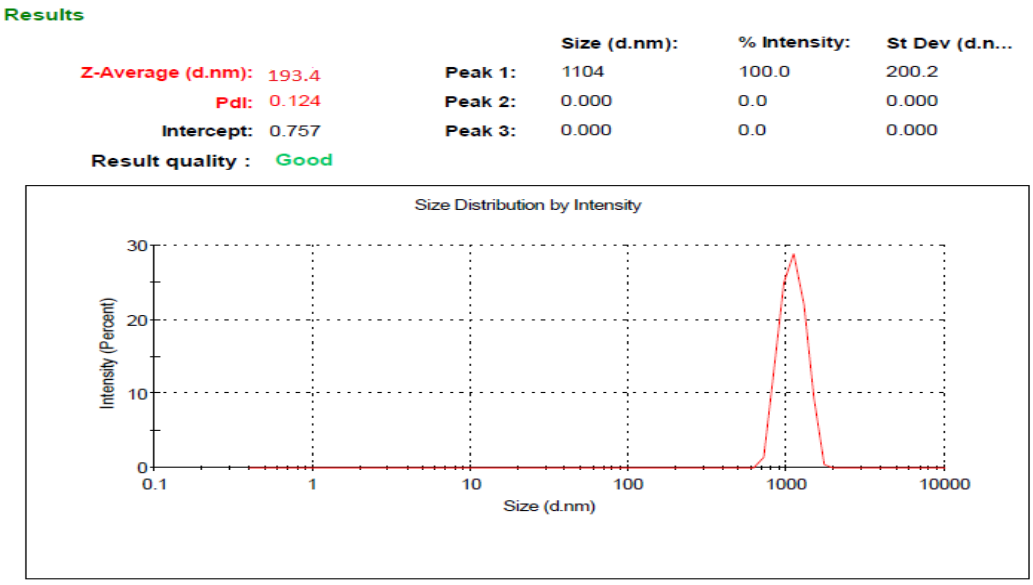

Fig.9: Particle size of nanomicelles in refrigerated condition

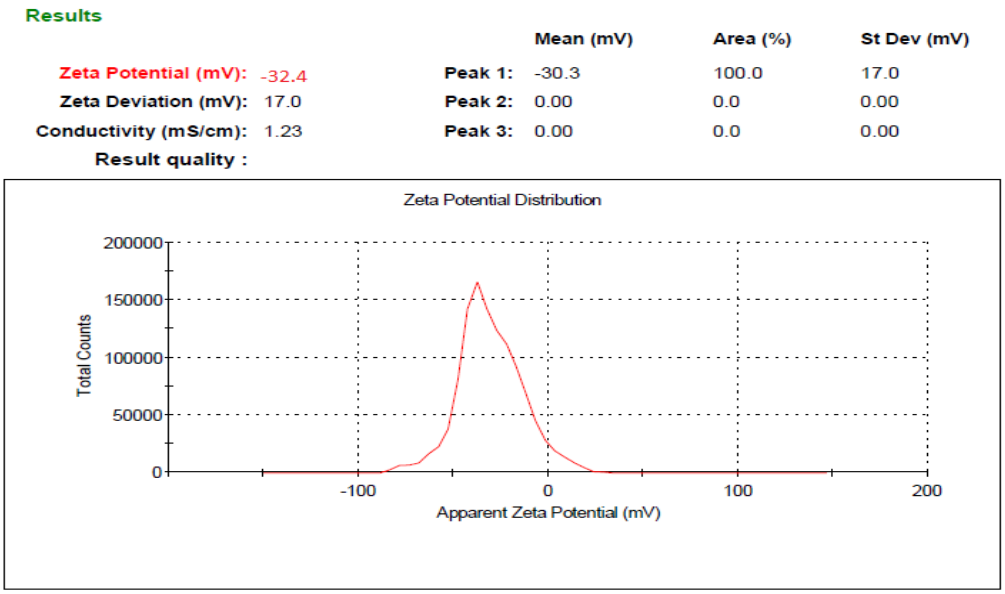

Fig.10: Zeta potential of nanomicelles in refrigerated condition

DETERMINATION OF PARTICLE MORPHOLOGY BY SCANNING ELECTRON MICROSCOPY (SEM):

The morphology of the nanomicelles using casein was found out by scanning electron microscope (SEM). SEM image in figure reveals that the particles are in micelle shape with rough surface.

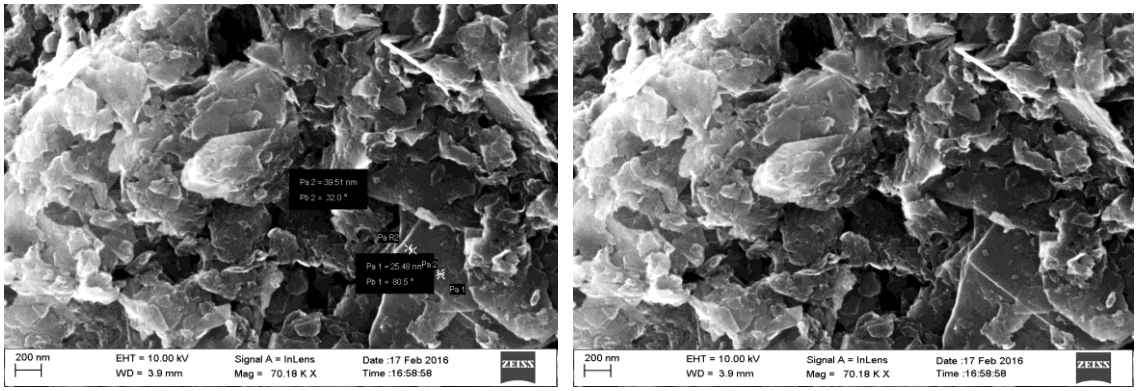

Fig.11: SEM image of efavirenz loaded nanomicelle formulation

CONCLUSION:

This research study aimed to formulate Efavirenz loaded nanomicelles to enhance the solubility. Twelve batches of efavirenz loaded nanomicelles was prepared by sonication method following homogenization method by varying the concentration of polymers. Intially prepared nanomicelles were evaluated for particle size, Zeta potential, Entrapment efficiency. Then best three formulation F1, F2, F3 with least particle size and high entrapment efficiency were selected for In vitro release studies. Considering overall parameters formulation F3 with a particle size of 146.9 ± 167.0 nm and 87.2 % of the drug in nanoparticles was chosen as the best nanomicelle formulation for liquid oral preparation. Thus it can be concluded that efavirenz loaded nanomicelles possesses promising future in delivery of oral formulation of drugs for pediatrics.

REFERENCES:

1. Anthony S. Fauci,1988; The Human Immunodeficiency Virus: Infectivity and Mechanisms of Pathogenesis; 10.1126/science.3277274.
2. R. Gurnya, F. De Jaeghere, 2000; Oral bioavailability of a poorly water soluble HIV-1 protease inhibitor incorporated into pH-sensitive particles: effect of the particle size and nutritional state; 10.1016/s0168-3659(00)00272-8.
3. Tewodros Mamo, E Ashley Moseman, Nagesh Kolishetti, 2010; Emerging nanotechnology approaches for HIV/AIDS treatment and prevention; https://doi.org/10.2217%2Fnnm.10.1
4. Diego A Chiappetta, Christian Hocht, 2010; Efavirenz-loaded polymeric micelles for pediatric anti-HIV pharmacotherapy with significantly higher oral bioavailability; 10.2217/nnm.09.90.
5. Viviana Simon, David D Ho, 2006; HIV/AIDS epidemiology, pathogenesis, prevention, and treatment; 10.1016/S0140-6736(06)69157-5
6. Diego A. Chiappetta, Graciela Facorro, 2011; Synergistic encapsulation of the anti-HIV agent efavirenz within mixed poloxamine/poloxamer polymeric micelle; 10.1016/j.nano.2011.01.017
7. Ali Akbar Moosavi-Movahedi,Mansoore Esmaili, S. Mahmood Ghaffari, 2011; Beta casein-micelle as a nano vehicle for solubility enhancement of curcumin food industry application; 10.1016/j.lwt.2011.05.023
8. Dganit Danino, Michal Bachar a , Amitai Mandelbaum, 2012; Development and characterization of a novel drug nanocarrier for oral delivery, based on self-assembled β-casein micelles; 10.1016/j.jconrel.2012.01.004.

9. Alejandro Sosnik , Diego A. Chiappetta, 2009; Drug delivery systems in HIV pharmacotherapy: What has been done and the challenges standing ahead; 10.1016/j.jconrel.2009.05.007.
10. Kyung T. Oh, Tatiana K. Bronich, Alexander V. Kabanov, 2004; Micellar formulations for drug delivery based on mixtures of hydrophobic and hydrophilic PluronicR block copolymers ; 10.1016/j.jconrel.2003.10.018
11. Lingbing Li, Ye Bang Tan, 2008; Preparation and properties of mixed micelles made of Pluronic polymer and PEG-PE; 10.1016/j.jcis.2007.09.053
12. Carlo Giaquinto, Erika Morelli, Federica Fregonese, 2008; Current and Future Antiretroviral Treatment Options in Paediatric HIV Infection; 10.2165/00044011-200828060-00005.