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DEVELOPMENT OF FORMULATION AND EVALUATION OF ZIDOVUDINE ORAL NANOSUSPENSION

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ABSTRACT

The aim of the present research was to development of formulation & evaluation of Zidovudine oral Nanosuspension. Zidovudine is a potent in vitro inhibitor of human immune deficiency virus belongs to the category of anti-retro viral drug. Zidovudine has the short biological half life and poor bioavailability due to solubility problems. The solubility of Zidovudine in various solvents, surfactants and cosurfactants were checked to optimize the components of Nanosuspension. The Nanosuspension was formulated by using methanol as an organic solvents and water, as the anti-solvent. Poloxamer-188, HPMC K-30, Eudragit S 100, PVP K-30, used to be polymers, Tween-80 as a surfactant prepared by High pressure homogenization technique. The formulated Nanosuspension were subjected for various evaluation parameters like particle size & shape, drug content, SEM, zeta potential, viscosity, saturation solubility, In vitro release, treatment of kinetic data & stability studies. Particle size, polydispersity index and zeta potential of optimized formulation were found to be 826.5 d.nm, 0.283pdi, 4.79(mv) so it is the stable one .On the basis of *in vitro* release study the formulation F2 & F1has been shown the highest amount of drug release respectively 98.06%, 97.35% based on the n value 0.5240 it was follows the Non- fickian diffusion mechanism. The stability studies we also conducted in two different on room temperature and refrigerator temperature it showed that the refrigerator is the more suitable for storage of nanosuspension. These results demonstrate the potential use of Nanosuspension for improving the bioavailability of poor water soluble compounds like Zidovudine.

Key Words:- Zidovudine; high pressure homogenizer; oral route of nanosuspension; SEM; zeta potential.

INTRODUCTION

The advances in pharmaceutical research work, there are thousands of new compounds are synthesized every year but, 40% of these compounds show solubility problem which further makes their processing difficulties. Poor aqueous solubility not only produces irreproducible therapeutic response but also leads to the wastage of large amount of drugs (Sarita Kumari Yadav *et al*., 2012) These are poor water soluble drugs having a criteria slow drug absorption it leads to the inadequate and variable bioavailability and it show the gastrointestinal mucosal toxicity. For orally administered drugs solubility is the most important one to achieve their desired concentration in systemic circulation for pharmacological response. Problem of solubility is a major challenge for formulation exports. The poor solubility and low dissolution rate in gastrointestinal fluids often cause insufficient bioavailability. It is a major problem for those drugs belonging to the biopharmaceutical classification system (BCS) classes II and IV. As for the BCS class II drugs are the rate limiting step is drug release from the dosage form and solubility in the gastric fluid so that it leads the absorption problem in GIT fluids, that's why increasing the solubility in turn increases the bioavailability for BCS class II drugs, various techniques are employed. They are chemical modifications and physical modification of the

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drug substance, and other techniques. Chemical Modifications, use of buffer, derivatization, complexation salt formation, and Change of the PH. Miscellaneous Methods Supercritical fluid process, use of adjuvant like, cosolvency, hydro trophy, Solubilizer surfactant and novel excipients. Physical Modifications Particle size reduction like nanonisation and Micronization, modification of the crystal habit like co crystallization, amorphous form and polymorphs, drug dispersion in carriers like eutectic mixtures, solid dispersions, solid solutions and cryogenic techniques.

Main limitation to therapeutic effectiveness of Zidovudine is sparingly soluble in water its dose dependent toxicity, short biological half-life and les bioavailability. Thus, to overcome the problem Nanosuspension was prepared.

Zidovudine is a potent in vitro inhibitor of human immunodeficiency virus (HIV) with varying efficacy against other retroviruses. The exception of Epstein Barr virus, Mechanism studies show that Zidovudine is phosphorylated to the monophosphate and diphosphate derivatives by the host cell cytosolic thymidine kinase and thymidylate kinase, as follow. The identity of the enzyme that phosphorylated Zidovudine diphosphate is didn't known, but is believed to be the cellular nucleoside diphosphate kinase. The triphosphate of Zidovudine appears has to be active form of the drug. Zidovudine triphosphate has competes well with thymidine 5-triphosphate for binding to the HIV reverse transcriptase and also functions as an alternative to the substrate. Incorporation of Zidovudine monophosphate led's of chain termination.

A pharmaceutical nanosuspension it may be defined as the very finely colloid Biphasic, dispersed, solid drug particles in an aqueous vehicle. The particle size below the 1μm, without any matrix material, it stabilized by the with help of the surfactants and polymers, prepared by suitable methods for Drug Delivery applications, through various routes of administration like, topical, ocular, parenteral, pulmonary routes, and oral. The nanosuspension not only solves the problem of poor solubility and bioavailability but also alters the pharmacokinetics of drug and that improves drug efficacy and safety. In a nanosuspension technology, the drug is maintained in the required crystalline state with reduced particle size, leading to an increased the dissolution rate and therefore to maintain the bioavailability of drugs for this purpose high pressure homogenization technique is selected (Ethiraj T *et al*., 2013).

MATERIALS

Zidovudine as a gift sample from Hetero pharmaceutical Pvt limited, Hyderabad, HPMC K-30, Tween-80, Methanol was purchased from Himalaya pharmaceutical Nellore, PVP K-30 was purchased from drug India Hyderabad, Poloxomer-188, Eudragits-100 was gift sample from Thorab pharma & research laboratory, Puducherry.

METHODS

Solubility study

The solubility of Zidovudine in 10 mg/10 ml of solvent was carried out with different solvent.

Melting point

Melting point of Zidovudine was determined by taking a pinch of the drug into a capillary tube, closed at one end. It was placed in glycerin, the temperature of the glycerin is gradually increased until the drug is melted at certain temperature it was noted as the melting point of the Zidovudine drug.

Drug and excipients interaction (FTIR) study

The compatibility between pure drug and polymers like HPMC K-30, PVP K-30, poloxomer-188, Eudragit S100 were detected by FTIR spectroscopy (Bruker Pvt. Ltd, Germany). The potassium bromide pellets were prepared on the KBr press. To prepare the KBr pellets the solid powdered sample were ground together in a mortar with 100 times quantity of KBr. The finely grounded powder was introduced into a stainless steel die. The powder was pressed in the die between polished steel anvils at a pressure of about 10t/in2. For liquid samples thin film of sample liquid is made on pellet76. The spectra were recorded over the wave number of 8000 cm-1 to 500 cm -1.

Preparation method of Nanosuspension

High pressure homogenization

The process can be explained in three steps they are:

Step 1: Drug was dissolved in methanol to prepare an organic solution and fixed Amount as given in table no-1 the polymers and surfactant are dissolved in mentioned quantity of water it is the aqueous phase,

Step 2: The aqueous water is kept under high pressure homogenizer (Remi RQ-127) on room temperature,

Step 3: The organic solution is added drop wise through syringe to aqueous solution. Under the process of high pressure homogenizer at rotation speed of 100 Rpm up to the 8 hours Nanosuspension was formed, the organic solution was evaporated under room temperature.

Evaluation parameters

Scanning Electron Microscopy

The shape and surface morphology of Nanoparticles were investigated using scanning electron microscopy (SEM) Iact Iso cell Hyderabad. The samples for SEM study were prepared by lightly sprinkling the formulation on a double adhesive tape stuck to an aluminum stub. The stubs were then coated by the gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in a high vacuum generating evaporator. The gold coated samples were then properly scanned and photomicrographs were taken randomly with a scanning electron microscope (Vijay Kumar Singh *et al*., 2014).

Measurement of Particle Size

The particle size was measured by photon correlation spectroscopy (PCS) using a Malvern instrument Hyderabad.

Zeta Potential of Nanosuspension

The prepared Nanosuspension was to isolate the 5ml of sample consisting 100 µg it was diluted with the 5 ml of double distilled water and Zeta potential of the diluted dispersions was measured using Malvern instrument Hyderabad. Sign of charge on the drug particles and their mean Zeta Potential values were obtained from the instrument. (Sandhya J. *et al*., 2013).

Viscosity

Viscosity of the Nanosuspension was performed by the using the Brookfield viscometer set the spindle no-60 at 100rpm Nanosuspension was kept under the Brook field viscometer on room temperature the values are displayed on digital meter obtained values was noted.

Saturation Solubility Studies

Saturation solubility measurements were carried out through UV absorbance determination at 268 nm using UV-Visible spectrophotometer (model UV-2600plus). The saturation solubility studies were done both the unprocessed pure drug and different batches of the lyophilized Zidovudine Nanosuspension. By the way for 10 mg of the unprocessed pure drug compound and Nanosuspension equivalent to 10 mg were weighed and measured separately and introduced into 25 ml Stoppard conical flask Containing 10 ml distilled water. The flasks were sealed and placed in rotary shaker (RemiRQ-127A) for 24 hrs at the room temperature and equilibrated for 2 days. The samples were collected after the specified time period of intervals, and it is filtered through the filter paper and then analyzed. The diluted samples were analyzed using UV spectrophotometer at 268nm (Ethiraj T *et al*., 2013).

Drug content

0.5ml of each one preparation were utilized for the purpose of dissolve in 10ml isotonic solution and kept overnight and also 10 mg of drug were utilized for the purpose of dissolved in 10 ml of the isotonic solution and kept overnight. From that all preparations along with the drug were filtered dilutions made in the concentration of one microgram per millimeter. These dilutions were estimated their content uniformity with the help of UV spectrophotometer at the wave length 268nm. The spectrophotometer produced the absorbance, based on that the calculations were made with the help of calibration curve consisting slope and intercept values (Neha Tyagi *et al*., 2011).

***In vitro* release study**

The *In vitro* release studies were preferred for all the formulations by the way of utilizing the USP category II dissolution equipment under the following circumstances.

Dissolution medium 900ml of 0.1N HCL rotating speed is50 rpm Temperature kept constant at 37 ± 0.5°C sampling with drawing time is followed 1 to 8 hours at programmed time interval aliquot samples (5ml) were collected and replenish by the same quantity of fresh medium. The aliquot sample (5 ml) was filtered with the help of 0.45 µm restricted membrane filter paper and the filtrate was to be diluted properly through the fresh medium and was predictable using UV-Vis spectrophotometer (model UV-2600plus) at wave length 268 nm (Sandhya J *et al*., 2014).

Treatment of dissolution data with different kinetic model

The quantity of drug released from Zidovudine Nanosuspension was analyzed by the way of the square root of point in time must be performed with a flexible model that can identify the contribution to overall kinetics, an equation proposed by Ritger and Peppas. For finding out the mechanism of drug release from the Zidovudine Nanosuspension

Stability Study of Nanosuspension

The stability studies for Nanosuspension were performed at the two different storage conditions for 90 days as follows:

1. Room temperature
2. Refrigerated temperature (2-8˚c)

Optimized batch F2 Nanosuspension was utilized for each condition, the particle size, physical appearance dissolution studies and drug content are the most important parameter for the activity & physical stability of any nanosized formulations.

RESULT AND DISCUSSION

Preformulation Studies

Solubility study: The solubility of Zidovudine was carried out of 10 mg of drug in 10 ml of different solvents like ethanol, methanol, and water it was revealed that it was freely soluble in ethanol, methanol, but sparingly Soluble in water.

Melting point of Zidovudine: Melting point of Zidovudine was found to be113-115°C.

Drug and excipients interaction (FTIR) study: The FTIR studies reviled that there was no interaction between the drug and polymers.

EVALUATION OF NANOSUSPENSION

Estimation of % saturation solubility drug in different formulations: The saturation solubility of drug and Nanosuspension was performed in a 10 ml distilled water in this result formulation F2 so the more amount of drug saturation solubility 96% of drug compared to the pure un processed drug and other formulations.

Drug content: The % Drug content of Zidovudine Nanosuspension was performed in this result F2 show the drug content range is 86% to 97% it is the maximum drug content This indicated that the F2 formulation was considered to be the best formulation.

Viscosity measurement: The Brook field viscometer to determine the viscosity of Zidovudine Nanosuspension of different formulations was measured they show the result within the satisfactory limits.

Particle size, poly dispersivity index and Zeta potential measurement

Polydispersity and zeta potential are the impotent evaluation parameters are responsible for the stability of nanosuspensions. Polydispersity index give the degree of particle size distribution it ranged from the values 0.211 to 0.671. It depending on the formulation variables higher the value of Polydispersity index indicates the broad particle size distribution. A narrow in size distribution is essential step to prevent the crystal growth due to the Ostwald ripening and maintain the stability of the nanosuspensions which formulations have the lower the Polydispersity values showed the long term stability and it was preferred for the studies.

Particle size and poly dispersivity index measurements of different formulations was shown in the figure no 1 in this figure F2 formulation possess the less particle size as compare to other formulations. The formulation F2 had shown the average particle size value 826.5 d.nm with the 0.283 poly dispersity index so the F2 have poses the long term stability.

Zeta potential of the formulation F2 was showed in figure No2 It was showed the value 4.79(mv) which indicates the prepared formulation was stable.

Scanning electron microscopy

The Scanning electron microscopy scans the Zidovudine Nanosuspension it given the images at resolution of 500x, 1000x and 5000x this are showed in figure no 3 it was indicating the size and shape of the nanoparticles these are found to be spherical in shape, smooth surface and less aggregates.

In vitro drug release study was carried out over the Zidovudine Nanosuspension different proportion of HPMC K30, Eudragit s100 and Poloxamer-188 the effect of polymers was observed on drug release. From the observation it was found that F2 has shown drug release range of 38.34-98.6% among its proportions, F1 has shown drug release range of 35.16-97.35 among its proportions, these show the highest drug release among all Zidovudine Nanosuspension, comparison of drug release pattern of all Zidovudine Nanosuspension was showed in the Figure No-4

Treatment of dissolution data with kinetic model

Dissolution data of all formulations were subjected to the treatment of different kinetic equations it was found to be that the drug release pattern were best fitted with zero order release equation and involves combination of polymer relation. The 'n' value obtained with the application of Korsmeyer and Peppas equation was found to be 0.5240 for F2. This value indicates a non-Fickian release mechanism. The R^2 values was found in the range of 0.5240 to 0.9979 the corresponding the plot of ("cumulative % drug release vs time) for the first order equation indicated good linearity. The plot of the Higuchi's model was founded to be linear. The R^2 values were founded in the ranges of 0.5241 to 0.9954. The prepared formulation was followed the Higuchi's model.

The optimized Zidovudine Nanosuspension F2 was introduced to the Stability studies up to the 90 days at the Room temperature and Refrigerated temperature. It

reveals that the no physical changes like color, odor, taste and the physical appearance at the Room temperature and Refrigerated temperature. It show the effect on the particle in size at the Room temperature initially value of 277.3± 5.41 after 30 days it shows the particle in size 298.2 ±3.12, after 60 days it shows the particle in size 318 ± 3.15 after 90 days it shows the particle in size 347± 6.98 so it increase. It show the effect on the particle in size at Refrigerated temperature initially 277.3± 5.41after 30 days it show the particle in size 285.1 ± 2.11 after 60 days it show the particle in size 291.1 ± 2.13 after 90 days it show the particle in size 300.00 ± 1.21. it show the effects on Dissolution rate on room temperate initially 98.06% after 30 days it show the results 95.05 after 60 days it show the results 90.06 after 90 days it show the results 87.2% in refrigeration condition initially 98.06 % after 30 days it show the results 96.01 after 60 days it show the results 94.05 after 90 days it show the results 93.01 it show the effect on the drug content initially on room temperature it have the drug content 95% after 30 days it show the results 93% after 60 days it show the results 91% after 90 days it show the results 90% is compared to the room temperature and refrigerated temperature the Refrigerated temperature show the less increment in particles, Dissolution rate less decreases and drug content also less decreases so the ideal storage condition for the Nanosuspension is Refrigerated temperature.

Table 1. Formulation of oral nanosuspension

Ingredient	F1	F2	F3	F4	F5	F6
Zidovudine	2gm	2gm	2gm	2gm	2gm	2gm
Poloxomer-188	100mg	1500mg	-	-	-	-
HPMC k-30	-	-	100mg	1500mg	-	-
Eudragit	-	-	-	-	100mg	1500mg
PVP k-30	2gm	2gm	2gm	2gm	2gm	2gm
Tween-80	0.8ml	0.8ml	0.8ml	0.8ml	0.8ml	0.8ml
Methanol	50ml	50ml	50ml	50ml	50ml	50ml
Water	100ml	100ml	100ml	100ml	100ml	100ml

Table 2. *In vitro* drug release studies

Hours	Cumulative % drug release					
	F1	F2	F3	F4	F5	F6
1	35.6	38.34	30.90	36.05	19.6	20.5
2	48.96	49.60	46.80	55.78	30.5	35.7
3	58.67	59.40	52.90	64.62	46.0	48.23
4	65.60	67.90	65.70	78.40	50.5	55.30
5	78.96	79.40	76.72	84.64	55.5	59.55
6	87.27	89.70	85.40	89.01	60.0	67.40
7	90.68	95.30	88.36	90.50	75.6	78.6
8	97.35	98.06	91.40	93.01	85.1	87.2

Table 3. Stability study data after 90 days for F2

Stability condition	Parameters	Initial	After 30 days	After 60 days	After 90 days
Room temperature	Particle size	277.3± 5.41	298.2 ±3.12	318 ± 3.15	347± 6.98
	Appearance	No change	No change	No change	No change
	Dissolution	98.06%	95.05%	90.06%	87.2%
	Drug content	95%	93%	91%	90%
Refrigerator	Particle size	277.3± 5.41	285.1 ± 2.11	291.1 ± 2.13	300. ± 1.21
	Appearance	No change	No change	No change	No change
	Dissolution	98.06%	96.01%	94.05%	93.01%
	Drug content	95%	94%	93%	92%

Figure 1. Particle size and poly dispersity index measurements for optimized formulation

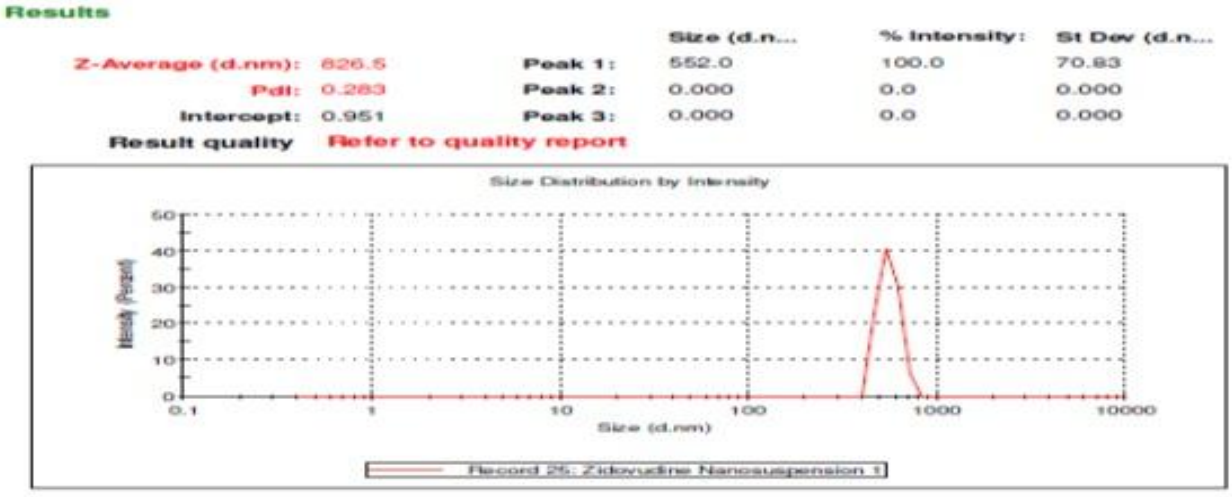


Figure 2. Zeta potential measurements for optimized formulation

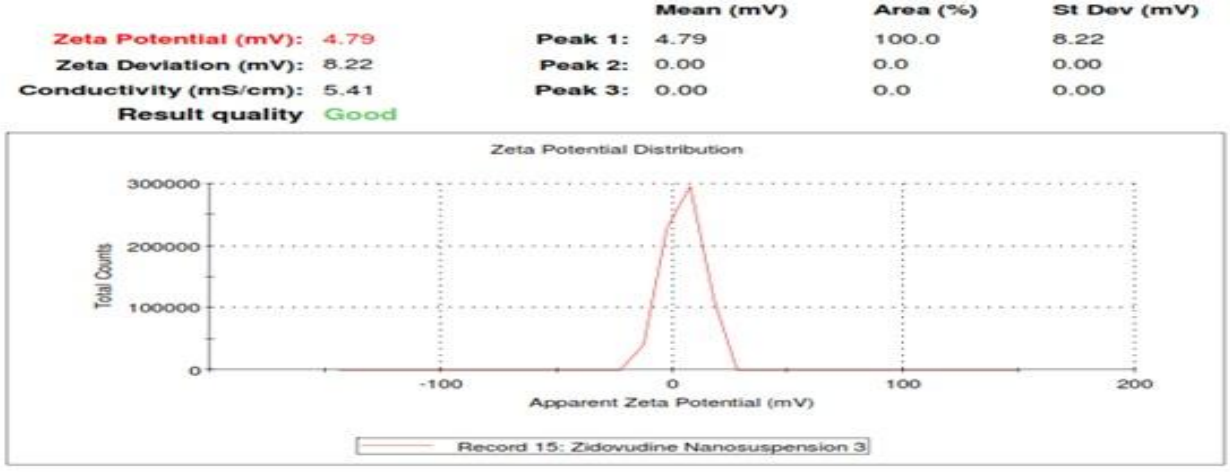


Figure 3. Scanning Electron microscopy image of optimized formulation

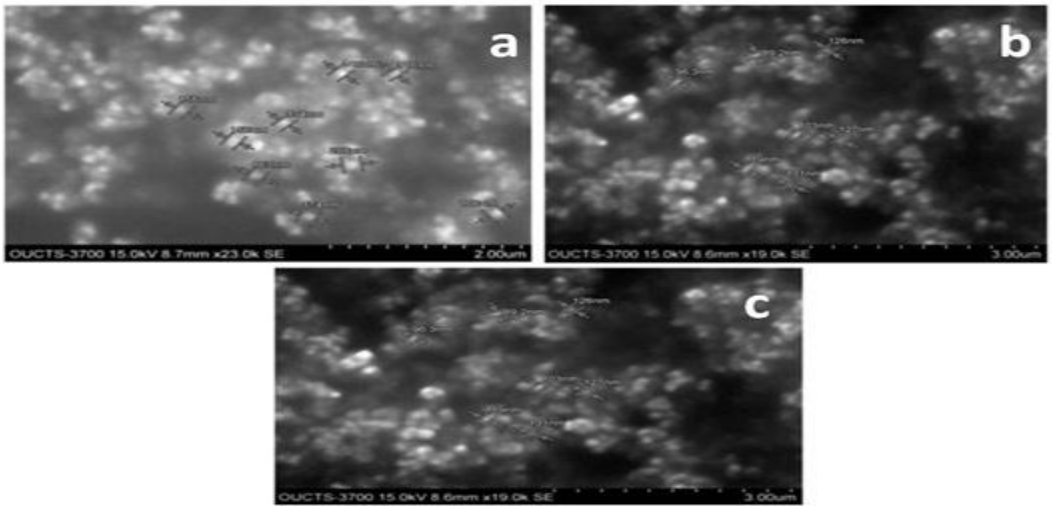
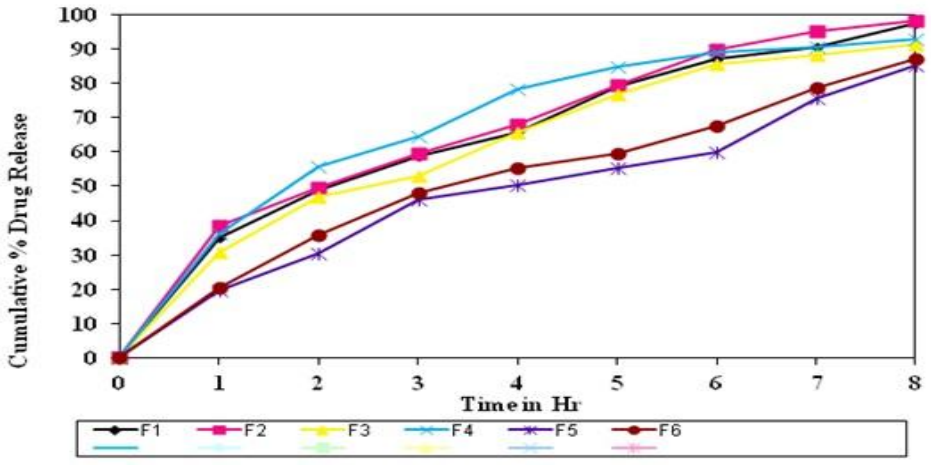


Figure 4. Comparative cumulative percent drug release plot for F1-F6



CONCLUSION

From the experimental result, it was concluded that, the poloxomer-188 used as a polymer in F2&F1 has shown the effective cumulative drug release up to 8 hours compared to the other formulations. The kinetic study shown in the F2 it revealed that the exponent "n" value is a within the permissible limits. It indicated that the release mechanism for F2 may be diffusion mechanism followed by the non-fickian transport so the F2 selected as the best formulation. The evaluation of the nanosuspension it reveal that the following parameters for the optimized formulation F2 as follows saturation solubility 96%, % Drug content uniformity 97%, average particle size 826.5 d.nm, poly dispersity index 0.283, Zeta potential 4.79(mv). These are indication of the stability of the nanosuspension. The stability studies indicate that the nanosuspension suspension more stable in a refrigeration condition. Form the above studies it is evident that a promising drug delivery system of the Zidovudine nanosuspensions.

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