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DESIGN OF BIODEGRADABLE POLYMER NANOPARTICLES FOR ORAL DRUG DELIVERY OF STAVUDINE: *IN- VITRO* DISSOLUTION STUDIES AND CHARACTERIZATION

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ABSTRACT

Chitosan as a natural biodegradable nanoparticular carrier, have important potential application for administration of therapeutic molecules. Chitosan based nanoparticles have attracted a lot of attention upon their biological properties such as biocompatibility, bioadhesivity, antimicrobial property and immunostimulatory activity. The aim of present investigation was to describe formulation and characterization of novel biodegradable nanoparticles based on chitosan for encapsulation of Stavudine. To achieve this objective solvent evaporation method, *in- situ* nanoemulsion polymer cross linking method were used drug containing nanoparticles were prepared with different drug polymer ratio at ambient temperature and freeze drying of nanoparticles formulation for long term stability. The resulting nanoparticles loading efficiency is 55.19% to 90.60% and loading capacity is 25.16% to 42.27% and dissolution studies were done by dialysis bag method with ph 7.4 (phosphate buffer)as a dissolution medium. In Characterization the obtained particle size of nanoparticles is 65.5-176nm, size distribution and shape were done by using scanning electron microscopy, zeta potential is for the best formulation. According to release characteristics following the zero order release kinetics and release 16 hours.

Key words: Chitosan, Stavudine, poly vinyl alcohol, *in-situ* nanoemulsion polymer cross linking method, solvent evaporation method.

INTRODUCTION: Novel drug delivery systems present an opportunity for formulation scientists to overcome the many challenges associate with anti retroviral (ARV) drug therapy. The currently available anti –HIV drugs classified into the nucleoside reverse transcriptase inhibitors, non nucleoside reverse transcriptase inhibitors, protease inhibitors and recently fusion , integration inhibitors. Most of these drugs bear some significant draw backs such as relatively short half life, low bioavailability, poor permeability and un desirable side effects. So the efforts have been made to design drug delivery systems for anti retroviral therapy as reducing dosing frequency, increase bioavailability, decrease degradation/metabolism in GIT, improve CNS penetration and inhibit CNS efflux, delivery them to target cells and selectively minimal side effects. ARV delivery systems that have been developed by achieving designed drug release kinetics specifically targeting drugs to macrophages , brain , gastric mucosa and for addressing formulation difficulties such as solubility ,stability and drug entrapement[1]. In this trends nanoparticles show a fastest development from out of total novel drug delivery systems

The essential material characteristics of an ideal drug carrier The essential material characteristics of an ideal drug carrier include (1)biocompatibility and bioacceptability of the carrier and its degradation products,(2) ability to be loaded with effective dosage, (3) acceptable stability during preservation, (4) satisfactory drug-release rate from the drug-loaded composite, (5)suitable for regular clinical administration, and (6) economically feasible for manufacture.[2]The hydrophilic nanoparticles have received considerable attention to deliver therapeutic peptide, protein, antigen, oligonucleotide and genes by intravenous ,oral and mucosal administration. The information has emphasized and revealed the advantages of nanoparticles over the microspheres. Chitosan is non toxic and soft tissue compatible in a ranges of toxicity tests[3].It posses antimicrobial activity and absorbs toxic metals like mercury, calcium, lead, etc. in addition, its good adhesion, coagulation ability, inexpensive [4].It possess positively charge and exhibits absoption enhancing effect[www.pharmainfo.net]. Chitosan nanoparticles are obtained by two methods solvent evaporation method and in- *situ* nanoemulsion polymer cross linking method.

Stavudine is one of the most essential nucleoside reverse transcriptase inhibitors for AIDS treatment with its oral bioavailability over 80%. In clinical study, Stavudine can appreciably increase CD4 cell counts and reduce

mean serum P24 antigen levels and infectious HIV titers. However the duration of the above responses is inadequate Furthermore, D4T is slightly hydrophilic with its log *D*oct=−0.84, where *D*oct is the distribution coefficient between octanol and phosphate buffered saline.

MATERIALS AND METHODS

Chitosan(viscosity 100 cps) was purchased from Paras pharmachem suppliers (Pune). Stavudine was obtained as a gift sample from Cipla Ltd., Patalganga, Mumbai. The chemicals used for synthesis nanoparticles include poly vinylalcohol, calcium chlori from Loba chem, Mumbai, tween-80, glycerol (Quligens, Mumbai). Equipments are for SEM(model) in Anna university Chennai , freeze drying (model) in SRM university potheri , zeta potential (model) in pune university pune and sonicator(sonica-hz), magnetic stirrer(2MLDX-), rotary shaker(RS-12R) Remi equipment ltd vasai. The dialysis bag from hi-media laboratories pvt ltd, Mumbai

Nanoparticles preparation:

Chitosan nanoparticles were synthesized by *in-situ* nanoemulsion-polymer cross linking method (F1, F2, and F3), solvent evaporation method (F4, F5, and F6).

IN -SITU NANOEMULSION POLYMER CROSS LINKING METHOD

Stavudine was encapsulated in chitosan by this method. thus 10 mg of the drug Stavudine taken in 10ml of drug loading solvent and emulsified under sonication 20KHz in 30 ml of 0.1%m/V aqueous solution of chitosan(2%acetic acid),using polyoxyethylene sorbitan mono-oleate(TWEEN-80)as a stabilizer, glycerol(~30ml) was then added drop wise to produce the emulsion . Calcium chloride solution (2mol L^{-1}, 1.5ml) was added into the reaction mixture to effect cross-linking of the nanoparticles produced. The reaction mixture was cured for 24 hrs at room temperature (25^0C).[5]

SOLVENT EVAPORATION METHOD:

Briefly 20 mg of stavudine, and 60mg of polymer were taken in f3 formulation ratio was(1:3) by using sonication drug dissolved in methanol (solvent), polymer dissolved in acetic acid2%.Drug and polymer phases were

mixed by drop by drop on sonicator and at the last PVA solution 2% was added then go for magnetic stirring for 24hrs. The resulting emulsion kept store night go to ultra centrifugation 30000 rpm for 20min.[6]

EVALUATION OF NANOPARTICLES:

FT IR STUDY:

For the FTIR studies a specified quantity of potassium bromide and samples was blended uniformly. The resultant blend was then comperssed to prepare the pellet as desired. The pellet was subjected to analysis.

DSC:

Samples of drug , drug-polymer physical mixture were heated from 20^0c to 400^0c in crimped aluminium pans to produce thermograms at a heating rate of 10^0c min^{-1}hr. alumina (2mg) in crimped aluminium pans supplied by the instrument manufacturer was used as the standard reference material to calibrate the temperature and energy scales.

Drug loading efficiency, drug loading capacity:

Drug loading efficiency and loading capacity of nanoparticles with different formulation were determined by ultra centrifugation of samples at 30000 ×g and for 30min. the amount of stavudine was determined in clear supernatant by UV spectrophotometry at 265.5 nm using solvent methanol as blank. The stavudine loading capacity (LC) of nanoparticles and stavudine loading efficiency (LE) of the process were calaculated from the equations 1 and 2 indicated below.

LC= (A-B)\C×100--------1

LE=(A-B)\A×100---------2

Where A is the total amount of stavudine, B is the free amount of stavudine, C is the nanoparticles weight.

Morphological characterization

The surface morphology (roundness, smoothness and formation of aggregates) and the size of nanoparticles formulation were studied by SEM (Scanning Electron Microscopy). The solid sample for SEM-analysis was coated with a thin layer of platinum or gold using the physical vapour deposition (PVD) process at a 30MA current from the distance of 50nm during 180 seconds.

Zeta potential: The zeta potential value of the sample was measured by zeta potential probe model DT-300. Zeta potential can help to under standing how individual colloids interact with one another. Each colloid carries a "Like" electrical charge which produces a force of material electrostatic repulsion between adjacent particles. If charge is high enough, the colloids remain discrete, disperse and in suspension. [7]

***In- vitro* drug release;**

Drug release from nanoparticles *in-vitro* was carried out in dialysis bag method. Each bag filled with 5ml of nanoparticles suspension in phosphate buffer saline (pH 7.4). While the received chamber containing the buffer with out nanoparticles. These total beakers were placed on a rotary shaker rotating at 110rpm at 37^0 c. at several pre determined time intervals, the entire content of each receiver chamber was removed and replaced with fresh 5ml PBS, the amount of stavudine that diffused into the receiver chamber was quantified by UV method.

Kinetic modeling of stavudine nanoparticles:

In order to investigate the mode of release from the microcapsules, the release data were analyzed with the following mathematical models.

$q_t = k_0 t$ (zero order kinetics)

$\text{Log} (q_t / q_0) = - k_1 t / 2.303$ (first order kinetics)

$q_t = k_{kp} t_n$ (korsmeyer and peppas equation)

$q_t = k_h t_{1/2}$ (higuchi's equation)

where, q_t is the percent of drug released at time't', k_0, k_1, k_{hc}, k_{kp} and k_h are the coefficients of zero order, first order, korsmeyer-peppas and higuchi's equations

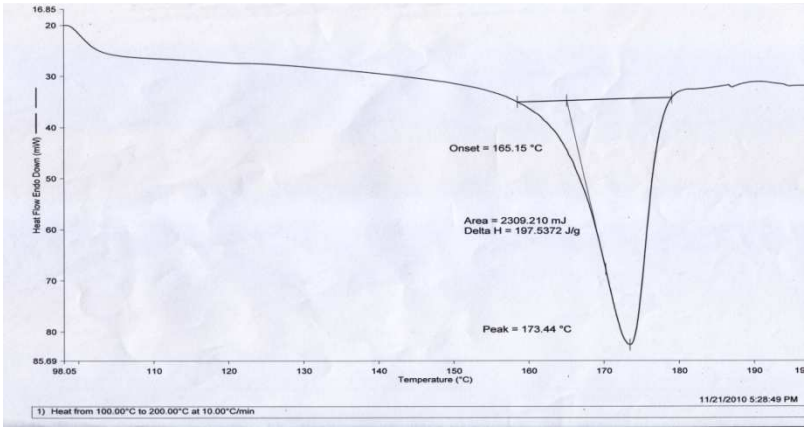
RESULTS AND DISCUSSIONS:

DSC and FT-IR studies:

These studies were carried out determine whether stavudine was incorporated in formulation F3 nanoparticles in crystalline, amorphous or bound form. DSC studies revealed that the pure stavudine was amorphous

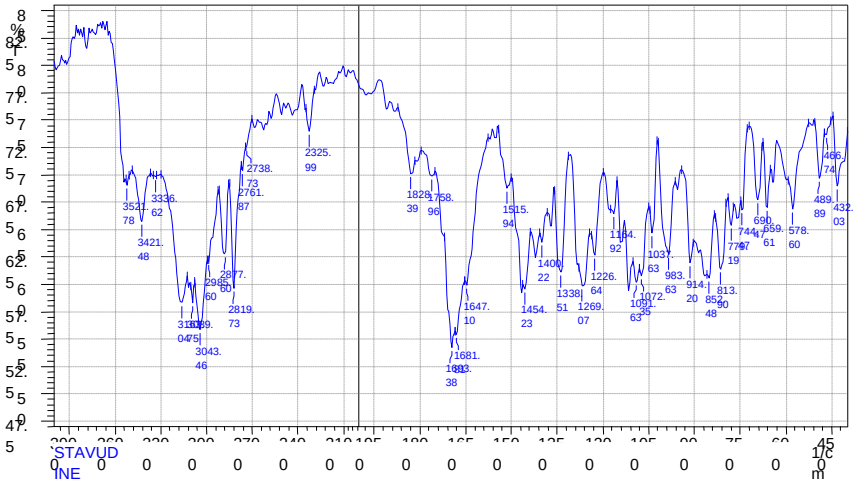
with sharp melting point 175.05^{0}c (figure1) and stavudine combine with polymer was showing a decomposition 173.44^{0}C (figure 2).The drug was there considered encapsulated in its amorphous form.

Figure-2:



In FT-IR studies were recorded for pure stavudine (figure 3), stavudine with polymer chitosan(figure 4) and formulation of stavudine nanoparticles(figure 5). In FT-IR studies , the characteristic stavudine N-H stretching at around 3043.46 cm^{-1}, O-H stretching at 3421cm^{-1} was clearly not observed in the stavudine loaded formulation F3 and additionally, C=C stretching of stavudine at 1681.81cm^{-} was also observed unchanged in formulation F3, suggesting no drug-polymer inter actions in loaded nanoparticles.

Figure-3:



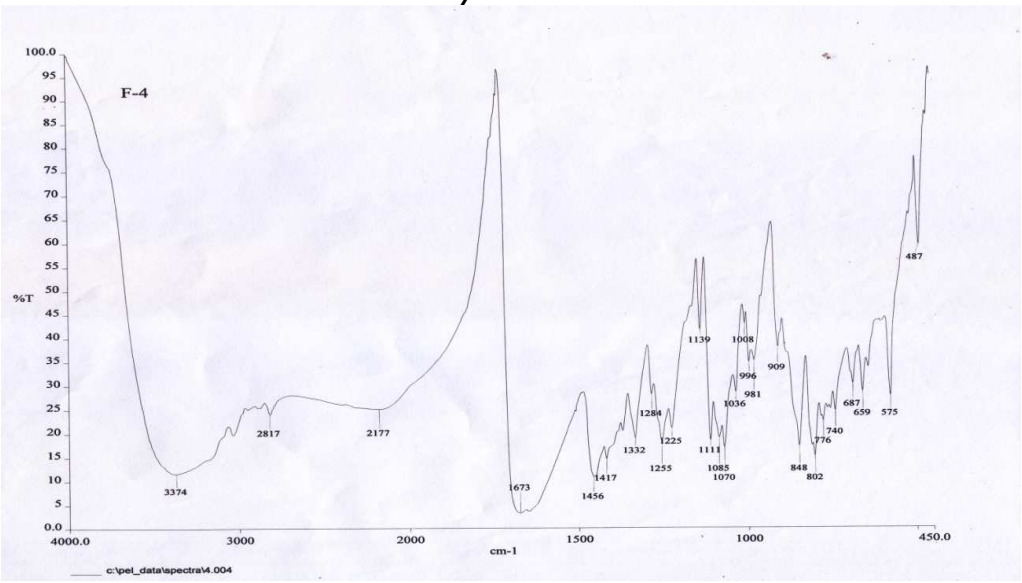


Figure-4

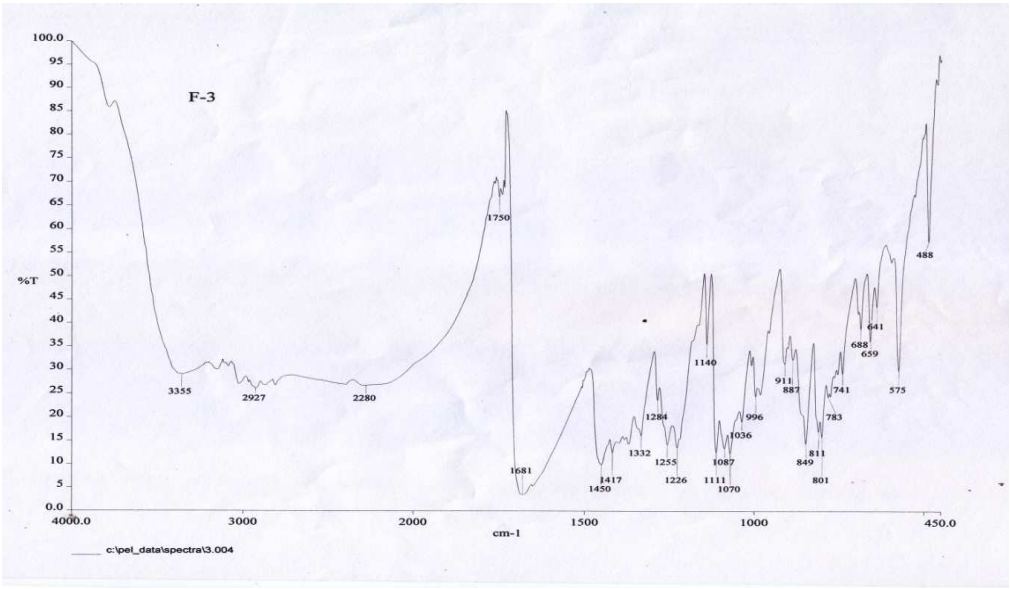


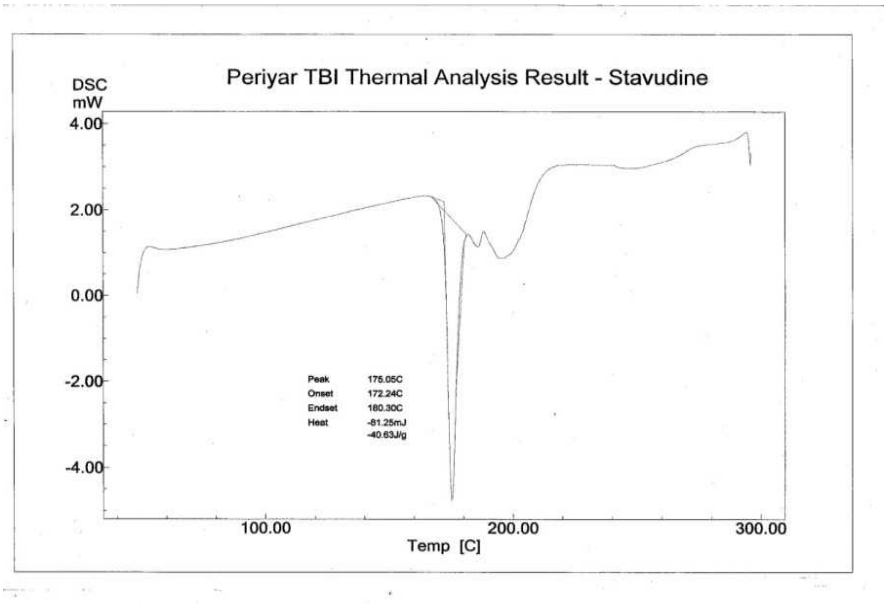
Figure-5

Stavudine nanoparticles were prepared by following *in-situ* nanoemulsion polymer cross linking method and solvent evaporation method. Six different formulations were designed using different drug polymer ratio of stavudine and chitosan 100cps with methanol as a solvent. The different ratios of drug and polymer showed a distinct impact on the drug loading efficiency and drug loading capacity. The formulation F3 shows better drug loading efficiency than other formulations (table 1).

S.No	Formulation	Chitosan sol.mg/ml	Drug Loading efficiency (%)	Drug loading capacity (%)
METHOD A		***IN -SITU* POLYMER CROSS LINKING METHOD**		
1	F1	10	55.193	40.73
2	F2	20	84.58	33.16
3	F3	30	90.60	25.16
METHOD B		**SOLVENT EVAPORATION METHOD**		
4	F4	10	78.12	42.27
5	F5	20	82.83	36.01
6	F6	30	89.94	26.44

The SEM study was done for the formulation showing better drug loading efficiency is F3. (Figure 1) shows that SEM study of chitosan loaded stavudine spherical nanoparticles were formed by using drug polymer ratio (1:3). In this the particles observed were size range from 65.5 to 176 nm.

Figure-1:



Zeta potential of the formulation F3 is the positive only the value is the 33.26±s.d. zeta potential is an important physicochemical parameter, which can influence factors like stability of a nano-drug carrier formulation. Extremely positive or negative zeta potential values cause larger repulsive forces, while electrostatic repulsion between particles with the same electric charge prevents aggregation of the particles [8].

In-vitro studies were carried with all formulations for their *in-vitro* studies release pattern across cellophone membrane. The *in-vitro* release studies of stavudine –chitosan nanoparticles were shown in (table 2) and comparision of different formulations shown in (figure 8). Intial release of the drug is associated with those drug molecules dispersing close to the nanoparticle surface the drug release many depends upon the drug polymer concentration. Both the size and the amount of drug loading were known to influence the nanoparticular drug release profile[9]. In general, the drug release mechanism of formulations followed an over all zero order kinetics dependent only. Figure.3 shown that in F1formulation showed drug release of 60.305 % with in 13hour give release pattern in controlled manner; F2 formulation showed drug release 87.64% with in 14hour give release pattern in controlled manner; F4 formulation showed drug release 68.48% with in 16hour give release pattern in controlled manner; F5 formulation showed drug release 70.38% with in 16hour give release pattern in controlled manner; F6 formulation showed drug release 80.35% with in 16hour give release pattern in controlled manner. One comparison of the release profile of the six formulations it was observed that release from formulation F3 was found to be slow and constant manner. From this observed data it shown that increase the chitosan concentration increases the cumulative percentage drug release. It was observed that cumulative drug diffusion is the order F3>F2 >F6 >F5 > F4>F1 formulations give best drug release pattern than other formulation. According to t_{25}, t_{50}, t_{90} (table 3) formulation F3 shows a 93.71 release in 15.18 hrs than other formulations

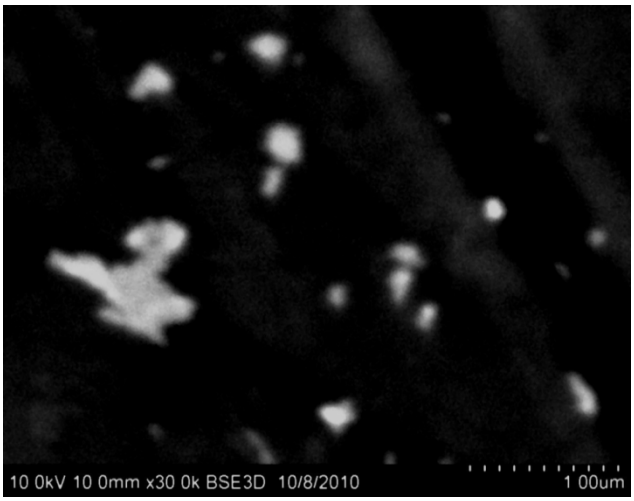


Figure 6: SEM photograph

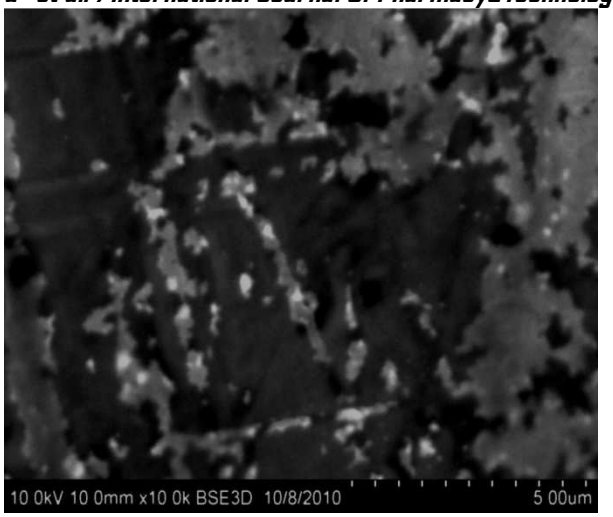


Figure 7: SEM photograph

Figure 8: Comparative study of different formulations drug release

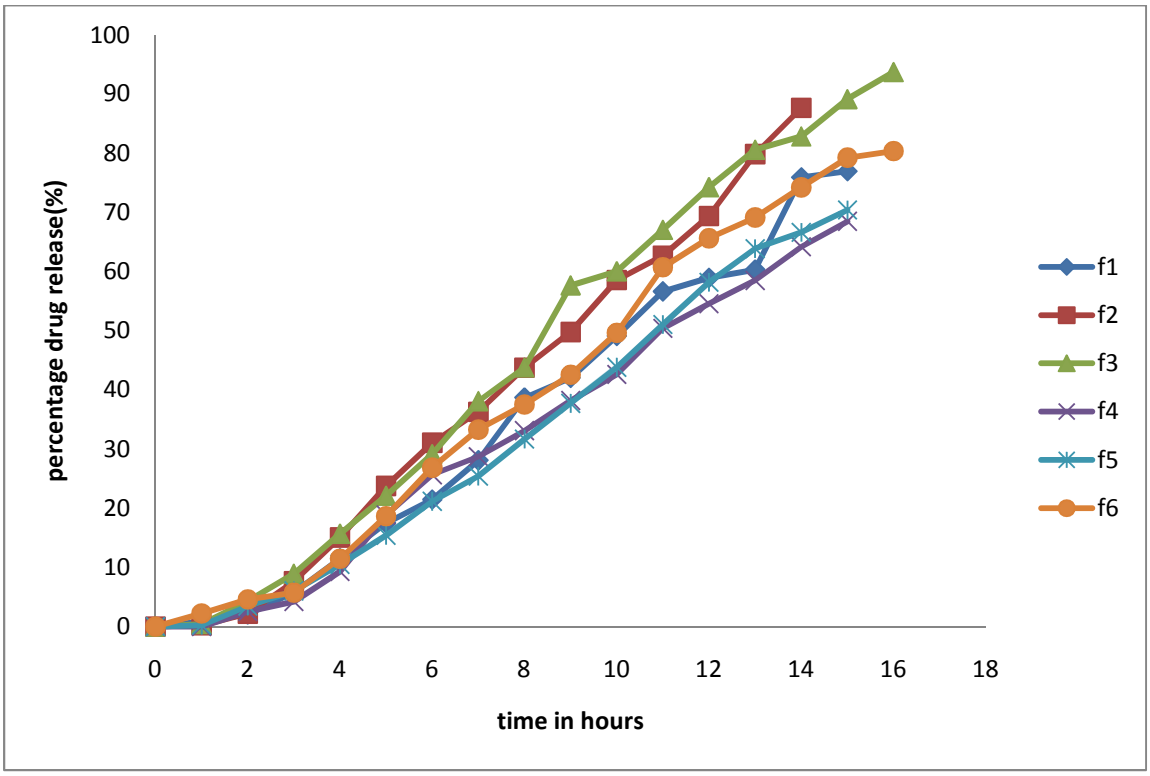


Table-2: Percentage drug release from stavudine nanoparticles:

S.No	Time in hours	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)
1	1	0.86	0.236	0.455	0.0336	0.227	2.24
2	2	2.05	2.20	4.360	2.55	3.49	4.61
3	3	5.77	7.656	8.921	4.24	6.03	5.74
4	4	11.54	15.066	15.691	9.31	10.52	11.45
5	5	17.48	23.772	22.098	18.86	15.43	18.67
6	6	21.465	31.063	29.129	25.66	21.16	26.86
7	7	28.087	36.272	38.09	28.72	25.40	33.29
8	8	38.690	43.750	43.78	33.11	31.69	37.53
9	9	42.038	49.776	57.66	38.20	37.72	42.55
10	10	49.107	58.549	60.97	42.59	43.82	49.62
11	11	56.622	62.686	67.03	50.40	51.04	60.71
12	12	58.928	69.382	74.25	54.57	58.18	65.62
13	13	60.305	79.83	80.58	58.48	63.86	69.12
14	14	_	87.64	82.84	64.17	66.62	74.21
15	15	_	_	89.13	68.48	70.38	79.24
16	16	_	_	93.71	_	_	80.35

S.NO	FORMULATION	t_{25}(hours)	t_{50}(hours)	t_{90}(hours)
1	F1	6:30	10:06	_
2	F2	5:06	9:18	_
3	F3	5:30	8:30	15:18
4	F4	5:54	10:54	_
5	F5	6:44	10:54	_
6	F6	5:48	10:03	_

CONCLUSION:

Clinical efficiency is required for any novel drug delivery system. Stavudine loaded chitosan nanoparticles herald a Novel controlled drug delivery, which offers several potential benefits .Stavudine nanoparticles had shown an excellent drug loading efficiency with polymer chitosan. Stavudine is an anti –Retroviral drug ,was selected as drug candidate for present study because it possesses the requisite properties necessary for formulation chitosan nanoparticles drug delivery system which has relatively short half life (1.5 hrs). Comparatively based upon the method of preparation *in -situ* nanoemulsion polymer cross linking method and solvent evaporation method , the *in-situ* method has produced a good results like drug loading efficiency, *in-vitro* release studies , $t_{90,}$ Zeta potential and the corresponding formulation produced good results was found to be F3.

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