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Formulation and *in vitro* evaluation of lamivudine loaded nanoparticles

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ABSTRACT

Nanoparticles represent a promising drug delivery system of sustained and targeted drug release. They are specially designed to release the drug in the vicinity of target tissue. The aim of this study was to prepare and evaluate PLGA nanoparticles containing Lamivudine in different drug to polymer ratio. SEM indicated that nanoparticles have a discrete spherical structure. FT-IR studies indicated that there was no chemical interaction between drug and polymer and stability of drug. The *in vitro* release behavior from all the drug loaded batches was found to be Higuchi release and provided sustained release over a period of 12 h. The developed formulation overcome and alleviates the drawbacks and limitations of Lamivudine sustained release formulations and could possibility be advantageous in terms of increased bioavailability of Lamivudine.

Keywords: Nanoparticles, Lamivudine, biodegradable

INTRODUCTION

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then to maintain the desired drug concentration. That is, the drug delivery system should deliver drug at a rate dictated by the needs of the body over a specified period of treatment. This idealized objective points to the two aspects most important to drug delivery namely spatial placement and temporal delivery of a drug. Spatial placement relates to targeting of drug to a specific organ or tissue, while temporal delivery refers to Sustainedling the rate of drug delivery to the target tissue. An appropriately designed Sustained release drug-delivery system can be a major advance towards solving these two problems. It is for this reason that the science and technology responsible for development of Sustained-release pharmaceuticals has been, and continues to be the focus of a great deal of attention in both industrial and academic laboratories.

Conventional drug therapy[1]

To gain appreciation for the value of Sustained drug therapy, it is useful to review some fundamental aspects of conventional drug delivery. Consider single dosing of a hypothetical drug that follows a simple one-compartment pharmacokinetic model for disposition. Depending on the route of administration, a conventional dosage form of the drug e.g.: A solution, suspension, capsule tablet etc. can produce a drug blood level versus time profile. The term drug blood levels refer to the concentration of drug in blood or plasma, but the concentration in any tissue could be plotted on the ordinate. Administration of a drug by either intravenous injection or an extra vascular route, e.g., orally, intramuscularly or rectally does not maintain drug blood levels within the therapeutic range for extended periods of time. The short-duration of action is due to the inability of conventional dosage forms to Sustained temporal delivery. If an attempt is made to maintain drug blood levels in the therapeutic range for longer periods by for e.g., increasing the initial dose of an intravenous injection, toxic levels can be produced at early times. This approach obviously is undesirable and unsuitable. An alternative approach is to administer the drug repetitively using a constant dosing interval, as in multiple-dose therapy. In this case the drug blood level reached and the time required to reach that level

depend on the dose and the dosing interval. There are several potential problems inherent in multiple dose therapy.

1. If the dosing interval is appropriate for the biological half-life of the drug, large peaks and valleys in the drug blood level may result. For e.g., drugs with short half-lives require frequent designs to maintain constant therapeutic levels.
2. The drug blood level may not be within the therapeutic range at sufficiently early times, an important consideration for certain disease states.
3. Patient non-compliance with the multiple-dosing regimens can result in failure of this approach.

In many instances, potential problems associated with conventional drug therapy can be overcome. When this is the case, drugs given in conventional dosage forms by multiple dosing can produce the desired drug blood level for extended period of time. Frequently, however these problems are significant enough to make drug therapy with conventional dosage forms less desirable than Sustained-release drug therapy. This fact, coupled with the intrinsic inability of conventional dosage forms to achieve spatial placement, is a compelling motive for investigation of Sustained-release drug delivery systems.

Terminology[2, 3]

Modified-release delivery systems may be divided conveniently into four categories:

1. Delayed release
2. Sustained release
3. Site-specific targeting
4. Receptor targeting.

Delayed-release systems are those that use repetitive, intermittent dosing of a drug from one or more immediate-release units incorporated into a single dosage form. Examples of delayed release systems include repeat-action tablets and capsules and enteric-coated tablets where timed release is achieved by a barrier coating.

Sustained-release systems include any drug delivery system that achieves slow release of drug over an extended period of time. If the systems can provide some Sustained, whether this is of a temporal or spatial nature, or both, of drug release in the body, or in other words, the systems is successful at maintaining constant drug levels in target tissue or cells, it is considered Sustained-release systems.

Site-specific and receptor targeting refer to targeting of a drug directly to a certain biological location. In the case of site-specific release, the target is adjacent to or in the diseased organ or tissues, for receptor release, the target are the particular receptor for a drug within an organ or tissue. Both of these systems satisfy the spatial aspect of drug delivery and are also considered to be Sustained drug-delivery systems.

Advantages of sustained release preparations

1. Decreased incidence and/ or intensity of adverse effects and toxicity.
2. Better drug utilization.
3. Sustained rate and site of release.
4. More uniform blood concentrations.
5. Improved patient compliance.
6. Reduced dosing frequency.
7. More consistent and prolonged therapeutic effect.
8. A greater selectivity of pharmacological activity.

Objectives

Sustained release systems include any drug delivery system that achieves slow release of drug over an extended period of time.

The objectives of oral sustained release formulations are:

1. Frequency of drug administration is reduced.
2. Patient compliance can be improved.
3. Drug administration can be made more convenient.
4. Better Sustained of drug absorption can be attained.

The concept of targeting

The concept of designing specified delivery system to achieve selective drug targeting has been originated from the perception of Paul Elrich, who proposed drug delivery to be as a “Magic Bullet”. It was the very first report published on targeting (Paul Elrich, 1902) describing targeted drug delivery as an event where a drug-carrier complex/ conjugate delivers drug(s) exclusively to the preselected target cells in a specific manner. Gregoriadis, 1981 described drug targeting using novel drug delivery system as ‘old drugs in new cloths.

New drug delivery system represents a means by which drug may be continuously delivered either locally or systemically or a larger site in an effective and repeatable manner. Sustained and targeted drug delivery systems have been receiving more and more attention as new methods of drug delivery.

One of the most exciting is the target-organ oriented drug delivery system. Presenting drugs into whole body is not only wasteful but also likely to lead to harmful effects that can be eliminated if the drug is delivered only to specific target organ. Targeted delivery is not restricted to any one route of administration. Oral formulations, parenterals, transdermal and pulmonary route and many other routes are available for effective drug targeting.

MATERIALS

Lamivudine Sura Labs, Dilsukhnagar, Hyderabad, PLGALactel, Durect corporation Birmingham Division, TPGSEastman company, UK, Acetone SRL, Dialysis membrane Himedia.

METHODOLOGY

Preparations of buffer

Preparation of 0.2M Potassium Dihydrogen Orthophosphate Solution

Accurately weighed 27.218 gm of monobasic potassium dihydrogen orthophosphate was dissolved in 1000 mL of distilled water and mixed.

Preparation of 0.2M sodium hydroxide solution

Accurately weighed 8 gm of sodium hydroxide pellets were dissolved in 1000 mL of distilled water and mixed

Preparation of pH 7.4 phosphate buffer

Accurately measured 250 mL of 0.2M potassium dihydrogen ortho phosphate and 195.5 mL of 0.2M NaOH was taken into the 1000 mL volumetric flask. Volume was made up to 1000 mL with distilled water.

Preparation of Standard Graph

100mg of Lamivudine pure drug was dissolved in 15ml of Methanol and volume make up to 100ml with 0.1N HCL (stock solution-1). 10ml of above solution was taken and make up with100ml by using 0.1 N HCL (stock solution-2 i.e. 100µg/ml). From this take 0.2, 0.4, 0.6, 0.8 and 1ml of solution and make up to 10ml with 7.4 phosphate buffer to obtain 2, 4, 6, 8, and 10 µg/ml of Lamivudine solution. The absorbance of the above dilutions was measured at 270 nm by using UV-Spectrophotometer taking pH 7.4 phosphate buffer as blank. Then a graph was plotted by taking Concentration on X-Axis and Absorbance on Y-Axis which gives a straight line Linearity of standard curve was assessed from the square of correlation coefficient (R^2) which determined by least-square linear regression analysis.

Method of preparation of lamivudine loaded nanoparticles

Solvent dispersion (Nanoprecipitation)

The nanoparticles are prepared by dissolving the drug in organic phase along with the Poly(lactic-co-glycolic acid) polymer (PLGA) and added to the aqueous solution containing TPGS((d-alpha-tocopheryl polyethylene glycol 1000 Succinate) which acts as an emulsifier. The solution of organic phase was added in drop wise into aqueous phase under homogenization at 11,000 rpm. The dispersion was kept under magnetic stirring for 4hrs at room temperature. The solution is kept under reduced pressure for about 2-3min. This process forms nanoparticles loaded with drug.

Table1: Composition of the Nanoparticles

Ingredients	Batch no							
	F1	F2	F3	F4	F5	F6	F7	F8
PLGA (50:50)(mg)	25	50	75	100	50	100	150	200
TPGS (%g/ml)	0.015	0.03	0.06	0.09	0.15	0.24	0.39	0.63
Lamivudine (mg)	150	150	150	150	150	150	150	150
Acetone (ml)	5	5	5	5	5	5	5	5
Water (ml)	10	10	10	10	10	10	10	10

RESULTS AND DISCUSSION

Preparation of Standard Graph

Determination of absorption maxima

The standard curve is based on the spectrophotometry. The maximum absorption was observed at 270 nm.

Calibration curve

Graphs of Lamivudine was taken in pH 7.4 Phosphate buffer

Table 2: Calibration curve data for Lamivudine at 270 nm

Concentrations [µg/mL]	Absorbance
0	0
2	0.128
4	0.261
6	0.387
8	0.491
10	0.618

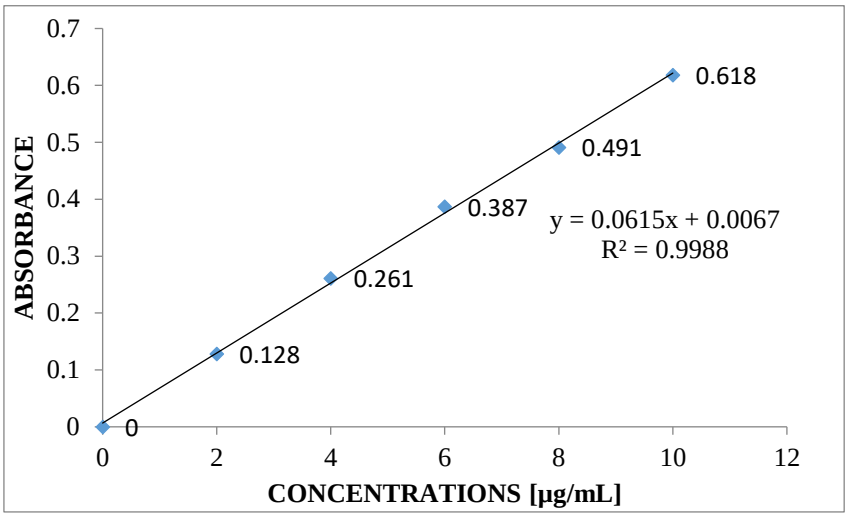

Fig 1: Standard graph of Lamivudine in 7.4 Phosphate buffer

Evaluation of lamivudine loaded nanoparticles

Table 3: Evaluation of Nanoparticles

Batch No	Mean Particle size (nm)	%Yield	Drug encapsulation efficiency	PDI	Zeta Potential (mV)
F1	96.07	90.36	90.91	0.668	-26.12
F2	100.92	93.51	92.35	1.268	-24.81
F3	121.07	95.28	94.17	1.153	-23.52
F4	153.09	**97.10**	**97.76**	**0.168**	**-28.25**
F5	100.24	88.35	86.42	0.277	-16.55
F6	145.21	91.51	90.30	0.309	-20.83
F7	160.64	94.62	92.91	0.698	-22.59
F8	171.06	94.02	95.35	0.385	-12.11

Quality control parameters for tablets

Table 4: *In vitro* Drug release studies of Lamivudine F1, F2, F3, F4

TIME (hr)	CUMULATIVE PERCENT OF DRUG RELEASED			
	F1	**F2**	**F3**	**F4**
0	0	0	0	0
1	27.42	29.69	32.41	22.26
2	34.39	40.09	47.69	28.78
3	47.60	46.16	58.34	35.36
4	56.51	57.65	64.61	57.23
5	67.62	65.19	70.08	66.98
6	78.37	78.67	78.39	77.46
7	85.26	81.76	84.56	85.68
8	96.78	89.54	87.98	93.14
10	99.82	95.34	93.18	98.13
12		97.54	97.14	99.37

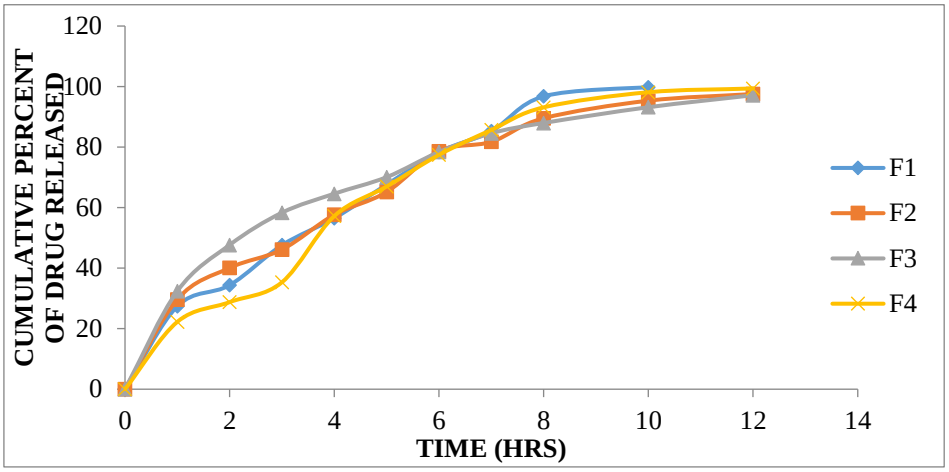

Fig 2: Dissolution study of Lamivudine Nanoparticles

Table 5: *In vitro* Drug release studies of Lamivudine F5, F6, F7, F8

TIME (hr)	CUMULATIVE PERCENT OF DRUG RELEASED			
	F5	**F6**	**F7**	**F8**
0	0	0	0	0
1	17.92	20.92	27.93	16.85
2	22.65	34.36	41.62	22.76
3	33.89	42.61	48.02	30.50
4	44.32	54.53	60.47	49.11
5	52.87	61.88	66.85	61.78
6	65.90	72.46	78.68	76.89
7	73.36	81.87	87.39	83.43
8	79.77	89.29	98.77	97.14
10	90.53	99.14		
12	96.91			

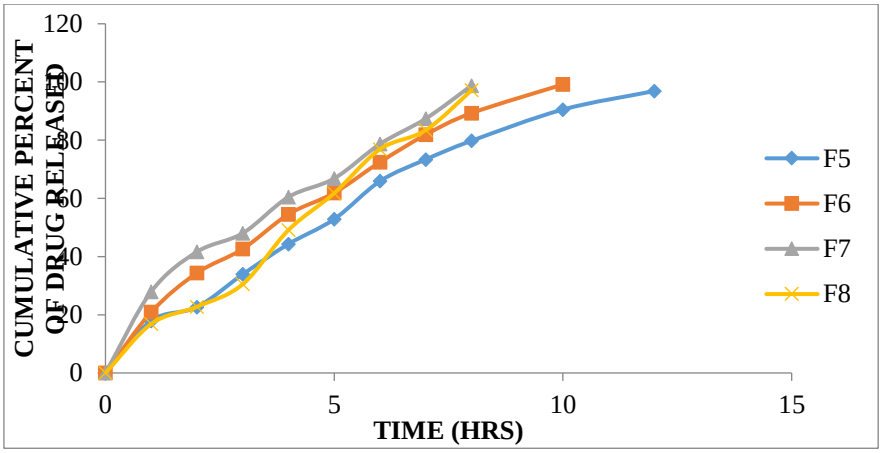

Fig 3: Dissolution study of Lamivudine Nanoparticles

Table 6: Release kinetics data for optimized formulation (F4)

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE / t)	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
22.26	1	1.000	1.348	0.000	1.891	22.260	0.0449	-0.652	77.74	4.642	4.268	0.374
28.78	2	1.414	1.459	0.301	1.853	14.390	0.0347	-0.541	71.22	4.642	4.145	0.496
35.36	3	1.732	1.549	0.477	1.811	11.787	0.0283	-0.451	64.64	4.642	4.013	0.628
57.23	4	2.000	1.758	0.602	1.631	14.308	0.0175	-0.242	42.77	4.642	3.497	1.144
66.98	5	2.236	1.826	0.699	1.519	13.396	0.0149	-0.174	33.02	4.642	3.208	1.433
77.46	6	2.449	1.889	0.778	1.353	12.910	0.0129	-0.111	22.54	4.642	2.825	1.817
85.68	7	2.646	1.933	0.845	1.156	12.240	0.0117	-0.067	14.32	4.642	2.428	2.213
93.14	8	2.828	1.969	0.903	0.836	11.643	0.0107	-0.031	6.86	4.642	1.900	2.741
98.13	10	3.162	1.992	1.000	0.272	9.813	0.0102	-0.008	1.87	4.642	1.232	3.410
99.37	12	3.464	1.997	1.079	-0.201	8.281	0.0101	-0.003	0.63	4.642	0.857	3.784

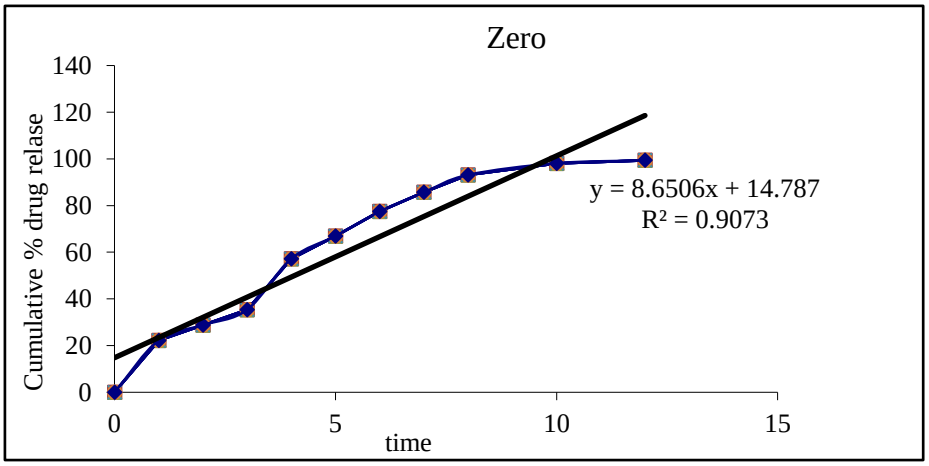

Fig 4: Graph of zero order kinetics

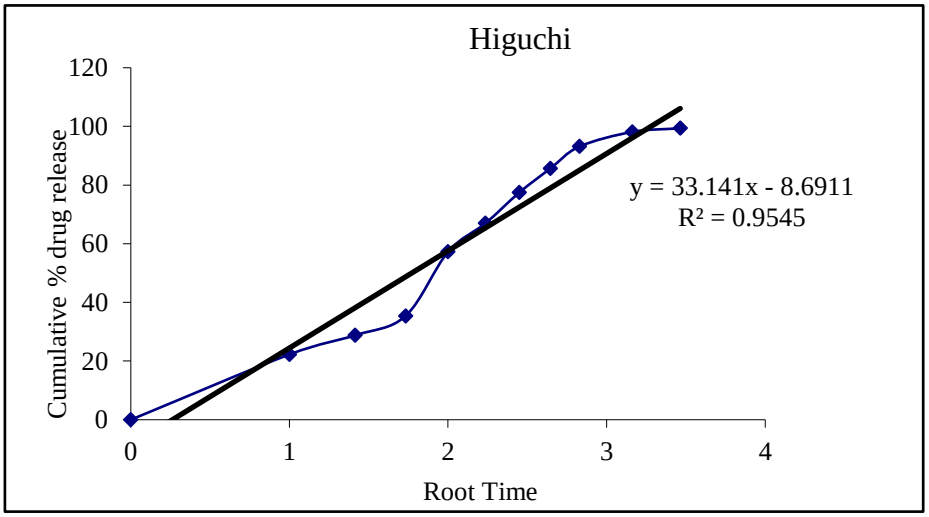

Fig 5: Graph of higuchi release kinetics

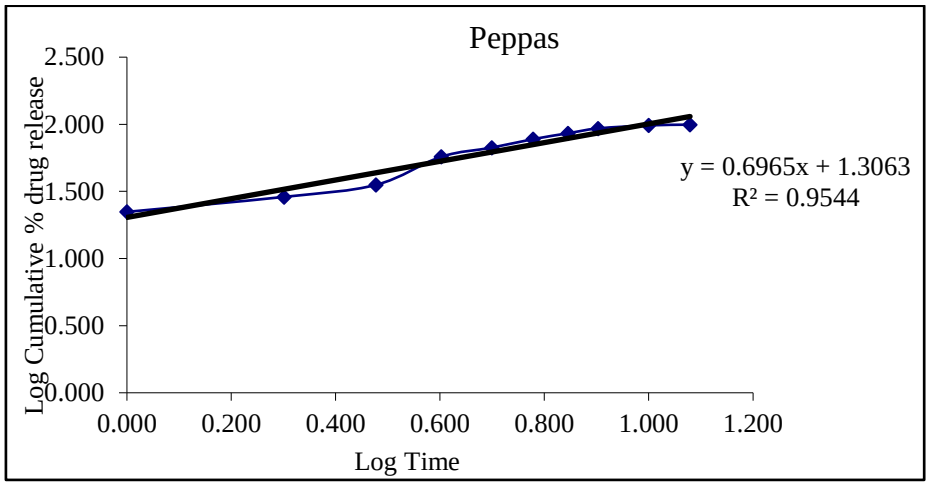

Fig 6: Graph of peppas release kinetics

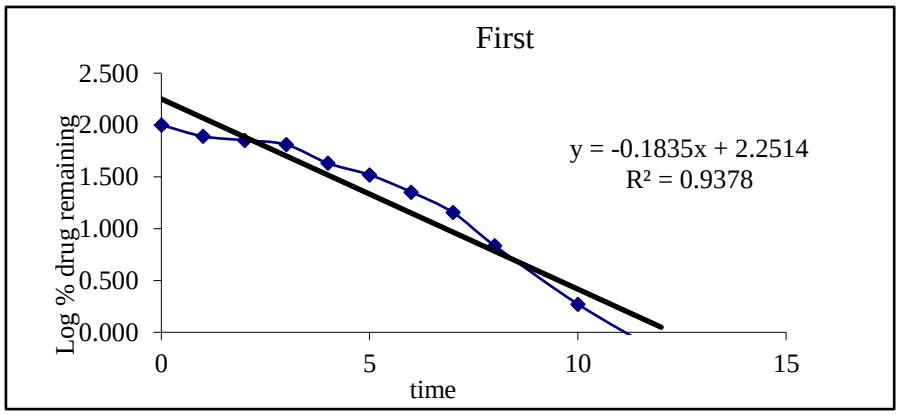

Fig 7: graph of first order release kinetics

Drug – Excipient compatibility studies

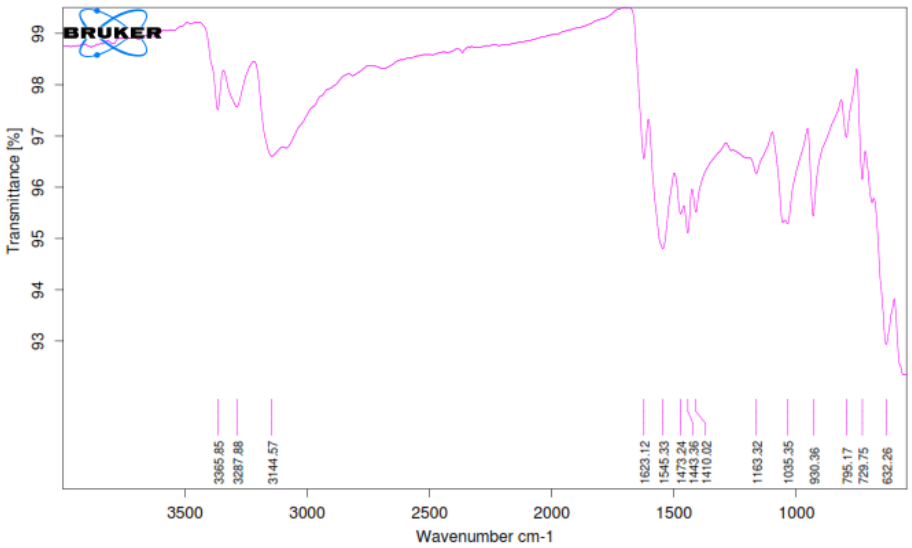

Fig 8: FT-TR Spectrum of Lamivudine pure drug

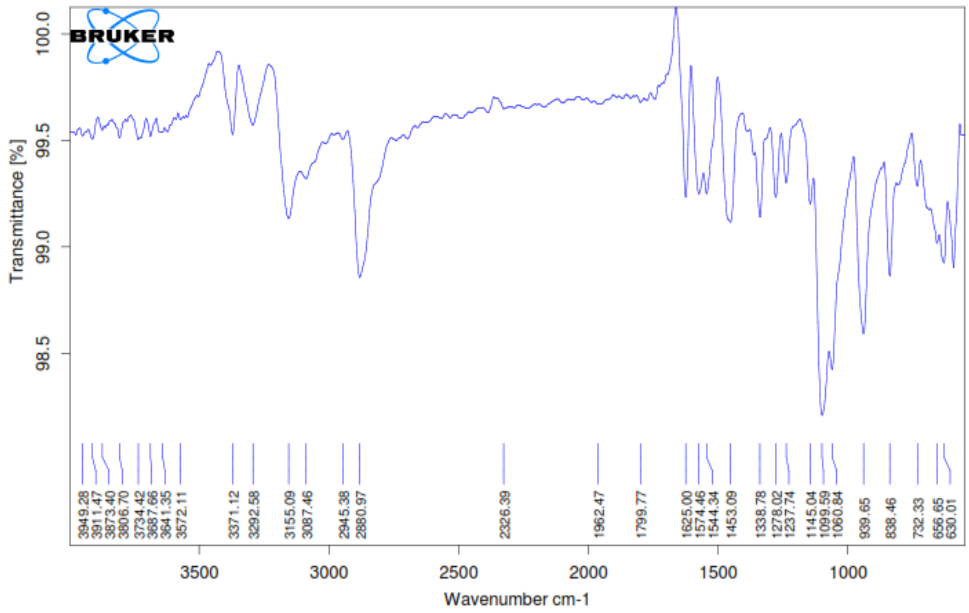

Fig 9: FT-IR Spectrum of Optimised Formulation

CONCLUSION

Site-specific and receptor targeting refer to targeting of a drug directly to a certain biological location. In the case of site-specific release, the target is adjacent to or in the diseased organ or tissues, for receptor release, the target are the particular receptor for a drug within an organ or tissue. Both of these systems satisfy the spatial aspect of drug delivery and areal so considered to be Sustained drug-delivery systems.

Lamivudine loaded nanoparticles were successfully formulated. Drug and excipient compatibility were studied by FTIR, and no incompatibility was observed.

Evaluation parameters revealed that the percentage of lipid and surfactant have significant effects on the particle size, %Yield, Drug encapsulation efficiency, Zeta Potential (mV) and *invitro* release from the nanoparticles formulation. The optimized Lamivudine loaded PLGA nanoparticles formulations (F4) were in nano size range (153.09nm) with high drug release (99.37%) adequate encapsulating efficiency exhibiting a homogenous, stable and effective.

Modified solvent evaporation technique involving homogenization and magnetic stirrer were used for preparing nanoparticles. A series of Nanoparticle sustained release systems were prepared: Lamivudine nanoparticles with PLGA (50:50). The formulated nanoparticles were evaluated for particle size and size distribution, entrapment efficiency and surface morphology. Based on the particle size, entrapment efficiency the formulation F4 (Lamivudine-PLGA) were selected for further *in vitro* study. The *in vitro* study showed the slow release of the drug from the nanoparticles and improved permeability. The improved bioavailability was observed. The *in vitro* studies showed the prolongation of drug release and improve permeability from the nanoparticulate systems. The formulations (F4) were reasonably safe after single dose oral administration. The formulations were able to improve the bioavailability. This prepared Lamivudine nanoparticles are take through the orally.

The results depict that the topical application of the Lamivudine nanoparticles system is an effective and safe alternative to the conventional for the possible management of abnormal lipids.

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