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Formulation and evaluation of lamivudine enclosed alginate microbeads

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ABSTRACT

The objective of the present study was to prepare and evaluate microbeads for the controlled release of lamivudine from the prepared microbeads using different polymers. The microbeads were prepared by ionic gelation method. The prepared microbeads were characterized for FTIR, scanning electron microscopy (SEM), the percentage drug content, entrapment efficiency, and in vitro dissolution studies. Accelerated stability studies were also carried out for the formulations. The microbeads were spherical in shape and free flowing. The entrapment efficiency was varying from 76 to 86%. The release of drug from the microbeads was extended up to 8 to 12 hours. FTIR studies showed the stable character of lamivudine in the microbeads. SEM studies revealed that the microbeads were porous in nature. The release kinetics studies revealed that the prepared microbeads were best fitted to the zero order for all eight formulations and peppa's model. The release kinetics data and characterization studies indicated that the drug release from microbeads was diffusion – controlled and the microbeads were stable in nature.

Keywords: Lamivudine, microbeads, controlled release, stability, ionic gelation, entrapment efficiency.

INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS), which is now a plague in several countries, was first identified in California in 1981. According to data published in the December, 2003 AIDS Epidemic Update, [1] an estimated 40 million people were living with HIV/AIDS at the end of 2003, of whom as many as 70% are living in Sub-Saharan Africa. In addition, an estimated 3 million people died of HIV/AIDS during 2003, of whom as many as 80% were living in sub-Saharan Africa. The World Health Organization (WHO) estimates that HIV/AIDS caused 2% of all deaths and 6% of deaths due to infectious diseases in India in 1998. If current HIV/ AIDS

policies continue, by 2033 AIDS will account for an estimated 17 percent of all deaths and 40 percent of deaths from infectious disease. Following the launch of generic antiretroviral drugs by Indian pharmaceutical companies in 2000 and the decline in the costs of these drugs, an increasing number of people with HIV/AIDS have been -using antiretroviral therapy. Of the estimated 550,000 people with AIDS in India, 370,000 reside in 60 major cities. Physicians in these cities are treating 90,000 of these people, 11,700 of whom (8,700 males and 3,000 females) are receiving antiretroviral therapy [2]. In such a way the antiretroviral therapy play essential role in the management of AIDS patients.

Lamivudine is a synthetic nucleoside analog that is being increasingly used as the core of an antiretroviral regimen for the treatment of HIV infection. Lamivudine, the (-)-enantiomer of 2′, 3′-dideoxy-3′-thiacytidine, is a nucleoside analog that inhibits HIV reverse transcriptase. [3] *In vivo*, nucleoside analogs are phosphorylated intracellularly by endogenous kinases to putatively active 5′- triphosphate (3TC-TP) derivatives that prevent HIV replication by competitively inhibiting viral reverse transcriptase and terminating pro viral DNA chain extension. [4,5] Lamivudine is rapidly absorbed after oral administration with an absolute bioavailability of 86% ± 16%, peak serum concentration of lamivudine (Cmax) of 1.5 ± 0.5 mcg/mL and mean elimination half-life (t½) of 5 to 7 hours, thus necessitating frequent administration to maintain constant therapeutic drug levels. [6]Therefore, the objective of the present work is paying attention to provide a long acting pharmaceutical formulation containing lamivudine in a modified release matrix as microbeads, to maintain the blood levels of the active ingredient for a prolonged period of time [7].

MATERIALS AND METHODS

Materials

Lamivudine was obtained as a gift sample from Milton Labs, Pondicherry. HPMC, Chitosan, Sodium Carboxyl methyl cellulose was Gift sample from novel drugs, Trichy. Sodium Alginate, Calcium Chloride, bought from S.D Fine chemicals, Mumbai. All the chemicals and reagents used in the study were of analytical grade.

Preparation of microbeads

The microbeads were prepared by the ionotropic gelation technique. The sodium alginate solution was prepared by dispersing the sodium alginate in de-ionized water under continuous stirring for 30 minutes. The weighed amount of the drug was thoroughly mixed with sodium alginate dispersion. By following the same procedure the alginate beads of different ratios of drug: polymer were prepared. The resulted homogeneous dispersion was extruded in to the 5% calcium chloride solution through hypodermic syringe with flat tip needle (20G) and stirred for 15 minutes at 100rpm using magnetic stirrer. The formed micro beads were allowed to cure for 30 minutes in the calcium chloride solution to complete the gelation reaction. The microbeads were then filtered and dried in hot air oven at 60ºC for 3 hr [8, 9, 16, 18, 19].

CHARACTERIZATION OF MICROBEADS

FTIR study

The lamivudine microbeads were subjected to FT-IR analysis by the following method [23], an approximately minimum quantity (less than 4mg) of sample was thoroughly blended with adequate quantity of IR grade KBr (less than 100mg) in mortar. The mix was then made into KBr pellets by hydraulic compression lever. The samples were the analyzed in a double beam IR spectrometer using KBr film as negative control (blank).

Scanning electron microscopy

Morphological details of the specimens were determined by using a scanning electron microscope (SEM).

Granulometric studies

Particle size distribution pattern was determined by sieve analysis on mechanical sieve shaker [16, 20], using different meshes (12, 16, 20 and 30) of American society of testing materials.

Micrometric properties of the beads

The mean particle size of the alginate microbeads was determined by optical microscopic method using a pre-calibrated stage micrometer [10].

Flow properties of Beads

The flow properties of prepared microbeads were investigated by measuring the Angle of Repose by using fixed funnel method [17]. Depends upon these values, the flow properties of the microbeads can be assumed. The Angle of Repose Values was mentioned in the Table-3. The value of angle of repose was calculated by using the following formula,

$$\text{Angle of repose } (\theta) = \tan^{-1} h/r$$

h=cone height, r=radius of circular base formed by the microbeads on the ground. The bulk and tapped densities were measured in a 10ml graduated cylinder as a measure of packability of the microbeads. Each experiment was carried out in triplicate. Results were shown in Table-3

Drug content (mg)

100 mg microbeads were powdered and transferred into a 100 ml volumetric flask and the volume was made upto the mark with 6.8 pH phosphate buffer and kept aside for 12 hrs with occasional shaking. Then the absorbance was analyzed spectrophotometrically at 270 nm. Three determinations were carried out for each formulation. The drug content was calculated by using the formula;

$$\text{Drug content} = \text{concentration} \times \text{dil factor} \times \text{conversion factor} \times \text{amt. of stock sol.}$$

Drug entrapment efficiency

Lamivudine content in the microbeads was estimated by a UV-spectrophotometric method [10, 11]. Accurately weighed 100mg of microbeads (100 mg) were powdered and suspended in 6.8 pH phosphate buffer. The resulting solution was kept for 24hrs. Next day it was stirred for 20min using ultra sonicator. The solution was filtered through a 0.45 μm membrane filter, after suitable

dilution, lamivudine content in the filtrate was analysed at 270nm using UV-Visible spectrophotometer. The obtained absorbance was plotted on the standard curve to get the exact concentration of the entrapped drug. Calculating this concentration with dilution factor the percentage of actual drug encapsulated in microbeads was calculated. The drug entrapment efficiency was determined using following relationship

The yield was calculated.

$$\text{Percentage yield} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

***In Vitro* Drug Release Studies**

In vitro dissolution studies were performed by using USP type I dissolution apparatus at 75 rpm [7]. The microbeads were weighed and filled in the empty capsule shells and placed in the basket. The dissolution medium (900ml) consisted of 0.1M hydrochloric acid for the first 2 hours and then changed to phosphate buffer pH 7.4 from 2nd to 10th hour. The temperature was maintained at 37°C ± 5°C. An aliquot (5 mL) was withdrawn at specific time intervals and replenished with an equivalent volume of dissolution fluid. Drug content was determined by UV – visible spectrophotometer at 270 nm.

Kinetics of drug release

The suitability of several equations to identify the mechanisms for the release of drug was tested with respect to the release data, and it was found that up to 50% of drug was released. The drug release data of the *in-vitro* dissolution study was analyzed with various kinetic equations like zero-order [12] (% release v/s time), firstorder [13] (Log % retained v/s time), Higuchi model [14] and korsmeyer and peppas equation [15]. Coefficient of correlation (r) values were calculated for the linear curves obtained by regression analysis of the above plots.

The value of 'n' gives an indication of the release mechanism; when n = 1, the release rate is independent of time (zero-order) (case II transport), n = 0.5 for Fickian diffusion and when 0.5 < n <1.0, diffusion and non-Fickian transport are implicated. Lastly, when n > 1.0 super case II transport is apparent. *K* values are release rate constants according to the models considered; R^2 values are determination Coefficients; and *n* is the exponent of the korsmeyer-peppas model.

Determination of stability of the microbeads

The microbeads prepared in the present study were filled in the hard gelatin capsules and stored in HDPE containers at 40°C/75% RH for 3 months as per ICH guidelines [22]. The samples were then characterized for % drug content.

RESULTS AND DISCUSSION

Preparation of microbeads

Microbeads of lamivudine were prepared (eight formulations) by ionotropic gelation technique and the formulations were evaluated for different parameters. The compositions of the alginate micro beads were given in the Table-1.

TABLE-1: The compositions of the alginate micro beads

Formulation code	Sodium alginate (w/v)	Calcium chloride (w/v)	Chitosan (w/v)	Sodium carboxy methyl cellulose (w/v)	HPMC (w/v)	Drug (Lamivudine) (w/v)
F1	3%	5%	_	_	_	1%
F2	4%	5%	_	_	_	1%
F3	3%	5%	_	0.5%	_	1%
F4	3%	6%	_	1%	_	1%
F5	3%	5%	_	_	0.5%	1%
F6	3%	6%	_	_	1%	1%
F7	3%	5%	0.5%	_	_	1%
F8	3%	6%	1%	_	_	1%

Characterization of microbeads

Granulometric studies

In the granulometric study, it was observed from the table that about 65 – 81 percent of microbeads were of 16 mesh size, which proves the flexibility of the method. The size distribution of microparticles was reported in Table-2.

Table - 2: Granulometric Study

Formulation code	#12 (1.68 mm) 1190-1680µm	#16 (1.19 mm) 840-1190µm	#20 (0.84 mm) 590-840 µm	#30 (0.59 mm) 297-590 µm
F-1	16.19±0.415	6.54±0.310	8.25±0.215	4.89±0.185
F-2	14.77±2.193	8.15±0.102	9.22±0.526	3.78±0.456
F-3	15.95±0.826	9.25±0.427	7.21±0.264	2.85±0.156
F-4	19.85±0.152	11.92±0.670	6.84±0.816	3.52±0.156
F-5	24.01±0.810	9.15±0.536	6.23±0.011	2.05±0.188
F-6	27.55±0.311	10.88±0.115	7.14±0.483	3.71±0.882
F-7	20.22±0.402	9.01±0.045	5.88±0.151	2.85±0.456
F-8	24.25±0.692	10.25±0.340	6.10±0.484	2.93±0.185

Values are mean±SD, n=3.

In this prepared lamivudine microbeads, with the increase in HPMC percentage the distribution of particle size shifts to the higher sieve size due to increase in the internal viscosity of the medium.

Micrometric properties of the beads

Lamivudine containing microbeads were in the size range of 0.77±0.76 to 0.98±0.94. Lamivudine -loading amount, stirring speed, curing time, polymer concentration and cross-linking agent seemed to affect the values of particle size. The results were shown in table No.3.

Flow properties of Beads

The flow property of the microbeads was checked by using the angle of repose method. Acceptable range of angel of repose was found to be 24 – 31˚. All the formulations angle of repose values were shown in the Table - 3.

Table - 3: flow property, entrapment efficiency, mean diameter of particle of microbeads

FORMULATION CODE	ANGLE OF REPOSE	ENTRAPMENT EFFICIENCY	DRUG CONTENT	MEAN DIAMETER OF PARTICLE (mm)
F1	31°.20' ±0.11	82.14±0.59	11.65±0.12	0.79±0.02
F2	28°.20'±0.17	89.60±0.16	11.96±0.58	0.77±0.12
F3	25 °.10'±0.22	90.19±0.86	12.01±0.28	0.97±0.02
F4	24 °.30'±0.25	86.16±0.15	13.25±0.35	0.98±0.08
F5	26 °.60'±0.28	91.61±0.22	12.22±0.62	0.86±0.18
F6	24 °30'±0.54	94.67±0.54	13.45±0.48	0.89±0.38
F7	24°30'±0.21	90.10±0.34	12.94±0.25	0.85±0.65
F8	26 °30'±0.57	92.23±0.28	14.22±0.12	0.93±0.28

Values are mean±SD, n=3.

Drug entrapment efficiency & Drug content

The drug content of prepared formulations was found to be in the range of 89.15 – 98.47%. The drug entrapment efficiency of all the formulations was in the range of 82.14– 94.67. The results of the each formulation drug entrapment efficiency values were shown in Table No: 03. Drug entrapment efficiency of microbeads values of the different formulations were observed and reported, as increases the concentration of sodium alginate and hydroxyl propyl methyl cellulose automatically drug entrapment efficiency also increases and drug content ranges from 11.65 to 14.22 mg were shown in table-3.

In Vitro Drug Release Studies

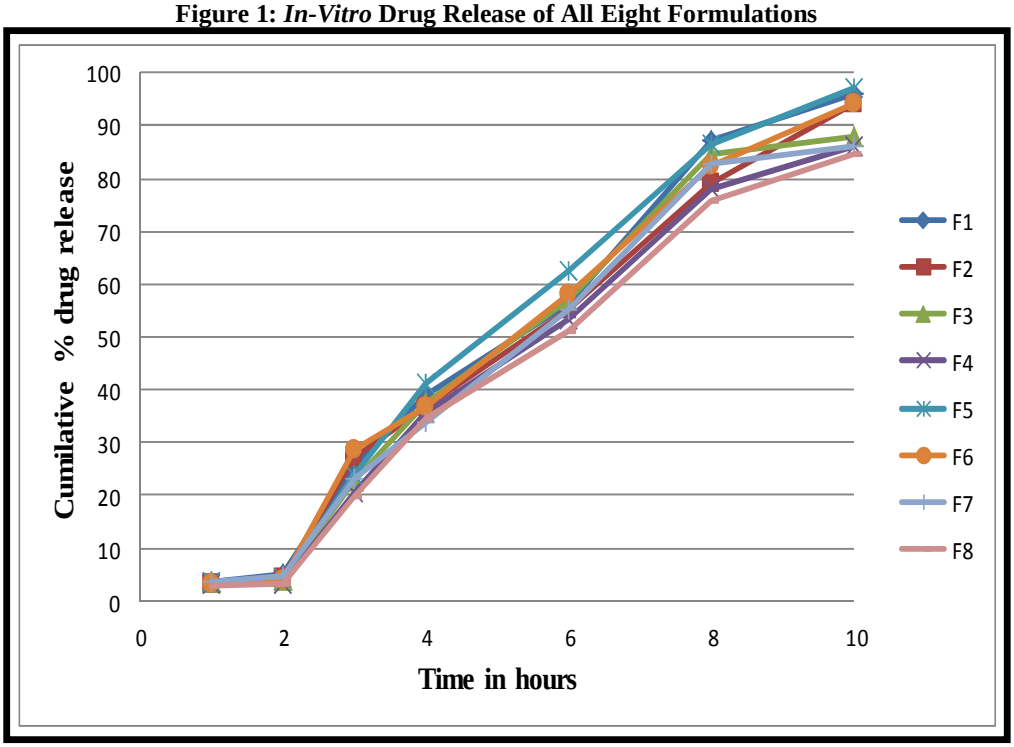
The In-Vitro drug release studies of the different formulations cumulative percentage drug release was observed in the range of 84.63– 97.17. The release studies were conducted in triplicate and the results were shown in Table 4 and in Figure-1. The formulations F1, F2 containing 1, 2% sodium alginate respectively showed a release of 96.12, 94.15% after 10 hours. This shows that more sustained release was observed with the increase in percentage of sodium alginate. The formulations F3, F4 containing 3% sodium alginate and 0.5, 1% sodium carboxy methyl cellulose showed 88.12, 86.17 % drug release respectively. The formulations F5, F6 containing 3% sodium alginate and 0.5, 1% hydroxyl propyl methyl cellulose showed a release of 97.17, 94.01% after 10 hours. Finally the formulations F7, F8 containing 3% sodium alginate and 0.5, 1% Chitosan showed a release of 97.17, 94.01% after 10 hours.

Table - 4: *In-Vitro* Dissolution Profile for Formulation F1 TO F8

Time (hrs)	% Drug Release							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
1	3.42±0.04	3.32±0.122	3.54±0.06	3.11±0.09	3.55±0.02	3.32±0.012	3.55±0.13	2.92±0.15
2	4.92±0.12	4.24±0.02	3.85±0.16	3.22±0.05	4.24±0.05	3.96±0.021	4.77±0.09	3.23±0.05
3	25.55±0.08	27.15±0.78	22.55±0.08	20.55±0.22	23.95±0.07	28.56±0.04	23.25±0.12	19.77±0.06
4	38.97±0.12	36.84±0.12	37.32±0.07	35.67±0.12	41.27±0.12	36.84±0.18	33.85±0.22	34.52±0.20
6	56.22±0.17	55.13±0.06	57.14±0.09	53.25±0.21	62.45±0.10	58.25±0.11	55.22±0.11	51.11±0.17
8	87.39±0.23	79.17±0.46	84.55±0.12	77.85±0.05	86.36±0.15	82.57±0.18	82.78±0.15	75.78±0.02
10	96.12±0.51	94.15±0.08	88.12±0.115	86.17±0.01	97.17±0.15	94.29±0.01	86.22±0.18	84.63±0.12

Values are mean±SD, n=3

Figure 1: *In-Vitro* Drug Release of All Eight Formulations



Maximum release of lamivudine from the various formulations was achieved in 12 hrs or longer, Figure1. Formulation F8 showed sustained drug release, when compared to all other formulations.

Kinetics of drug release

According to results obtained, the 'n' value Korsmeyer- Peppas equation was in range of 0.910 to 0.905 which suggest the drug release from mixture of polymers and Na cmc containing Microbeads were non-fickian diffusion mechanism. Similarly 'n' value for hydroxyl propyl methyl cellulose was in range of 0.917 to 0.904 which suggests the drug release was non-fickian diffusion mechanism; similarly 'n' value for chitosan was in range of 0.935 to 0.912 which suggests the drug release was non-fickian diffusion mechanism super case II transport evidenced with diffusion exponent values (n).

In vitro release data proved that formulation F8 having 1% Sodium alginate and 1% chitosan as polymers showed more optimum sustained release profile with maximum entrapment efficiency followed by zero-order kinetics. The release kinetics was shown in Table-5.

The values of diffusion co-efficient ranged between n=1.557 and 1.666 indicating the drug release from the microbeads followed the anomalous transport and super case-II transport mechanism controlled by swelling and relaxation of polymer chains.

Table-5: Drug Release Kinetics Data

Formulation	Zero order	First order	Korsermayer-Peppa's	
	R	R	r	N
F1	0.978	0.781	0.934	1.599
F2	0.984	0.867	0.915	1.603
F3	0.967	0.841	0.910	1.609
F4	0.976	0.872	0.905	1.657
F5	0.976	0.835	0.917	1.628
F6	0.978	0.893	0.904	1.626
F7	0.970	0.935	0.935	1.557
F8	0.979	0.853	0.912	1..666

Determination of stability of the microbeads

The stability of microbeads was determined. The samples were then characterized for % drug content and from the results it can be observed that there was no significant change in the % drug content of the formulations [22]. The results were summarized in Table-6.

Table- 6: results of assay for formulations F-1 to F-8 after accelerated stability studies.

Days	% Drug Release, 40°c							
	F1	F2	F3	F4	F5	F6	F7	F8
1	95.16±0.01	92.24±0.10	94.34±0.08	95.46±0.12	92.13±0.81	97.12±0.15	97.22±0.12	98.46±0.05
4	94.50±0.09	93.23±0.01	97.16±0.15	96.14±0.15	95.12±0.15	98.52±0.07	95.16±0.05	96.14±0.18
17	93.50±0.10	92.65±0.12	96.85±0.88	95.85±0.47	94.26±0.45	95.26±0.07	93.85±0.08	95.55±0.14
21	93.23±0.12	92.25±0.54	96.25±0.76	95.54±0.05	93.62±0.18	94.64±0.08	93.25±0.07	95.14±0.18
38	92.89±0.01	91.73±0.18	95.75±0.07	93.57±0.18	91.28±0.15	93.82±0.04	92.75±0.48	93.57±0.17
45	92.54±0.11	91.25±0.78	95.21±0.16	90.15±0.78	89.52±0.13	91.82±0.18	91.21±0.18	92.15±0.08

Values are mean± SD, n=3.

Scanning electron microscopy

Morphological details of the specimens were determined by using a scanning electron microscope (SEM). The morphology of the microbeads was shown in Figure 2. Scanning electron microscopy (SEM) results (Figure 2) show that the microbeads were spherical and that the microbeads prepared with Chitosan had a formed smooth surface.

FTIR study

The FTIR study revealed that the scanning range was 400-4000cm^{-1}, resolution was 4cm^{-1}. Spectra of the Lamivudine, and the drug with sodium alginate were obtained and compared for the compatibility. FTIR studies indicates four bands present in the lamivudine spectrum, namely; N-H, O-H, C=O, C=N linkages respectively. The same bands were also found in the spectra of the formulations, showing that no drug-polymer interaction occurred. The figure clearly shows that there was no significant compatibility between the drug and polymer used for the preparations. The FTIR graphs are shown in Figure 3 and 4.

FIGURE 2: SCANNING ELECTRON MICROGRAPHS OF LAMIVUDINE MICROBEADS 1(a), 1(b), AND 1(c) FORMULATIONS F3, F6, F7

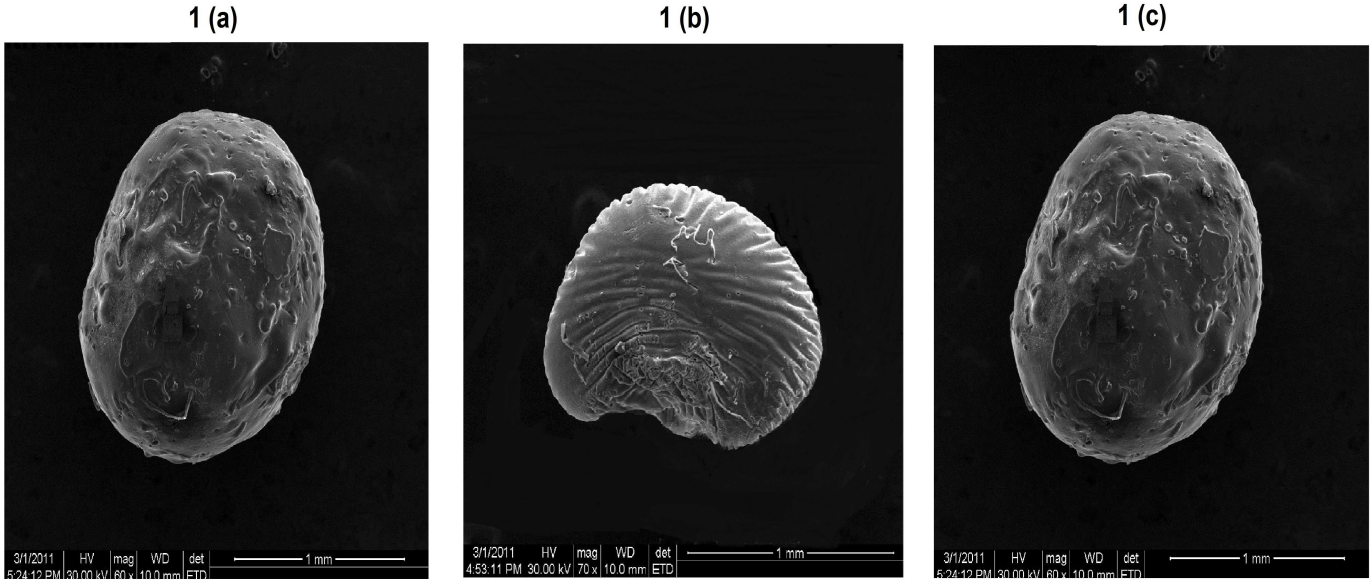


FIGURE 3: FTIR FOR PURE DRUG, SODIUM ALGINATE, HPMC AND NA CMC

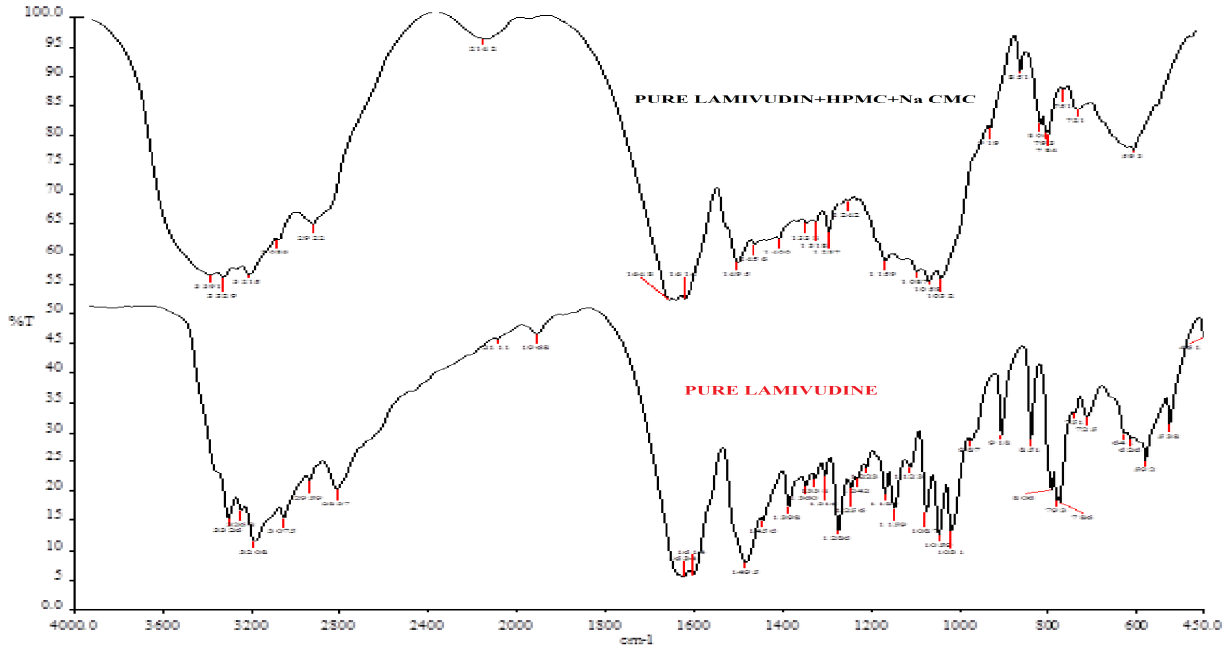
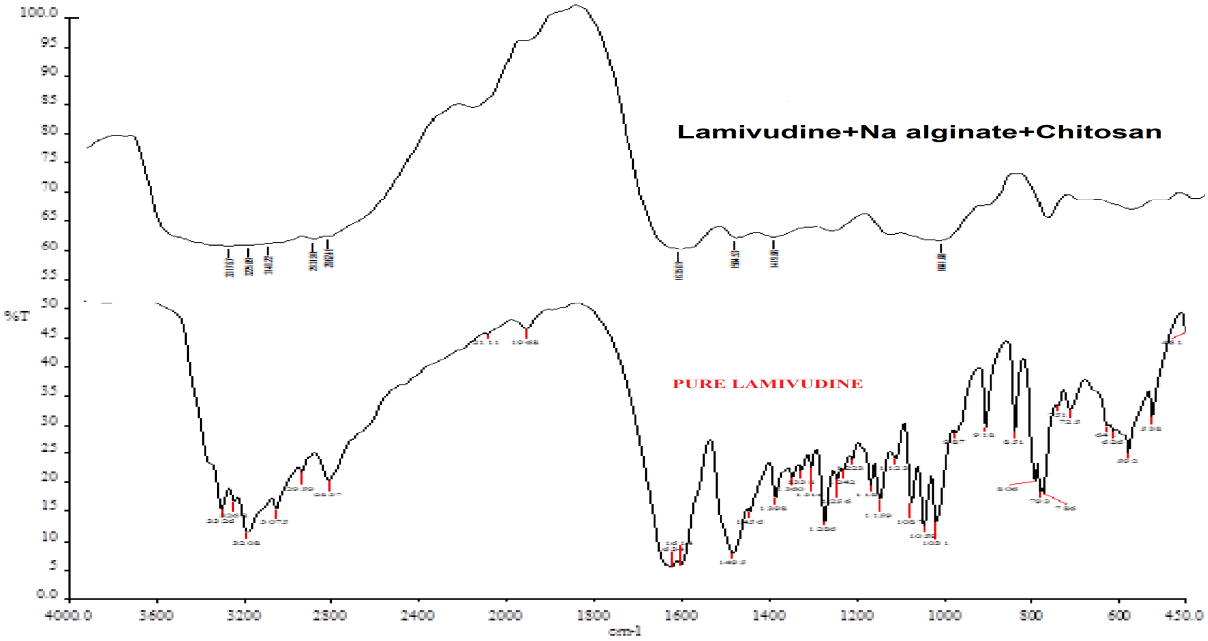


FIGURE 4: FTIR FOR PURE DRUG, SODIUM ALGINATE AND CHITOSAN



CONCLUSION

In the present study an attempt was made to prepare lamivudine microbeads by using Ionotropic gelation technique. The method was successfully used for preparation of microbeads using sodium alginate and with other coating polymers like Chitosan, Na CMC, HPMC, as drug release modifiers. The lamivudine drug release from microbeads formulated with sodium alginate with 1% Chitosan as coating polymer showed a satisfactory sustained release profile. As the formulation F8 was the best formulation based on better controlled release and good entrapment efficiency. High entrapment efficiency was observed in the microbeads prepared with combination of sodium alginate and Chitosan. The results of accelerated stability study on the microbeads revealed that the formulations were stable. The study further supported with FTIR study for absence of compatibility with drug and polymer. The results proved that the lamivudine microbeads can be best alternate for conventional tablets for HIV patients' treatment.

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