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FORMULATION, CHARACTERIZATION AND INVITRO EVALUATION OF LAMIVUDINE MICROSPHERES FOR SUSTAINE RELEASE

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ABSTARCT

In the present work, Microspheres of Lamivudine using Sodium alginate, Chitosan, Eudragit as polymers were formulated to deliver Lamivudine. The results of this investigation indicate that solvent evaporation method can be successfully employed to fabricate Lamivudine microspheres. FT-IR spectra of the Drug and optimised revealed that the drug is compatible. Micrometric studies revealed that the mean particle size of the prepared microspheres and are suitable for microspheres for oral administration. Increase in the polymer concentration led to increase in % Yield, % Drug entrapment efficiency, Particle size, % swelling. The invitro drug release decreased with increase in the polymer concentration. Analysis of drug release mechanism showed that the drug release from the formulations followed zero order release kinetics. Based on the results of evaluation tests formulation coded F4 was concluded as best. formulation.

KEYWORDS: Lamivudine, Sodium alginate, Chitosan, Eudragit, Microspheres.

INTRODUCTION

Oral route drug administration is by far the most preferable route for taking medications. However, their short circulating half life and restricted absorption via a defined segment of intestine limits the therapeutic potential of many drugs. Such a pharmacokinetic limitation leads in many cases to frequent dosing of medication to achieve therapeutic effect.[1]

Novel drug delivery systems (NDDS) offer many advantages, which include improved therapy by increasing the efficacy and duration of drug activity, increased patient compliance through decreased dosing frequency and convenient routes of administration, and improved targeting for a specific site to reduce unwanted side effects. The challenge for both drug and drug delivery companies is to deliver both existing and emerging drug technologies in a manner that improves the current benefits enjoyed by the patients.[2-3] Microencapsulation is used to modify and retard drug release. Due to small particle size of microspheres, they are widely distributed throughout the gastrointestinal tract which improves drug absorption and reduces side effects due to localized build-up of irritating drugs against the gastrointestinal mucosa. Microspheres are small spherical particles, with diameters 1 µm to 1000 µm. They are spherical free flowing particles consisting of proteins or synthetic polymers which are biodegradable in nature.[4-5] There are two types of microspheres; microcapsules and micromatrices, which are described as, Microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall. and micromatrices in which entrapped substance is dispersed throughout the matrix. Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Microsphere play an important role to improve bioavailability of conventional drugs and minimizing side effects. Ideal characteristics of microspheres.[6-7]

Lamivudine (2′,3′ -dideoxy-3′ -thiacytidine) is an antiretroviral medicine used to avoid and treat HIV/AIDS and used to treat perpetual hepatitis B. It is of the nucleoside simple opposite transcriptase inhibitor (NRTI) class. It can hinder both sorts (1 and 2) of HIV reverse transcriptase.[8] It is phosphorylated to dynamic metabolites that go after fuse into viral DNA. They hinder the HIV reverse transcriptase protein aggressively and go about as a chain eliminator of DNA blend. The absence of a 3'- OH bunch in the joined nucleoside simple keeps the arrangement of the 5' to 3' phosphodiester linkage crucial for DNA chain stretching, and in this manner, the viral DNA development is ended. Fundamental purpose behind determination of this medication is low biological half-life, less protein binding, reduce the harmful impacts, diminish the measurements and expansion the patient consistence.[9]

MATERIALS AND METHODS

Lamivudine was a gift sample from Karnataka Antibiotics, Bangalore. Sodium alginate, Chitosan, Eudragit, dichloromethane, sodium lauryl sulphate. Were purchased from A.R. Loba Chemical Pvt. Ltd, Mumbai. All other chemicals used were of L.R. grade.

Table 1: Formulation of Lamivudine Microspheres.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Lamuvidine	100	100	100	100	100	100	100	100	100	100	100	100
Sodium Alginate	25	50	75	100	-	-	-	-	-	-	-	-
Chitosan	-	-	-	-	25	50	75	100	-	-	-	-
Eudragit	-	-	-	-	-	-	-	-	25	50	75	100
DCM(ML)	15	15	15	15	15	15	15	15	15	15	15	15
Methanol	50	50	50	50	50	50	50	50	50	50	50	50
SLS	20	20	20	20	20	20	20	20	20	20	20	20

Solvent Evaporation Method

Lamivudine microspheres were prepared using Sodium Alginate, Chitosan, Eudragit and distilled water as continuous phase by solvent evaporation technique. Initially dichloromethane (DCM) and methanol was mixed uniformly at room temperature, then Sodium Alginate, Chitosan, Eudragit in various proportions was dissolved in the above solution. To this mixture, a drug solution corresponding was added and mixed thoroughly and injected drop wise in to the continuous phase consisting of 100mL of 0.2% (w/v) SLS (sodium lauryl sulphate) at 250 rpm.[10] The microspheres obtained was washed for 2-3 times with distilled water and dried at room temperature.

Characterization Of Microspheres

FTIR: The physical properties of the physical mixture were compared with those of plain drug. Samples was mixed thoroughly with 100mg potassium bromide powder and compacted under vacuum at a pressure of about 12 psi for 3 minutes.[11] The resultant disc was mounted in a. suitable holder. IR spectrum was recorded from 4000 cm-1 to 400 cm-1.

Bulk density: In this method microspheres are transferred to a measuring cylinder and is tapped till a constant volume obtained. This volume is bulk volume and it includes true volume of the powder.

Tapped density: The tapped density is measured as the transferred the microspheres in the cylinder and fix to the tap density apparatus and the tapping is performed about 100 times.[12]

Angle of repose: Angle of repose (θ) of the microspheres, which measures the resistance to particle flow, was determined by a fixed funnel method. The height of the funnel was adjusted in such a way that the tip of the funne just touches the heap of the blends. Accurately weighed microspheres were allowed to pass through the funnel freely on to the surface. The height and radius of the powder cone was measured and. Angle of repose was calculated.

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

Where, h = height; r = radius

Determination of % yield of microspheres: The dried microspheres were collected and weighed accurately. The percentage yield was then calculated using formula given below.

$$\% \text{ Yield} = \frac{\text{Mass of micro -spheres obtained}}{\text{Total weight of drug and polymer}} \times 100$$

Drug entrapment efficiency: Weighed amount of microspheres (100 mg) with phosphate buffer pH 7.4 (10 ml) was added in a vial. The solution was stirred vigorously for 24 hours with mechanical stirrer. Supernatant collected by centrifugation and drug content in supernatant was determined by using UV spectrophotometer at wavelength 270nm. The amount of drug entrapped in the microspheres was calculated by the following formula.[13]

$$\%\,\text{Drug Entrapment Efficiency} = \frac{\text{Experimental Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

SEM (Scanning Electron Microscope) studies: The surface morphology of the layered sample was examined by using SEM(JEOL Ltd., Japan). The small amount of powder was manually dispersed onto a carbon tab (double adhesive carbon coated tape) adhered to an aluminium stubs were coated with a thin layer (300A) of gold by employing POLARON - E 3000 sputter coater. The samples were examined by SEM with direct data capture of their mages on computer.

***Invitro* drug release study**: The dissolution studies were performed in a fully calibrated eightstation dissolution test. Apparatus (37± 0.50C, 50 rpm) using the USP type – I rotating basket method with pH 7.4 Phosphate buffer. A quantity of accurately weighed microspheres

equivalent to 100mg was employed in all dissolution studies. Aliquots of sample were withdrawn at predetermined intervals. For drug release by measuring the absorbance at 270nm. At the same time the volume withdrawn at each time intervals were replenished immediately with the same volume of fresh pH 7.4 Phosphate buffer maintaining. Sink conditions throughout the experiment.

RESULTS AND DISCUSSION

FTIR

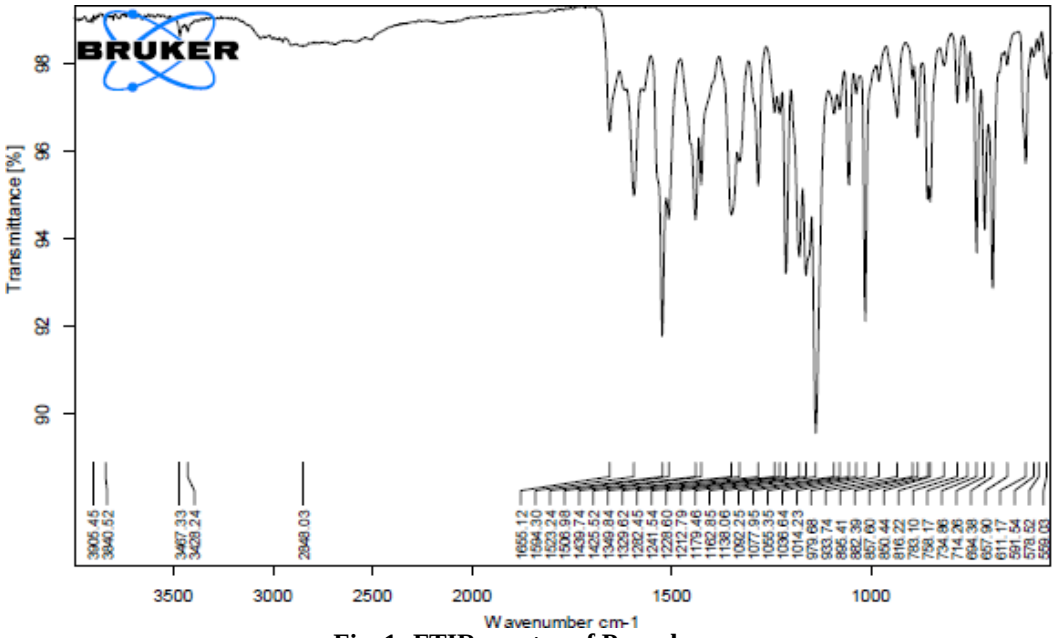

Fig. 1: FTIR spectra of Pure drug.

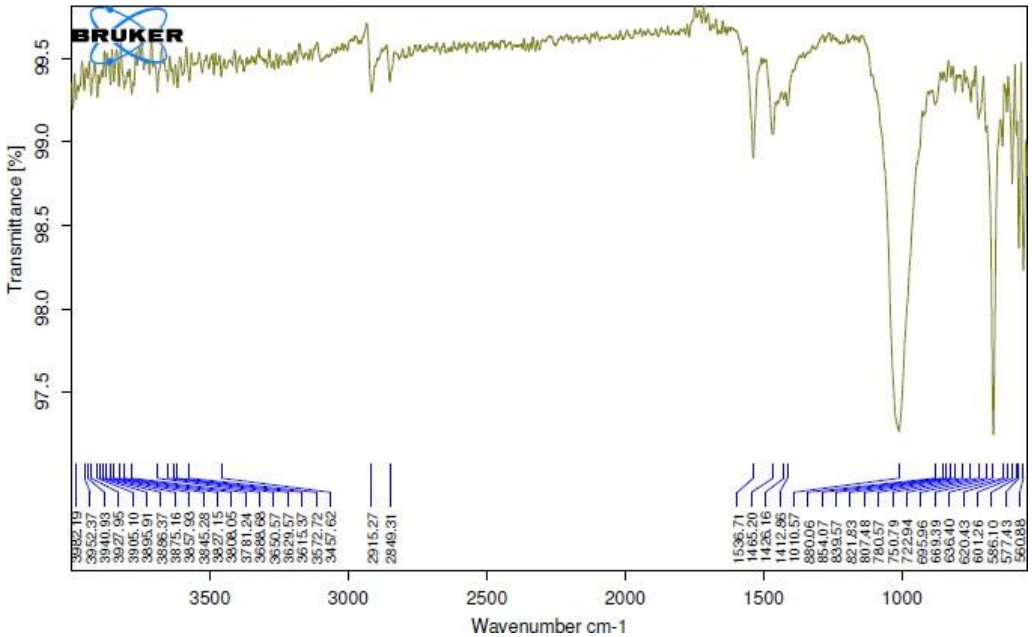

Fig. 2: FTIR spectra of Optimized formulation.

All the characteristic peaks of Lamivudine and were also found in the spectrum formulations. The results suggest that the drug is intact in the formulations and there is no interaction found between the drug and the excipients.

The mean size increased with increasing polymer concentration which is due to a significant increase in the viscosity, thus leading to an increased droplet size and finally a higher microspheres size. Microspheres containing Sodium Alginate as a polymer had a size range of 314.26±1.68 µm to 487.3±2.71 µm. microspheres containing Chitosan as polymer exhibited a size range between 410.31±1.42 µm to 461.2±1.17µm. Microspheres containing Eudragit as copolymer had a size range of 361.12±2.14 µm to 428.41±3.47 µm.

The particle size data is presented in Table 2. The particle size as well as % drug entrapment efficiency of the microspheres increased with increase in the polymer concentration. The bulk density of formulation F1 to F12 containing Sodium Alginate, Chitosan, and Eudragit formulation was in the range of 0.291 ±0.01 to 0.417 ±0.02 gm. /cm3 as shown in table 3, tapped density 0.304 ± 0.01 to 0.430 ± 0.02 and hausner's ratio 1.026 ± 0.01 to 1.045 ± 0.01. The Carr's index of formulation F1 to F12 containing different grades of Sodium Alginate, Chitosan, Eudragit 1.037 ± 0.01 to 4.309 ± 0.03 respectively. The angle of repose of formulation F1 to F12 containing Sodium Alginate, Chitosan, Eudragit formulation was in the range <23 respectively as shown. In table 2. The values of carr's index and angle of repose indicate good flow properties.

Table 2: Flow properties of Lamivudine microspheres.

Formul ation code	Mean partical size	Bulk. density ((gm./cm3))	Tapped density (gm./cm3)	Hauseners ratio	Carr's index	Angle of repose
F1	314.26±1.68	0.351 ±0.01	0.364 ± 0.01	1.037 ± 0.01	35.71 ± 0.02	20.321 ±0.16
F2	415.9±1.18	0.291 ±0.01	0.304 ± 0.01	1.045 ± 0.01	43.09 ± 0.03	23.942 ±0.15
F3	391.6±2.19	0.373 ±0.01	0.383 ± 0.01	1.026 ± 0.01	26.10 ± 0.01	20.120 ±0.12
F4	487.3±2.71	0.318 ±0.01	0.329 ± 0.01	1.034 ± 0.01	34.59 ± 0.02	17.108 ±0.15
F5	410.3±1.42	0.417 ±0.02	0.428 ± 0.02	1.026 ± 0.01	25.70 ± 0.01	16.926 ±0.13
F6	461.2±1.17	0.386 ±0.01	0.398 ± 0.01	1.031 ± 0.01	29.17 ± 0.02	17.181 ±0.13
F7	432.1±1.27	0.307 ±0.01	0.318 ± 0.01	1.037 ± 0.01	36.11 ± 0.01	21.170 ±0.12
F8	448.3±1.55	0.406 ±0.02	0.419 ± 0.02	1.032 ± 0.01	31.02 ± 0.02	16.812 ±0.12
F9	398.5± 2.64	0.383 ±0.01	0.397 ± 0.01	1.036 ± 0.01	34.29 ± 0.02	16.909 ±0.13
F10	378.1±1.25	0.327 ±0.01	0.339 ± 0.01	1.037 ± 0.01	35.39 ± 0.01	16.537 ±0.09
F11	428.4±3.47	0.416 ±0.02	0.430 ± 0.02	1.034 ± 0.01	32.58 ± 0.02	16.921 ±0.11
F12	361.1±2.14	0.372 ±0.01	0.387±0.01	1.040 ± 0.01	30.37 ± 0.01	17.103 ±0.12

It was observed that as the polymer ratio in the formulation increases, the product yield also increases. The low percentage yield in some formulations may be due to blocking of needle and wastage of the drug-polymer solution, adhesion of polymer solution to the magnetic bead and microspheres lost during the washing process. The percentage yield was found to be in the range. Percentage Drug entrapment efficiency of Lamivudine ranged from 62.15 to 95.18 % for microspheres containing sodium alginate, Chitosan and Eudragit polymer, The drug entrapment efficiency of the prepared microspheres increased progressively with an increase in proportion of the respective polymers. Increase in the polymer concentration increases the viscosity of the dispersed phase. The particle size increases exponentially with viscosity. The higher viscosity of the polymer solution at the highest polymer concentration would be expected to decrease the diffusion of the drug into the external phase which would result in higher entrapment efficiency. The % drug entrapment efficiency of the prepared microspheres was displayed in Table 3.

Table 3: % **Yield, drug entrapment efficiency of the prepared microspheres.**

S. No.	Formulation code	% yield	Drug Content (mg)	Drug entrapment efficiency
1	F1	91.88	98.12	74.75
2	F2	92.11	99.64	91.14
3	F3	91.69	96.15	70.16
4	F4	94.78	99.67	85.77
5	F5	95.41	97.48	92.68
6	F6	91.25	99.81	68.48
7	F7	90.23	99.34	95.18
8	F8	96.11	98.60	77.85
9	F9	92.17	99.11	62.15
10	F10	93.64	95.92	85.61
11	F11	90.12	98.29	90.49
12	F12	86.31	99.83	78.25

The microspheres were found to be discrete, spherical and free flowing. The nature of the microspheres indicates that the microspheres were multinucleated and monolithic type.

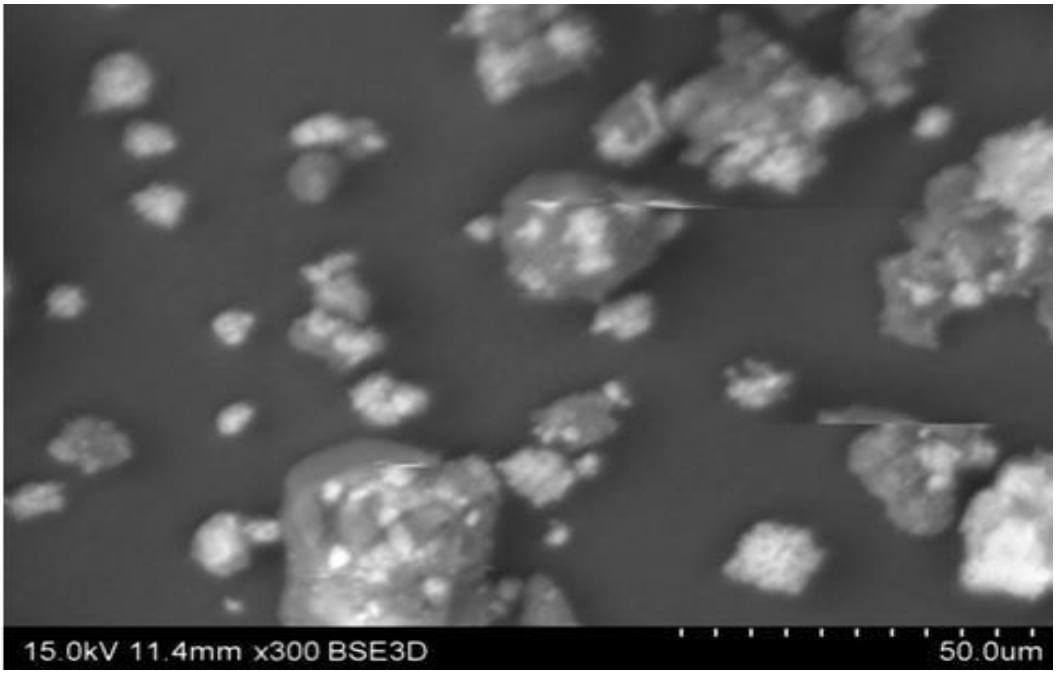

Fig. 3: SEM of Microspheres.

Dissolution studies of all the formulations were carried out using dissolution apparatus USP type I. The dissolutionstudies. Were conducted by using dissolution media, pH 1.2. The results of the in-vitro dissolution studies of formulations F1to F12 are shown in the plots. The formulations F1, F2, F3 and F4 containing Sodium Alginate showed a maximum releaseof 96.73% at 8hours and 91.47% after12 hours respectively. The formulations F5, F6, F7 and F8 containing Chitosan polymer showed maximum releaseof 95.18% and 94.68% after12 hours respectively. The formulations F9,F10,F11and F12 containing Eudragit polymer showed maximum release of 93.97% and 93.97% after 12 hours respectively. Invitro drug release from all the formulation was found to be slow and sustained over the period of 12 hours, among other formulation F4 showed better sustained release pattern.

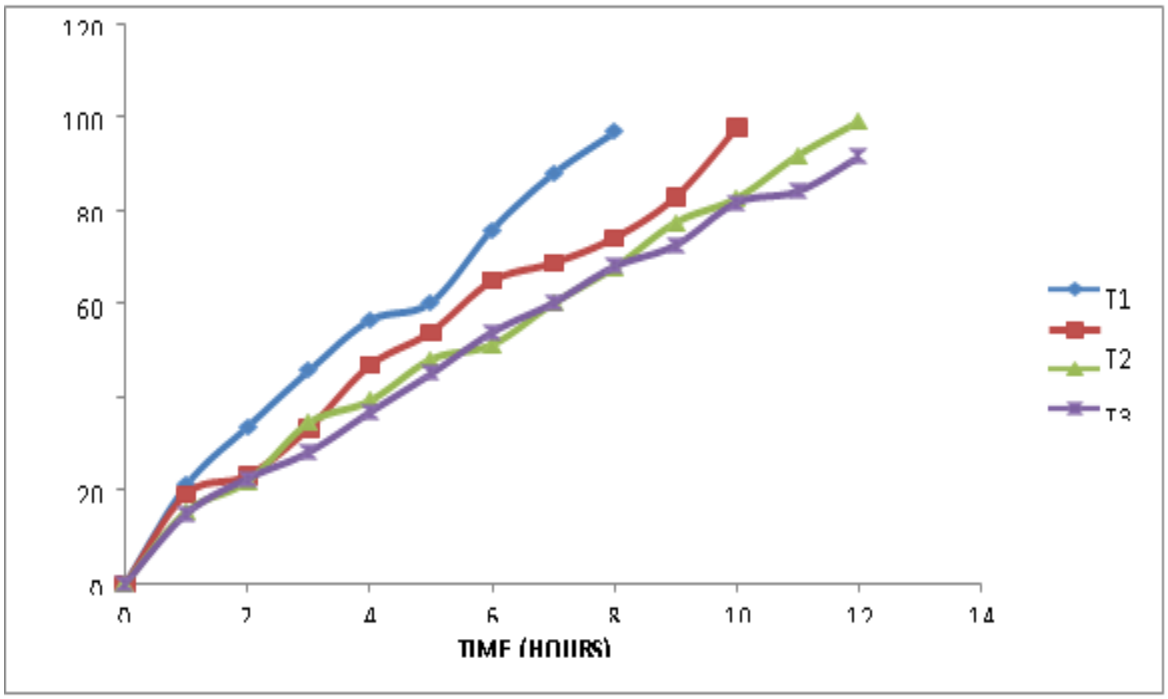

Fig. 4: Drug release profile of Lamivudine microspheres containing sodium alginate.

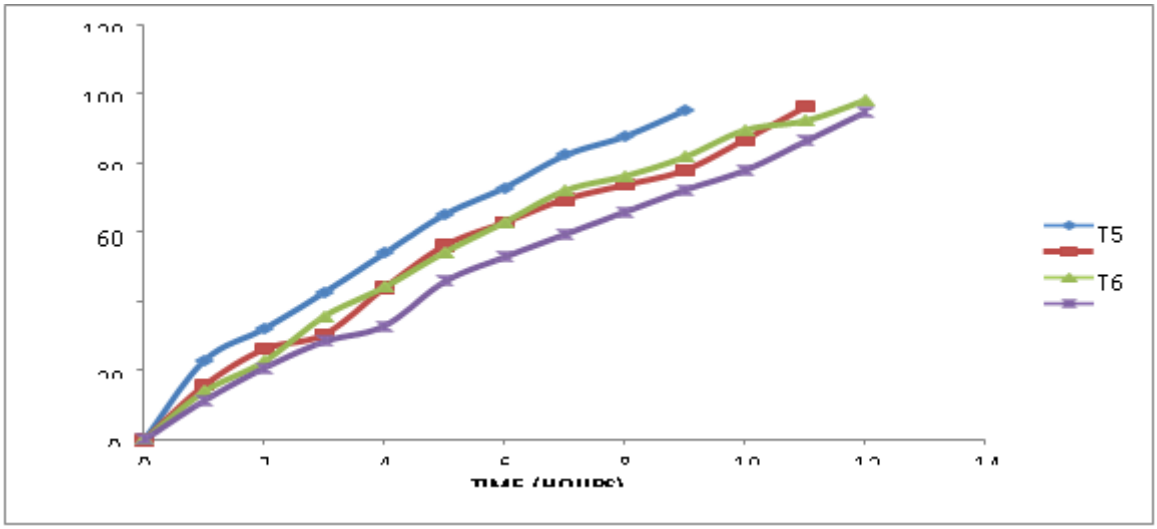

Fig. 5: Drug release profile of Lamivudine microspheres containing Chitosan.

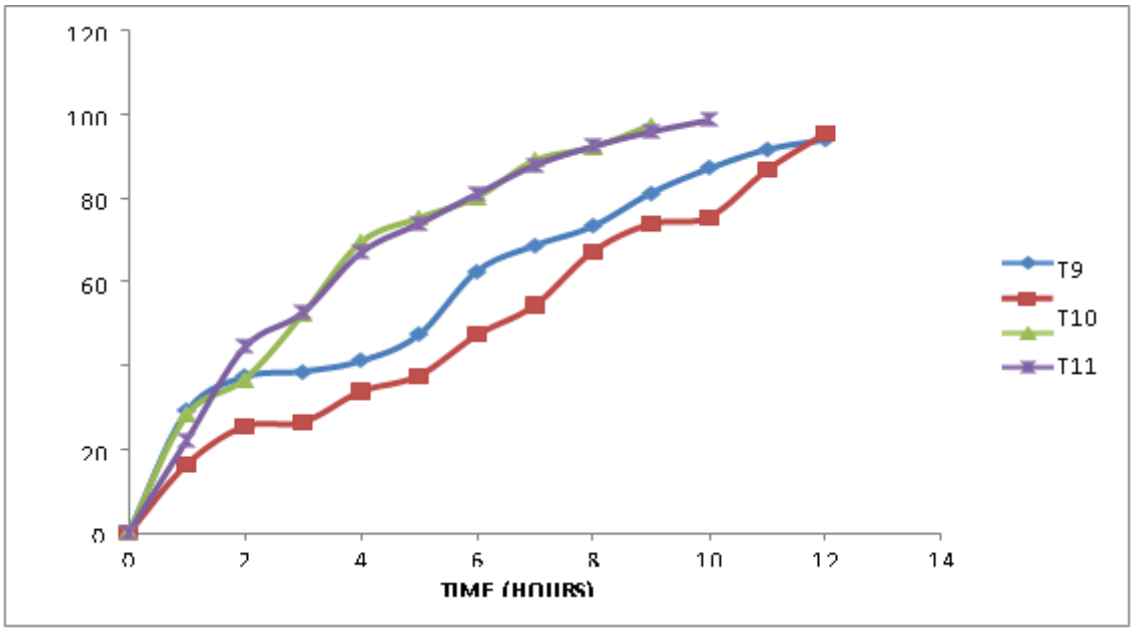

Fig. 6: Drug release profile of Lamivudine microspheres containing Eudragit.

CONCLUSION

In the present work, microspheres of Lamivudine using Sodium alginate, Chitosan, Eudragit as polymers were formulated for sustained drug release. Based on the results of evaluation tests formulation F4 was concluded as best formulation. Hence it can be concluded that Lamivudine loaded Sodium alginate microspheres may be useful to achieve sustained drug release.

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