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Research Article

FORMULATION AND EVALUATION OF STAVUDINE LOADED SODIUM ALGINATE BEADS BY IONOTROPIC GELATION METHOD

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ABSTRACT
The objective of the present study was to develop hydrophilic polymer and hydrophobic polymer based novel control release Stavudine beads for achieving the action up to 12 h and to characterize the efficacy and to analyze the effect of various polymers. The Stavudine beads were prepared by ionotropic gelation method from sodium alginate solution containing HPMC and ethyl cellulose in various ratios in order to get the required theoretical release profile. The result of FTIR spectra and DSC study indicated the stability and compatibility of the drug with the polymers used. The standard curve has been observed in both 0.1 N HCl and phosphate buffer pH 6.8 which showed a regression of 0.990 and 0.993 respectively. When observed through optical microscopy, the particle sizes ranged from 5.52 mm to 8.00 mm. The maximum amount of drug content was found to be 47.3 mg. From the results, it was concluded that the release rate of drug is slow and consistent in drug: polymer (HPMC K4M) ratio of 1:3 than the other formulations. Scanning electron microscopy showed that the beads were spherical with smooth surface. The percentage of encapsulation efficiency and *in-vitro* drug release of the best batch F4 was 94.62 % and 66.4 % in 12 h respectively showing a better controlled release of drug. The *in-vitro* release data was treated with mathematical equations, the drug release from the beads were ascertain and found to be First order and the mechanism of drug release followed Peppa's model with non-Fickian equation.
Keywords: Stavudine, sustained release, HPMC, ethyl cellulose.

INTRODUCTION

The most disastrous of its time is acquired immunodeficiency syndrome (AIDS). Along with the numerous efforts of scientific community and intervention of WHO to control the disease, the gross death due to human immunodeficiency virus (HIV) infection has increased up to 25 millions[1]. Stavudine is the FDA approved drug for clinical use for the treatment of HIV infection, AIDS and AIDS-released conditions either alone or combination with other antiviral agents. Stavudine is typically administered orally as a capsule and an oral solution. The virustatic drug has a very short half life (0.8 to1.5 h). However, patients receiving Stavudine develop neuropathy and lactic acidosis. The side effects of Stavudine are dose dependent and a reduction of the total administered dose reduce the severity of the toxicity[2]. The drug is freely soluble in water and hence selections of both hydrophobic and hydrophilic polymer matrix system are widely used in oral controlled drug delivery to obtain a desirable drug release, patient compliance and cost-effectiveness[3]. Hence in the present study work an attempt has been made to develop sustained release matrix tablet of Stavudine using hydrophilic (HPMC K4M) and hydrophobic (ethyl cellulose) polymers. The drug release for extended duration particularly for highly water soluble drug using hydrophilic matrix system is restricted because of the rapid diffusion of the dissolved drug through hydrophilic network. The objective of the present study was to develop hydrophilic polymer based Stavudine controlled release beads to enhance effectiveness of anti-retroviral therapy.

AIDS and HIV Infection

HIV is a lentivirus and like all viruses of this type, it attacks the immune system. Lentiviruses are part of a larger group of viruses known as retroviruses. They have been found in a number of different animals, including cats, sheep, horses, and cattle. However, as far as the origin of HIV is concerned, the most interesting lentivirus is the simian immunodeficiency virus that affects monkeys. Although HIV came to light in the early 80s, there is evidence that HIV infection was prevalent much earlier. The earliest known instances of HIV infection are as follows.

- A plasma sample taken in 1959 from an adult male living in the Democratic Republic of Congo has been found to be positive of HIV.
- HIV has been found in tissue samples from an American teenager who died in St. Louis in 1969.
- HIV has been found in tissue samples from a Norwegian sailor who died around 1976.[11,12]

History of HIV/AIDS in India

The AIDS pandemic began in India in the mid 1980s. In 1986, 12 commercial sex workers (CSW) tested HIV positive in (Chennai), Tamil Nadu, India. Most of the initial cases had occurred through heterosexual sex; but at the end of the 1980s, a rapid spread of HIV was observed among injecting drug users in Manipur, Mizoram and Nagaland, India.[14] In 1987, a National AIDS Control Programme was launched to coordinate national responses. Its activities covered surveillance, blood screening and health education[15]. In 1992, the government set up NACO (National AIDS Control Organization), to oversee the formulation of policies, prevention work and control programs related to HIV and AIDS. In 2001, the government adopted the National AIDS Prevention and Control Policy. 13 NACP III was launched formally on 6th July 2007.[16]

Table 1: Classification of viruses

Class	Method of Replication	Example
I	Double-stranded DNA viruses	Adenoviruses, Herpesviruses, Poxviruses
II	Single-stranded DNA viruses	Parvoviruses
III	Double-stranded RNA viruses	Reoviruses
IV	Single-stranded positive sense RNA viruses	Picornaviruses, Togaviruses
V	Single-stranded negative sense RNA viruses	Orthomyxoviruses, Rhabdoviruses
VI	Positive-sense single-stranded RNA viruses that replicate through a DNA intermediate	Retroviruses
VII	Double-stranded DNA viruses that replicate through a single stranded RNA intermediate	Hepadnaviruses

MATERIALS AND METHODS

Materials

Stavudine (Aurobindo Pharma Labs. Hyderabad, India), HPMC K4M (SKAN Research Labs (P) Ltd., Pondicherry, India), Ethyl cellulose (SD Fine chemicals Ltd.), Sodium Alginate (Loba Chemie, Mumbai, India), Aluminium sulphate (Qualigents Pvt, Mumbai, India) are used. All the ingredients were of analytical grade.

Methods

Infra red spectra analysis

Infrared spectrum of Stavudine was determined on Fourier Transform Infrared spectrophotometer using KBr dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and potassium bromide was run.[4,5]

Infrared absorption spectrum of Stavudine

The samples were crushed with KBr to make pellets under hydraulic pressure of 600 kg and then the FTIR spectra were recorded between 400 to 4000 cm^{-1}. IR spectrum shows all prominent peaks of Stavudine. The major IR peaks observed in Stavudine were 3423.76 (N-H), 31.69.15 (O-H), 3041.84 (C-H stitching), 2931.90 (C-H Stitching Alkane), 1261.49 (C-O) and 1226.77 (C-N).

Differential Scanning calorimetric analysis

The samples were heated from 0-300ºC at a heating rate of 10ºC/min under argon atmosphere using a micro calorimeter (DSC Q20 V24.4 BUILD 116.USA) and then thermo grams were obtained.

Preparation of beads by ionotropic gelation method[6]

Different bead formulations were prepared by using ionotropic gelation method. Sodium alginate solution was prepared by dissolving in de-ionized water (ions such as calcium, zinc, etc., will not be present which may act as a cross-linking agent) and heated at 60ºC. The pure drug stavudine was uniformly dispersed in sodium alginate solution with continuous stirring. Polymers such as HPMC and ethyl cellulose were added to the above solution in different ratios until a uniform dispersion was obtained. The resultant mixture was mixed thoroughly by continuous stirring to form a homogenous solution. This homogenous solution at a temperature of 40ºC was then introduced into 5 % w/v aluminium sulphate solution (cross-linking agent) using syringes with the needle diameter of 19G and 22G under continuous stirring by employing the magnetic stirrer to increase the mechanical strength of the beads. They were

allowed to rotate for about 1-2 h after which they were separated from the matrix solution and dried.

Evaluation Characteristics of Stavudine Beads

The developed beads were studied for various characterizations such as size and shape analysis, SEM, swelling study, drug content, drug entrapment efficiency, *in-vitro* drug release, and release kinetics as follows.

Morphology and Particle Size

The external morphology of the prepared bead was noted by the use of optical microscope. Particle size of prepared beads was determined using an optical microscope fitted with a stage assembled with a calibrated ocular/eyepiece micrometer. Mean diameter was calculated. About 20 dried beads were measured for calculation of mean diameter.

Table 2: Formulation table of stavudine sustained release beads

Batch Code	Drug (parts)	Sodium alginate (parts)	HPMC (parts)	Ethyl cellulose (parts)
F1	1	3	0	0
F2	1	3	1	0
F3	1	3	2	0
F4	1	3	3	0
F5	1	3	0	1
F6	1	3	0	2
F7	1	3	1	2

SEM Analysis

The beads were mounted onto stubs using double sized adhesive tape and sputter coated with platinum using a sputter coater. The counted beads were observed under SEM (model – JSM, 35 CF, jeol, Japan) at the required magnification at room temperature. The acceleration voltage used was 10 kV with the secondary electron image as a detector. The SEM image reveals that the beads are spherical in shape having smooth surface during dehydration.

Swelling Study

The extent of swelling was measured in terms of increase in particle size using optical microscopy. The swelling ratios of all the formulations of stavudine beads were studied. In this test, few of beads from each formulation were kept in petri dishes containing pH 6.8. Thus after 2nd hour swelling of the bead can be determined. The swelling behaviour was determined by measuring the change of the diameter of the bead using a microscope with a micrometer. The swelling ratio for each sample determined at time t was calculated using the following equation

$$Sw = \frac{Dt}{D0}$$

Where, Dt is the diameter of the beads at time (t), and
Do is the initial diameter of the dried beads

Drug content uniformity
Standard Preparation

About 50 mg of Stavudine was weighed accurately and transferred into a 50 ml volumetric flask. It was dissolved, and then it was suitably diluted and made up to volume with phosphate buffer solution at pH 6.8 and mixed.

Sample Preparation

Accurate quantity of stavudine beads of the formulation equivalent to 50 mg of Stavudine were transferred to a 50 ml volumetric flask and were soaked in phosphate buffer pH 6.8 and allowed to disintegrate for 4 hours. The resulting mixture was filtered.

Procedure

The absorbance of both standard preparation and the sample preparation after suitable dilutions were measured in a UV-visible Spectrophotometer at 266 nm using phosphate buffer solution pH 6.8 with blank.

Drug Content Calculation

The amount of Stavudine present in granules can be calculated using the formula,

$$\frac{A_t}{A_s} \times \frac{S_w}{50} \times 100$$

Where, A_t = Absorbance due to sample preparation,
A_s = Absorbance due to standard preparation,
S_w = Weight of Stavudine working standard (mg)

Encapsulation Efficiency

The encapsulation efficiency was calculated by the formula,

$$\% \ encapuslation \ efficiency = \frac{AC}{TC} * 100$$

Where, AC = Actual drug content of beads and
TC = theoretical drug content of beads

Table 3: Evaluation Characteristics of Stavudine Beads

Batch code	Particle shape	Average Particle size (mm)	% Swelling study	Actual Drug content (mg)	% encapsulation efficiency	Percentage of yield
F1	Spherical	5.52	282	38.5	77.15	80.35
F2	Spherical	6.64	301	41.27	82.54	87.56
F3	Spherical	7.40	336	44.18	88.37	91.01
F4	Spherical	8.00	213	47.31	94.62	93.61
F5	Spherical	6.38	434	39.7	79.41	88.54
F6	Spherical	7.18	456	42.8	85.73	92.31
F7	Spherical	7.78	478	45.6	91.25	87.34

In-vitro Drug Release Studies
Procedure

The release of Stavudine from the sustained release beads was studied first in 900 ml of 0.1N HCl for 2 h and continued in phosphate buffer solution pH 6.8 as dissolution medium using a USP dissolution paddle apparatus at 50 rpm and 37 ± 0.5°C. An aliquot (1 ml) was withdrawn at specific time intervals, filtered and diluted to 10 ml with medium and drug content was determined by UV-visible spectrophotometer at 266 nm. An equal volume of fresh dissolution medium was replaced to maintain the sink condition. Dissolution studies were performed for a period of 12 h. Cumulative percentage of drug release was calculated using an equation obtained from a standard curve.[4]

Table 4: Comparative % drug release

Time (h)	F1	F2	F3	F4	F5	F6	F7
0	0	0	0	0	0	0	0
15	10.01	8.3	6.4	4.2	7.08	4.3	1.01
30	16.5	12.6	9.05	7.2	10.01	7.5	4.1
60	20.02	16.5	13.6	10.4	13.6	11.02	8.1
90	26.32	21.03	18.2	14.2	17.1	14.5	11.1
120	31.2	27.2	22.6	18.4	21.4	14.1	15.1
150	37.4	32.1	27.1	23.7	26.4	22.1	19.2
180	42.3	38.3	31.2	27.2	32.7	27.1	24.3
210	48.2	43.5	37.2	32.2	37.5	32.06	28.2
240	54.2	49.3	42.1	38.3	42.4	38.1	33.3
270	59.2	53.7	48.8	43.2	48.4	43.08	37.01
300	65.02	58.3	53.4	49.1	54.3	49.3	42.07
330	70.4	64.2	59.1	54.1	59.4	54.5	49.2
360	76.8	70.3	64.4	60.1	65.3	60.5	54.1
720	83.7	77.7	71.5	66.4	72.2	64.01	60.07

Kinetic Analysis of *In-Vitro* Release Rates

The results of *in-vitro* release profile obtained for all the formulations were plotted in modes of data treatment as follows:

- Zero - order kinetic model - Cumulative percentage drug released versus time.
- First – order kinetic model - Log cumulative percent drug remaining versus time.
- Higuchi's model - Cumulative percent drug released versus square root of time.
- Korsmeyer equation / Peppa's model - Log cumulative percent drug released versus log time.[7,8]

Zero order kinetics

Zero order release would be predicted by the following equation: -

$$A_t = A_0 – K_0t$$

Where, A_t = Drug release at time 't', A_0 = Initial drug concentration,
K_0 = Zero - order rate constant (h^{-1})

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys Zero – order kinetics and its slope is equal to Zero order release constant K_0.

First Order Kinetics

First – order release would be predicted by the following equation: -

$$Log \ C = log \ C_0 – Kt / 2.303$$

Where, C = Amount of drug remained at time 't', C_0 = Initial amount of drug,
K = First – order rate constant (h^{-1})

When the data plotted as log cumulative percent drug remaining versus time, yields a straight line, indicating that the release follow first order kinetics. The constant 'K_1' can be obtained by multiplying 2.303 with the slope value.

Higuchi's Model

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation: -

$$Q = [D\varepsilon / \tau (2A - \varepsilon Cs) Cst]^{1/2}$$

Where, Q = Amount of drug released at time 't',
D = Diffusion coefficient of the drug in the matrix,
A = Total amount of drug in unit volume of matrix,
Cs = the solubility of the drug in the matrix, ε = Porosity of the matrix,
τ = Tortuosity, t = Time (h) at which 'q' amount of drug is released.

Above equation may be simplified if one assumes that 'D', 'Cs' and 'A' are constant. Then equation becomes: -

$$Q = Kt^{1/2}$$

When the data is plotted according to equation i.e. cumulative drug release versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'K' (Higuchi's 1963).

Korsmeyer Equation / Peppa's Model

To study the mechanism of drug release from the sustained-release matrix tablets of Stavudine, the release data were also fitted to the well-known exponential equation (Korsmeyer equation/ peppa's law equation), which is often used to describe the drug release behavior from polymeric systems.

$$M_t / M_a = Kt^n$$

Where, M_t / M_a = the fraction of drug released at time 't', K = Constant incorporating the structural and geometrical characteristics of the drug / polymer system, n = Diffusion exponent related to the mechanism of the release.

Above equation can be simplified by applying log on both sides,
And we get: -

$$Log \, M_t / M_a = Log \, K + n \, Log \, t$$

Table 5: Kinetic values obtained from different plots of formulations (F1-F7) of Stavudine beads

F. code	Zero-order plots	First-order plots	Higuchi's Plots	Korsmeyer et al's plots		Possible Drug Release mechanism
	Regression Coefficient (R^2)	Regression Coefficient (R^2)	Regression coefficient (R^2)	Slope (m)	Regression coefficient (R^2)	
F1	0.824	0.925	0.951	0.683	0.984	First order Non-fickian
F2	0.837	0.922	0.948	0.684	0.989	First order Non-fickian
F3	0.846	0.909	0.936	0.687	0.989	First order Non-fickian
F4	0.851	0.901	0.924	0.700	0.976	First order Non-fickian
F5	0.847	0.909	0.931	0.680	0.985	First order Non-fickian
F6	0.828	0.866	0.902	0.693	0.968	First order Non-fickian
F7	0.857	0.897	0.917	0.77	0.890	First order Non-fickian

RESULTS AND DISCUSSION

Stavudine with all evident advantages proved to be suitable candidates for development of an extended-release dosage form. The beads were prepared by ionotropic-gelation method using sodium alginate and aluminium sulphate solution as a cross-linking agent. In the present study, an oral sustained-release dosage form is developed of D4T an anti-HIV drug with using HPMC K_4M which is commonly used in hydrophilic matrix drug delivery systems. However, the use of hydrophilic matrix alone for extending drug release for highly water soluble drug is restricted due to rapid diffusion of the dissolved drug through the hydrophilic gel net work. For such drugs it becomes essential to include hydrophobic polymers in the matrix system. Hence in the present work, an attempt has been made to formulate the sustained release beads of D4T using hydrophilic matrix material in combination with hydrophobic polymer such as Ethyl Cellulose.

Morphology and particle size

The beads prepared by wet granulation method were first of all evaluated for morphology and size. The beads of all the batches exhibited almost spherical shape. The particle size ranged from 5.52 mm to 8.00 mm. The drug only with the sodium alginate exhibited the least size of 5.52 mm in F1. The size of the beads slightly increased about 1 mm with the addition of one polymer along with the sodium alginate in the batches F2 and F5 with 6.64 mm and 6.38 mm respectively. The size difference in the beads in F2 and F5 is due to the gelling capacity of the respective polymer which is more in HPMC than that of the ethyl cellulose. The size of beads is little more increased in the batches F3 and F6 since the parts of the polymer have been increased in their formulations as

7.40 mm and 7.18 mm respectively. The formulations F4 and F7 showed the particle size of 8.00 mm and 7.78 mm respectively. Though, the formulations F4 and F7 has equal parts of polymer content along with the sodium alginate, the bead size of F4 is high comparatively since because of higher gelling capacity of hydrophilic polymer (HPMC) than that of hydrophobic polymer (ethyl cellulose).

Swelling study

The prepared beads were subjected to swelling study which ranged from 213 % to 478 %. Although, the polymer parts are equal in F2 and F5 and F3 and F6, the formulations F2 and F3 have less percentage of swelling when compared to the formulations F5 and F6 because the former formulations were made with HPMC and later formulations with ethyl cellulose. Similarly, the polymer parts are equal in F4 and F7 but still F7 shows more swelling study than that of F4. This difference is because even though the gelling capacity of HPMC is more the swelling capacity is less. The ethyl cellulose has less gelling capacity but more swelling capacity. So, F4 shows very less swelling study and F7 shows very high swelling study due to this reason.

Drug content

The drug content of the beads ranges from 38.5 mg (F1) to 47.31 mg (F4).

In-Vitro studies

The prepared beads were subjected to dissolution test for evaluating the *in-vitro* drug release. The dissolution studies were carried out in 900 ml of 0.1N HCl for first 2 h and then continued with 900 ml of phosphate buffer pH 6.8 in USP XXIII dissolution apparatus (paddle type assembly) at 50 rpm

and 37 ± 0.5^{O}C. The result of dissolution studies indicates that the influence of hydrophilic and hydrophobic polymers showing the sustained and extended release of drug form the beads. The formulations F1-F7 include hydrophilic and hydrophobic polymers separately and in combination which was observed for drug release of 12 h.

The formulation F1 includes only drug and sodium alginate shows the release for 12 h with 83.7 % drug release.

The formulation F2 includes drug and polymer HPMC in ratio 1:1 showing the release for 12 h with 77.7 % drug release.

The formulation F3 includes drug and polymer HPMC in ratio 1:2 showing the release for 12 h with 71.5 % drug release.

The formulation F4 includes drug and polymer HPMC in ratio 1:3 showing the release for 12 h with 66.4 % drug release.

The formulation F5 includes drug and polymer ethyl cellulose in ratio 1:1 showing the release for 12 h with 72.2 % drug release.

The formulation F6 includes drug and polymer ethyl cellulose in ratio 1:2 showing the release for 12 h with 64.01 % drug release.

The formulation F7 includes drug and polymers HPMC and ethyl cellulose in ratio 1:1:2 respectively showing the release for 12 hours with 60.07 % drug release.

The formulation F1 shows highest drug release since it has no polymers in it. The F7 shows the lowest drug release which contains both the hydrophilic and hydrophobic polymer in it. As the polymer parts in the formulation increases, the % drug release decreases in case of both hydrophilic and hydrophobic polymers. To know the mechanism of drug release, the data was treated according to different model such as zero-order release, first-order release, Higuchi's and Korsmeyer/Peppa's equation. The drug release data of F1-F7 were fitted to all plots and found to best fit into First-order drug release. In the present study *in-vitro* release profile could be expressed by Korsmeyer-Peppa's for all the formulations showed good linearity indicates that diffusion is dominant mechanism of drug release with these formulations. In order to understand the complex mechanism of drug release from the beads the *in- vitro* stavudine release data were fitted to Korsmeyer-Peppa's release model and interpretation of release exponent values (n) enlightens us by understanding the release mechanism from the dosage form. The release exponent values thus obtained and based on these values we can conclude that the formulations F1 to F7 exhibited first-order and non-fickian transport. Polymeric system with low viscosity polymer (HPMC K4M) yielded a faster initial burst effect. Shoufeng Li (2003) has reported that increased polymer concentration resulted in a corresponding decrease in the drug release. Baumgartner *et al*. reported similar results, in which they have demonstrated that HPMC with higher viscosity resulted in thicker gel layer formation.
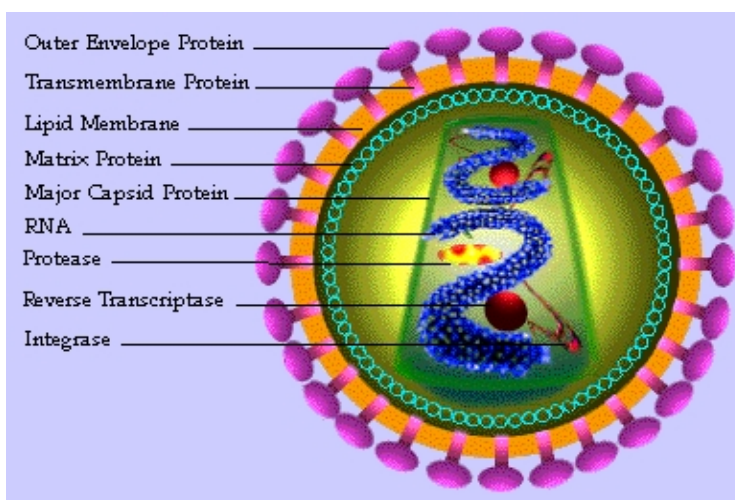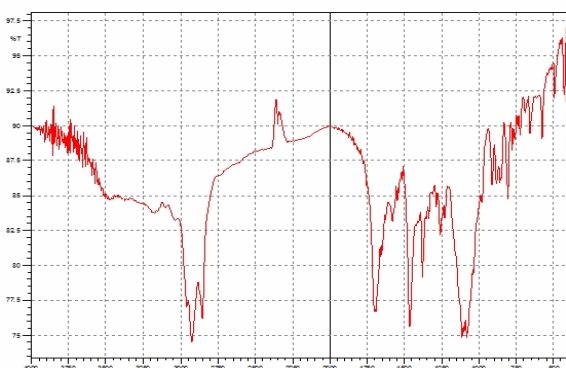

Figure 1: HIV Virus
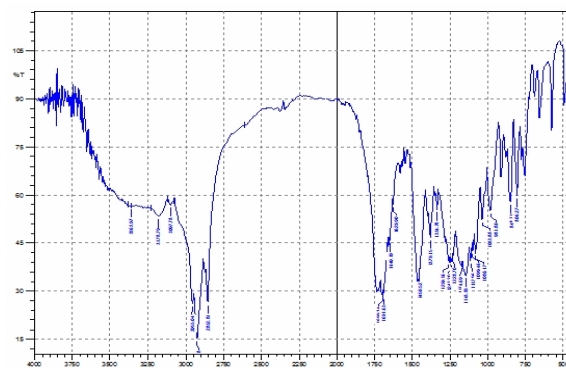

Figure 2: FT-IR Spectra of pure Stavudine

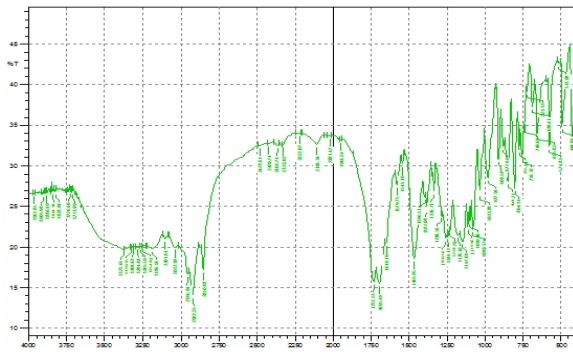**Figure 3: FT-IR spectra of Stavudine + HPMC K$_4$M**

Figure 4: FT-IR spectra of Stavudine + Ethyl cellulose
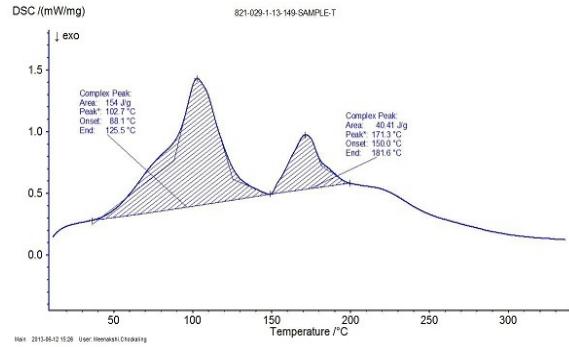
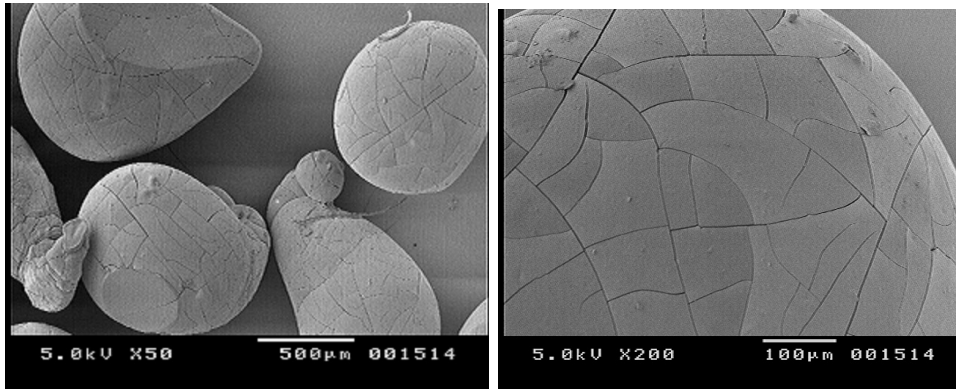**Figure 5: DSC of the best formulation F4**
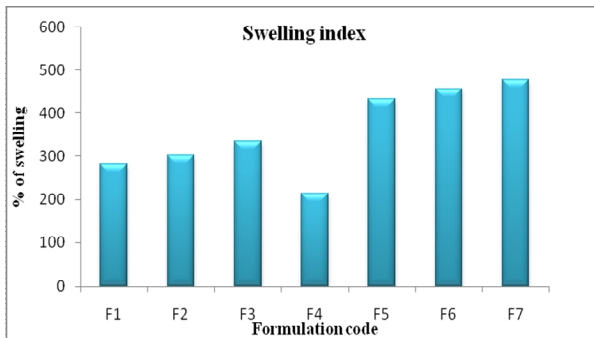

Figure 6: SEM images of drug loaded beads

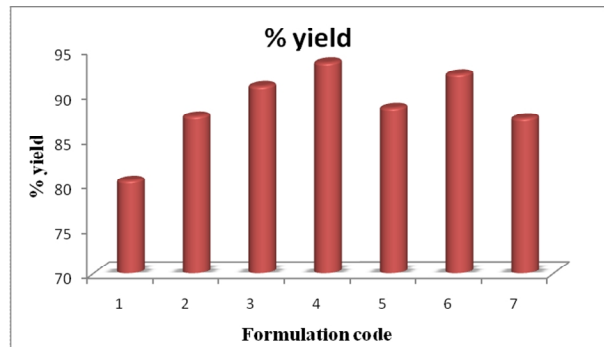**Figure 7: Swelling index of formulations F1-F7**

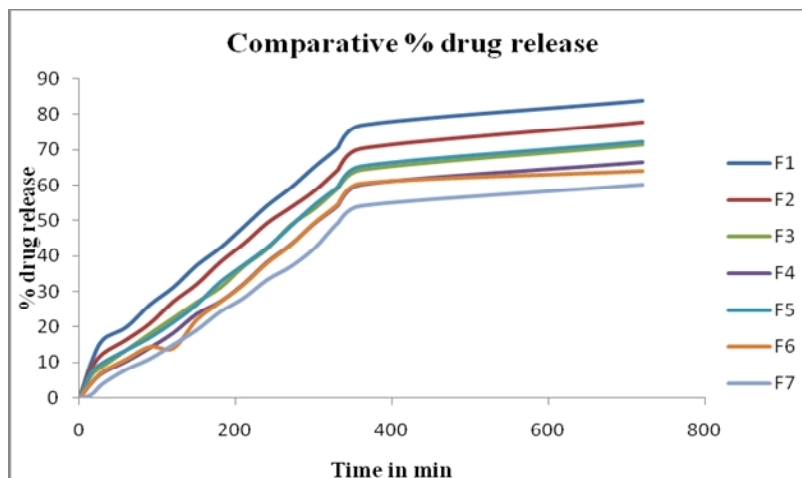**Figure 8: % yield of formulations F1-F7**

Figure 9: Comparative % drug release

CONCLUSION

The present study reports for the development of beads for sustained release of stavudine following oral administration. The results demonstrated that the release of the drug is dependent on the different ratio of drug to hydrophilic polymer HPMC K_4M or hydrophobic polymer ethyl cellulose. It can be conclusively stated that the F4 formulation appears to be promising system for the sustained release Stavudine for antiretroviral therapy based on actual drug content, % encapsulation efficiency, and *in-vitro* drug release data. From the results, it was also concluded that the release rate of drug is slow and consistent in drug: polymer (HPMC K_4M) ratio of 1:3 than the other formulations. The release kinetics of F4 indicates drug release by First order kinetics with non-fickian or anomalous transport with the release exponent ("n= 0.7000") indicates the drug release is controlled by more than one process including diffusion and erosion.

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