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Formulation and In-Vitro Evaluation of Stomach Specific Drug Delivery System of Stavudine

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ABSTRACT

The purpose of the study was to prolong the gastric residence time of stavudine by designing its floating tablets and to study the influence of different polymers on its release rate. Nine formulation of stavudine containing varying concentrations of polymers were designed by optimization. The floating matrix tablets of stavudine were prepared by direct compression method. The prepared tablets were evaluated for physicochemical parameters such as hardness, weight variation, percent friability, floating properties (floating lag time, total floating time and matrix integrity), swelling studies, drug content, stability study and In vitro drug release. The drug polymer interaction was studied by DSC thermal analysis. The physicochemical parameters of formulated tablets were found to be within normal range. The floating lag time of all the formulations was within the prescribed limit (<10 minutes). All the formulations showed good matrix integrity and retarded the release of drug for twelve hours except the formulations F1 and F4. The release pattern of stavudine was fitted to different models based on coefficient of correlation(r). The swelling studies of all the formulations showed that formulations containing xanthan gum has higher swelling indices than HPMC K100M and HPMC K15M. Optimized formulation (F5) of stavudine floating tablet showed no significant change in hardness, drug content, total buoyancy time and in vitro dissolution pattern after storage at 40°C/75% relative humidity for 3 months.

Keywords: Stavudine, HPMC, floating tablet, in vitro buoyancy.

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INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS), which threatens to cause a great plague in the present generation, was first identified in California in 1981. AIDS is a disease in which the body's immune system breaks down and is unable to fight off infections caused by human immunodeficiency virus (HIV). HIV infects human cells and uses the energy and nutrients provided by those cells to grow and reproduce, so it is necessary to take lot of medicines for longer periods of time. This can lead to an increase in non compliance of drugs. This problem is very serious in case of drugs having shorter biological half life because they must be taken more no of times. It is crucial for the success of AIDS therapy to maintain systemic drug concentration consistently above its target antiretroviral concentration throughout the course of the treatment. One method to solve such problems is by having sustained release of the drug. [1-3].

Stavudine is the FDA-approved drug for clinical use for the treatment of HIV infection (Katlama et al., 1998, Merrill et al., 1996), AIDS and AIDS-related conditions either alone or in combination with other antiviral agents. Stavudine, a nucleoside analogue of thymidine, is phosphorylated using cellular kinases to the active metabolite stavudine triphosphate. Stavudine triphosphate inhibits the activity of HIV-1 reverse transcriptase by competing with the natural substrate thymidine triphosphate and by causing DNA chain termination following its incorporation into viral DNA [4].

Stavudine is typically administered orally as a capsule and an oral solution. The drug has a very short half-life (1.5 h) thus necessitating frequent administration to maintain constant therapeutic drug levels. 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 reduces the severity of the toxicity (Goodman et al., 1985). Hence one of the methods of fabricating controlled release formulations is incorporation of the drug in the floating matrix containing a hydrophilic rate controlling polymers and gas generating agent was used to formulate the stomach specific drug delivery of stavudine [5,6].

MATERIALS AND METHODS

Materials

Stavudine was obtained from CIPLA Ltd., (Mumbai, India). HPMC K15M and HPMC K100M were received as gift samples from Colorcon Asia Pvt. Ltd., (Goa, India). Micro-crystalline cellulose was received as gift samples from Griffon Pvt. Ltd., (Mumbai, India). Other materials Xanthan Gum, Sodium Bicarbonate, Polyvinylpyrolidone, Talc and Magnesium Stearate were purchased from Qualigens fine chemicals, (Mumbai, India).

Methods

Preparation of floating tablets: The composition of different formulations of stavudine floating tablets is shown in Table 1. Different tablet formulations were prepared by direct

compression technique. All the powders passed through 40/60 mesh sieve. The required quantity of drug, various polymers and other ingredients were mixed thoroughly. Talc and magnesium stearate were finally added as a glidant and lubricant respectively. The blend was directly compressed (9 mm diameter, round flat faced punches) using multiple punch tablet compression machine (Cad mach Machinery Ltd., Ahmedabad, India). Each tablet contained 80 mg of stavudine.

Characterization of powder blend: The tablet blend were evaluated for their bulk density, tapped density, compressibility index, angle of repose and Hausner ratio. The tapping method was used to determine the bulk density, tapped density, percent compressibility index and Hausner ratio.

$$\text{Compressibility index} = [\rho_t - \rho_b / \rho_t] \times 100$$

$$\text{Hausner ratio} = \rho_t / \rho_b$$

Where ρ_t = tapped density

ρ_b = initial bulk density of tablet blend.

Angle of repose θ of the tablet blend measures the resistance to particle flow and was determined by fixed funnel method [7,8].

Differential scanning calorimetry (DSC): The DSC analysis of pure drug, drug+ HPMC K15M, drug+ HPMC K100M and drug+ Xanthan gum were carried out using a Shimadzu DSC 60, (Japan) to evaluate any possible drug-polymer interaction. The 2 mg sample were heated in a hermetically sealed aluminum pans in the temperature range of 25-300ºc at heating rate of 10ºc /min under nitrogen flow of 30ml/min [9].

Evaluation of floating tablets: The prepared floating tablets were evaluated for Dimension (Diameter and Thickness) using 6 tablets (vernier calipers), uniformity of weight using 20 tablets (Shimadzu BL-220H analytical balance), hardness using 6 tablets (Monsanto hardness tester), friability using 20 tablets (Roche type friabilator) [10,11].

Drug content

The drug content in each formulation was determined by triturating 20 tablets and powder equivalent to 25 mg was added in 100ml of 0.1N hydrochloric acid followed by stirring for 10 minutes. The solution was filtered through a 0.45µ membrane filter, diluted suitably and the absorbance of resultant solution was measured by using Shimadzu-1700 Pharmaspec UV-VISIBLE spectrophotometer at 266 nm using 0.1N hydrochloric acid as blank.

In-vitro buoyancy

The *in vitro* buoyancy was determined by floating lag time, per the method described by Rosa *et al.* The tablets were placed in a 100 ml beaker containing 0.1N hydrochloric acid. The time required for the tablet to rise to the surface and float was determined as floating lag time.

The duration of time the dosage form constantly remained on the surface of medium was determined as the total floating time [12].

Determination of swelling index

The swelling behavior of a dosage unit was measured by studying its weight gain. The swelling index of tablet was determined by placing the tablets in the basket of dissolution apparatus using dissolution medium 0.1 N HCL at 37±0.5ºC. After every 0ne hour up to 12 hours, each dissolution basket containing tablet was withdrawn and blotted with tissue paper to remove the excess water and weighed on the analytical balance (Shimadzu BL-220H). The experiment was performed in triplicate for each time point. Swelling index (SI) was calculated by using the following formula [13];

$$\text{Swelling Index (S.I.)} = \{(Wt-Wo)/Wo\}$$

Where, Wt = weight of tablet at time t.
Wo = weight of tablet before immersion.

In- vitro release studies: The release rate of stavudine from floating tablets was determined using United States Pharmacopeia (USP) Dissolution Testing Apparatus 2 (paddle method; Veego Scientific VDA-8DR, Mumbai, India). The dissolution test was performed using 900 ml of 0.1N hydrochloric acid, at 37 ± 0.5°C and 50 rpm. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus hourly and the samples were replaced with fresh dissolution medium. The samples were filtered through a 0.45µ membrane filter and diluted to a suitable concentration with 0.1N hydrochloric acid. Absorbance of these solutions was measured at 266 nm using a Shimadzu-1700 Pharmaspec UV-VISIBLE spectrophotometer. For each formulation, the experiments were carried out in triplicate. The release data were analyzed to study the release kinetics using zero order, first order, matrix, Korsmeyer-Peppas and Hixson Crowell equations by using PCP disso V3 software [14, 15].

Stability study: Stomach specific tablets of stavudine formulated and accelerated stability studies were carried out as per ICH guidelines. The prepared stavudine floating tablets containing HPMC K100M (F5) were selected for stability study on the basis of in vitro buoyancy and in vitro drug dissolution studies. The floating tablets were stored at 40°C/75% RH in closed high density polyethylene bottles for 3 months. The samples were withdrawn after periods of 1 month, 2 month and 3 month. The samples were analyzed for its hardness, floating characteristics, drug content and In vitro drug release[16].

RESULT AND DISCUSSION

The prepared floating tablets were evaluated for thickness, weight variation, hardness, friability, drug content, swelling index, in vitro buoyancy studies, in vitro drug dissolution studies and stability studies. All the studies were performed in triplicate, and results are expressed as mean ± SD.

Characterization of powder blend

The powders prepared for compression of floating tablets were evaluated for their flow properties, the results were shown in Table 2. Angle of repose was in the range of 20.29±0.21º to 21.39±0.47 which indicates excellent flow of the powder for all formulations. The bulk density of the powder formulation was in the range of 0.732±0.01 to 0.745±0.01 gm/ml; the tapped density was in the range of 0.822±0.01 to 0.838±0.00 gm/ml, which indicates that the powder was not bulky. The Carr's index was found to be in the range of 10.86±0.06 to 11.14±0.05, the Hausner ratio was found to be in the range of 1.121±0.005 to 1.125±0.005, indicating compressibility of the tablet blend is good. These values indicate that the prepared granules exhibited good flow properties.

Differential scanning calorimetry (DSC)

Any possible drug polymer interaction can be studied by thermal analysis. Stavudine exhibits a sharp endothermic peak at 175.05 °C shown in figure 1a, which corresponds to its melting point. The stavudine+ HPMC K15M exhibit a sharp endothermic peak at 173.87ºC, stavudine+ HPMC K100M exhibit a sharp endothermic peak at 172.30ºC and stavudine+ Xanthan gum exhibit a sharp endothermic peak at 172.15ºC shown in figure 1b, 1c and 1d respectively. Hence DSC study shows that there is no any drug polymer interaction.

Physicochemical evaluation of floating tablets

The floating stavudine tablets were off-white, smooth, and flat shaped in appearance. The results of physicochemical characterizations are shown in Table 3. The thickness of floating tablets was measured by vernier caliper and was ranged between 3.13±0.10 and 3.31±0.11 mm. The weight variation for different formulations (F1 to F9) was found to be ±1.75% to ±2.46%, showing satisfactory results as per Indian Pharmacopoeia (IP) limit. The hardness of the floating tablets was measured by Monsanto tester and was controlled between 5.5±0.44 and 5.8±0.25 kg/cm2. The friability was below 1% for all the formulations, which is an indication of good mechanical resistance of the tablet. The percentage of drug content for F1 to F9 was found to be in between 98.24±0.6 to 99.81±1.4 of stavudine, it complies with official specifications.

Floating characteristic

All the tablets were prepared by effervescent approach. The results of floating study were shown in table 3. On immersion in 0.1N HCL solution pH (1.2) at 37ºC, the tablets floated, and remained buoyant without disintegration. The study shows that the batches containing HPMC K100M polymer showed less floating lag time than the batches containing HPMC K15M and batch containing Xanthan gum. All the prepared batches shows the total floating time more than 24 hours except the F1 batch shows only more than 12 hours. From the results it can be concluded that the concentration of polymer increased, decrease the floating lag time and

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increase the total floating time. Hence the HPMC K100M polymer shows the good floating characteristic.

Table 1: Composition of Stavudine floating tablet

Ingredients (mg/tab)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Stavudine	80	80	80	80	80	80	80	80	80
HPMC K15M	40	80	120	-	-	-	-	-	-
HPMC K100M	-	-	-	40	80	120	-	-	-
Xanthan gum	-	-	-	-	-	-	40	80	120
Sodium bicarbonate	50	50	50	50	50	50	50	50	50
Polyvinylpyrolidone	24	24	24	24	24	24	24	24	24
Microcrystallne Cellulose pH 102	94	54	14	94	54	14	94	54	14
Talc	9	9	9	9	9	9	9	9	9
Magnesium Stearate	3	3	3	3	3	3	3	3	3

Table 2: Flow properties of powder

Formulation Code	Angle of repose (θ)*	Bulk density (gm/cm3)*	Tapped density (gm/cm3)*	Hausner ratio (HR)*	Carr's index (IC)*
F1	21.17±0.21	0.745±0.01	0.838±0.00	1.124±0.001	11.12±0.06
F2	21.19±0.58	0.732±0.01	0.822±0.01	1.122±0.00	10.93±0.05
F3	20.54±0.49	0.743±0.00	0.836±0.01	1.125±0.00	11.11±0.05
F4	22.11±0.21	0.743±0.02	0.836±0.02	1.124±0.001	11.11±0.06
F5	20.82±0.11	0.732±0.00	0.822±0.01	1.121±0.005	10.86±0.06
F6	20.29±0.21	0.733±0.01	0.823±0.02	1.122±0.001	10.96±0.07
F7	21.39±0.47	0.745±0.01	0.838±0.00	1.124±0.005	11.12±0.06
F8	20.59±0.50	0.732±0.01	0.822±0.01	1.121±0.001	10.89±0.06
F9	20.76±0.78	0.744±0.02	0.837±0.01	1.125±0.005	11.14±0.05

*All the values are expressed as mean± SE, n=3.

Table 3: Physico-Chemical Characterization of Stavudine Floating Tablets

Code	Thickness (mm)*	Weight variation test (%)	Hardness (kg/cm^2)*	Friability (%)*	Drug content (%)*	Floating lag time (S)*	Total floating time (h)
F1	3.18±0.14	±2.11	5.8±0.25	0.41±0.05	99.81±1.4	193±1.73	>12
F2	3.16±0.12	±2.16	5.7±0.27	0.31±0.08	99.67±1.7	161±2.08	>24
F3	3.15±0.12	±2.02	5.5±0.44	0.36±0.03	98.75±0.5	132±2.64	>24
F4	3.28±0.11	±1.75	5.6±0.41	0.37±0.01	99.47±1.3	113±1.52	>24
F5	3.13±0.10	±2.46	5.7±0.27	0.36±0.08	100.07±0.5	93±1.52	>24
F6	3.23±0.16	±1.85	5.58±0.62	0.28±0.06	100.38±0.8	66±1.15	>24
F7	3.18±0.14	±1.89	5.58±0.37	0.41±0.03	100.01±1.7	227±0.57	>24
F8	3.21±0.14	±1.86	5.5±0.44	0.36±0.12	98.24±0.6	215±2.08	>24
F9	3.31±0.11	±1.90	5.66±0.32	0.34±0.10	99.39±1.5	181±1.00	>24

*All the values are expressed as mean± SE, n=3.

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Table 4: In vitro release and swelling index data of optimized formulation F5

Time (hours)	% Drug release (%)*	Swelling index*
0	0	0
1	28.70±0.58	1.59±0.05
2	41.62±0.86	1.91±0.02
3	52.56±0.86	2.19±0.01
4	57.42±0.73	2.4±0.02
5	66.35±0.77	2.53±0.01
6	72.54±0.85	2.78±0.01
7	80.39±0.72	2.92±0.01
8	86.69±0.72	2.99±0.01
9	91.59±1.29	3.21±0.01
10	95.06±1.02	3.03±0.01
11	97.53±0.53	2.87±0.02
12	99.59±0.39	2.71±0.02

*All the values are expressed as mean± SE, n=3.

Table 5: Different Kinetic models for Stavudine floating tablets (F1 to F9)

Code	Zero order		First order		Higuchi		Korsemeyer-Peppas		Best fit model
	R^2	K_0 (mg/h^{-1})	R^2	K_1 (h^{-1})	R^2	K ($mg\ h^{-1/2}$)	R^2	n	
F1	0.7653	0.2060	0.8648	0.0072	0.9903	4.3504	0.9947	0.4335	Peppas
F2	0.8305	0.1722	0.9086	0.0059	0.9945	3.9429	0.9949	0.5040	Peppas
F3	0.8805	0.1486	0.9947	0.0030	0.9977	3.3831	0.9970	0.5119	Higuchi
F4	0.7761	0.1899	0.9406	0.0064	0.9916	4.1901	0.9938	0.4542	Peppas
F5	0.8618	0.1684	0.8979	0.0055	0.9983	3.8121	0.9981	0.5113	Higuchi
F6	0.9031	0.1449	0.9936	0.0029	0.9952	3.2893	0.9963	0.5459	Peppas
F7	0.8706	0.1631	0.9693	0.0043	0.9988	3.7182	0.9988	0.5225	Higuchi
F8	0.8766	0.1517	0.9970	0.0032	0.9979	3.4572	0.9968	0.5389	Higuchi
F9	0.9149	0.1393	0.9978	0.0026	0.9949	3.1577	0.9983	0.5900	Peppas

Table 6: Stability studies of optimized formulation of stavudine floating tablet (F5)

Characteristic	Initials	1 Month	2 Month	3 Month
Hardness (kg/cm2)*	5.7±0.27	5.83±0.288	5.66±0.288	5.83±0.288
Drug content (mg/tablet)*	100.07±0.5	100.04±1.5	99.39±1.5	99.47±1.32
Floating lag time (s)*	93±1.52	98±2.64	95±2.00	97±2.64
Total floating time (h)*	>24	>24	>24	>24
In vitro drug release at 12 hour*	99.59±0.39	99.21±0.15	98.16±0.36	98.07±0.11

*All the values are expressed as mean± SE, n=3.

Swelling index

Swelling study was performed on all the batches (F1 to F9) for 12 hours. The result of swelling index were shown in figure 2, it shows the plot of swelling index as a function of time for different formulation. It was observed that the swelling indices were increased with increase in polymer concentration. Formulation containing xanthan gum showed higher swelling indices as compared with other formulation containing the same amount of HPMC K15M and HPMC K100M. This is because during dissolution a tablet containing xanthan gum instantly forms a viscous gel layer that slows down in sweep of dissolution fluid towards the core of matrix tablet. Swelling was strong enough to avoid premature disintegration as well as burst effect and retarded the release of pure drug for a long period of time. Complete swelling was achieved by the end of seven to nine hours for different formulation. Swelling index values starts decreasing when polymer erosion starts in medium. The result of swelling index for optimized formulation F5 were shown in table 4.

In- vitro release study

In-vitro dissolution studies of all the formulations of floating tablets of Stavudine were carried out in 0.1 N HCl. The study was performed for 12 hours, and percentage drug release was calculated at 1hours time intervals. The results of in vitro dissolution studies of all formulations were shown in Figure 3. The higher initial drug dissolution was observed in tablets containing HPMC K15M (F1) and HPMC K100M (F4). This showed that in less concentration HPMC hydrated more rapidly in the presence of 0.1 N HCl. The variation in drug release was due to different types of polymers and different concentrations of polymer in all the nine formulations.

It is expected that the developed formulation should have the following theoretical drug release profile, *i.e*., 20 to 25 % in 1 h, 25 to 45 % in 2 h, 55 to 75 % in 4 h, 65 to 85 % in 6 h and 85 % after 8 h. Formulations F1 to F4 and F6 to F9 failed to meet the needed theoretical drug release profile. Formulation F5 meet the needed theoretical drug release profile and floated with a lag time of 93±1.52 seconds; for these reasons, it was considered that the formulation F5 was best formulation among all the nine formulations of this series. The percentage drug release data of formulation F5 were shown in table 4.

Kinetic modeling of drug release

The data obtained from in vitro dissolution studies were fitted in different models viz. zero order, first order, Higuchi and Korsemeyer- Peppas equation, the results were shown in Table **5**. The first order plots were found to be fairly linear as indicated by their high regression values (r^2 = 0.7653 to 0.9149). To confirm the exact mechanism of drug release from these tablets, the data were fitted according to Korsemeyer- Peppas equation. Slope values (n= >0.5) suggested that the release of stavudine from the floating tablets followed the non- Fickian transport mechanism. This means that water diffusion and also the polymer relaxation had an essential role in drug release. When n takes the value of 0.5, it indicates diffusion controlled

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drug release. The value of n in case of optimized formulation F5 is close to 0.5 indicating a diffusion controlled drug release mechanism [17].

Figure 1a: DSC thermal analysis of stavudine

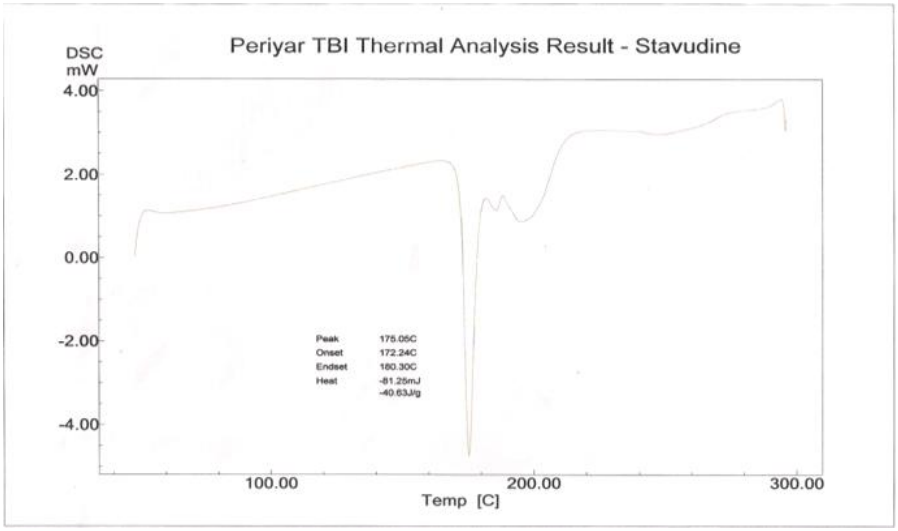

Figure 1b: DSC thermal analysis of stavudine+ HPMC K15M

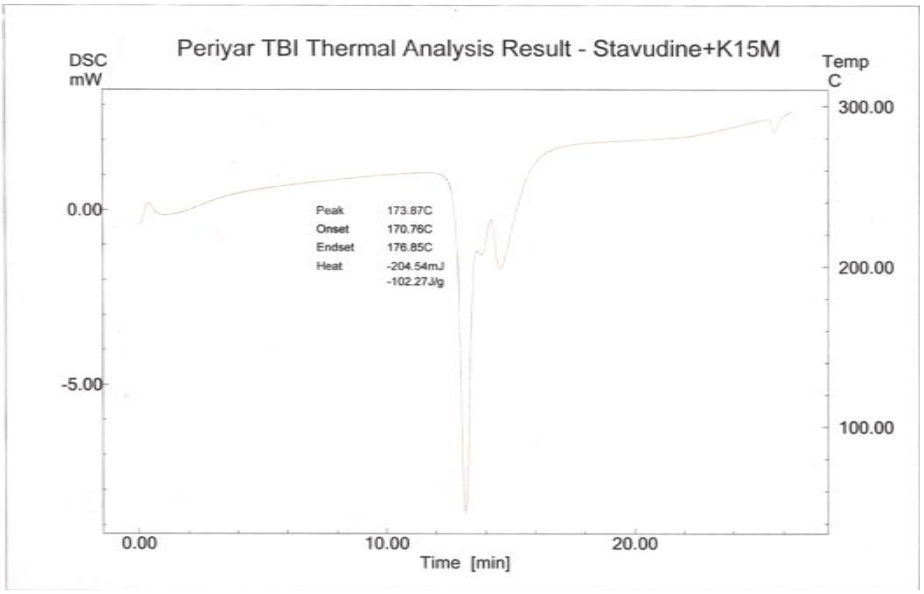

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Figure 1c: DSC thermal analysis of stavudine+ HPMC K100M

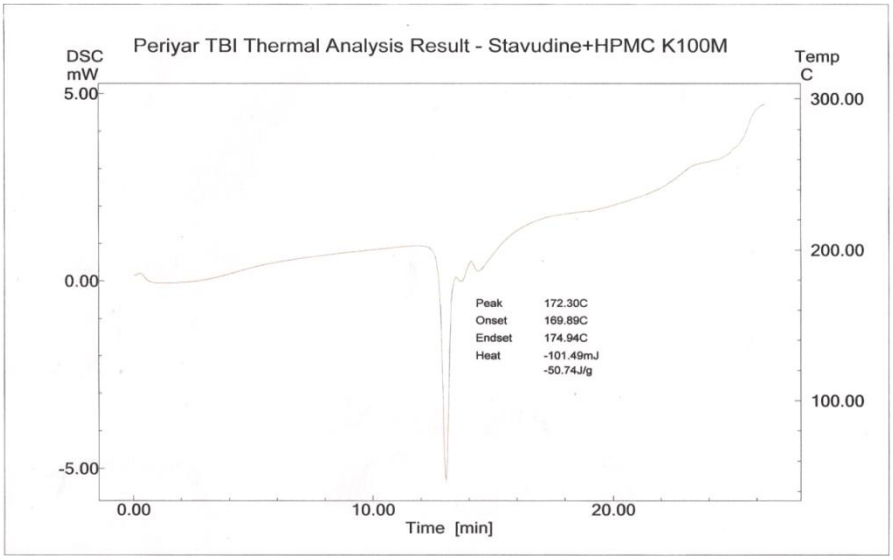

Figure 1d: DSC thermal analysis of stavudine+ Xanthan gum

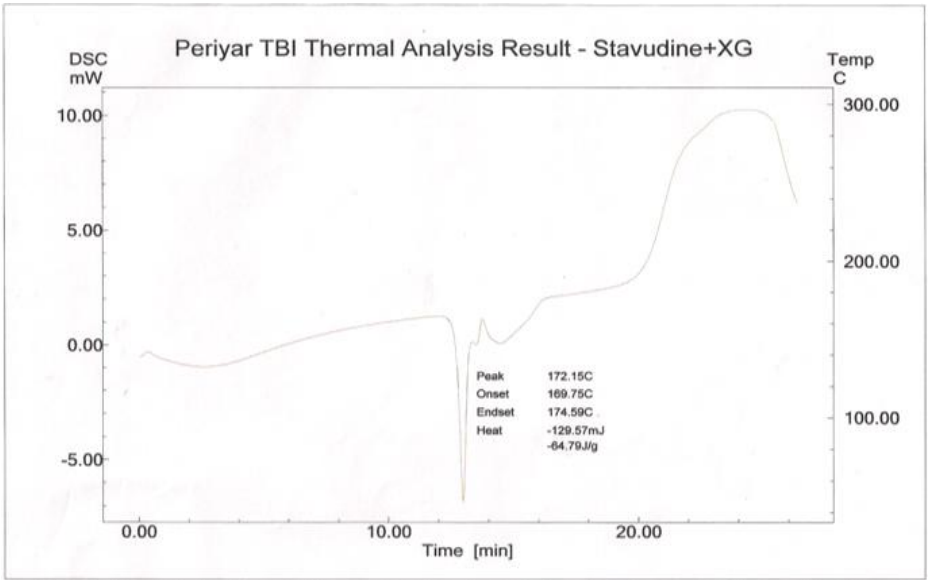

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Figure 2: Swelling index of stavudine floating tablet (F1 to F9)

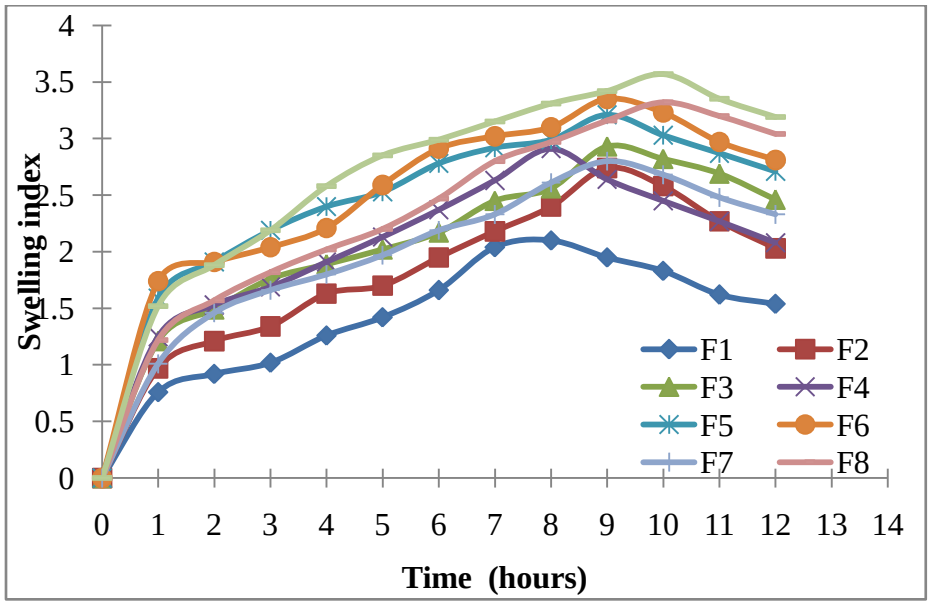
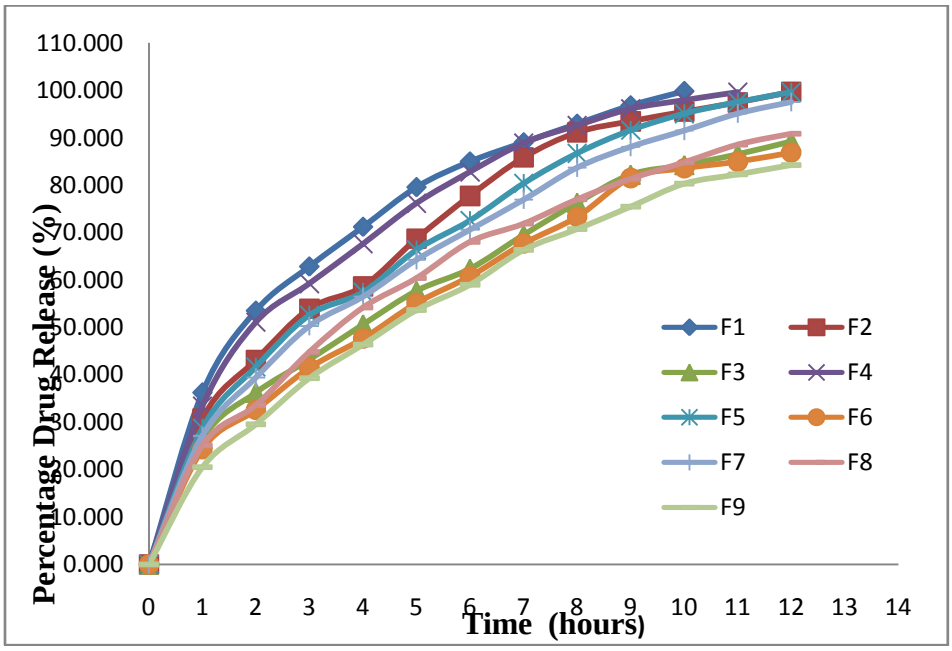


Figure 3: In Vitro drug release profile of all formulation



Stability study

The stability study results obtained were shown in Table 6. The stavudine floating tablets did not show any significant change in physicochemical parameters and other tests

(Table 6). Thus, it was found that the stomach specific tablets of stavudine (F5) were stable under these storage conditions for at least 3 months.

CONCLUSION

The aim of the study was to study the effect of various hydrophilic polymers on in vitro release rate from gestroretantive floating tablet of Stavudine based on a low density polymer. The effervescent-based floating drug delivery was a promising approach to achieve *in vitro* buoyancy. Different types of matrix forming polymers- HPMC K15M, HPMC K100M and Xanthan gum were studied. The use of gel-forming polymer methocel K100M and gas-generating agent sodium bicarbonate was essential to achieve *in vitro* buoyancy. Formulation F5 showed controlled drug release and adequate floating properties. The kinetics of drug release was best explained by Higuchi equation. The drug release from the tablets was sufficiently sustained and non- Fickian transport of the drug from tablets was confirmed. The stavudine floating tablets were stable at 40°C/75% RH up to 3 months.

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