Research Article

Design and *In-Vitro* Evaluation of Gastro Retentive Floating Microbeads of Zidovudine Hydrochloride

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ABSTRACT

The present work is an attempt to prepare floating microbeads of Zidovudine as model drug to achieve an extended retention in upper GIT which results in enhanced absorption, thereby improves bioavailability and avoiding multiple dosing for hospital acquired infections. In the present investigation, microbeads were prepared by ion-gelation method using polymers like sodium alginate, hydroxy propyl methyl cellulose (K100M), gas forming agent like sodium bicarbonate, and curing agents such as calcium chloride and Barium chloride solutions. Prepared microbeads were evaluated for micromeritic property, particle size and morphology, in-vitro buoyancy study, drug loading and encapsulation efficiency and in-vitro drug release kinetic studies. All the formulations produced optimal flow properties as represented in terms of compressibility. Result showed barium chloride cross linked formulations showed the excellent flow ability as compared to calcium chloride cross linked formulations. SEM study revealed irregular surfaces with pores. The alginate floating microbeads were shown good floating ability. The prepared microbeads exhibited prolonged drug release for 12 hrs and remained buoyant up to 8 hrs. Barium chloride linked microbeads showed better drug release of 91.78 percentage and drug content of 87.14 percentages as compared to calcium chloride linked. Thus, it can alternatively be used to avoid multiple dosing, thereby reducing the chance of dose dumping.

Keywords: Buoyancy, curing agent, floating microbeads, non-fickian diffusion, zidovudine

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INTRODUCTION

A pandemic of AIDS continue to spread relentlessly throughout the world. According to the United Nation Acquired immune deficiency syndrome (UN AIDS) and World health organisation (WHO) updates, estimates released at the end of 2007, 42.5 million people are living with HIV/AIDS. According to the data more than 95% of cases remain in developing countries. Beyond the death tool and human suffering, AIDS continue to roll back hard on developmental gains in many region of the world [1]. Hence to counteract the above problem, a considerable number of researches are carried out to develop the new entities of anti-retroviral having wide spectrum of activities. Among such agents especially the Nucleoside reverse transcriptase inhibitors (NRTIs) groups are found to be more effective. Hence there is a need to develop more precise and suitable dosage form in terms of sustained release oral dosage form in-order to avoid the undesirable side effect associated with frequent dosing. Several difficulties are found while designing controlled release systems, especially to obtain better absorption and enhanced bioavailability [2, 3]. Floating drug delivery system (FDDS) or Hydro dynamically balanced system (HBS) are among the several approaches of controlled drug delivery systems that have been developed in order to increase the gastric retention time of dosage. Gastro

retentive floating drug delivery system (GRFDDS) has bulk density lower than gastric fluids and thus remains buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time [4]. Zidovudine hydrochloride (Azidothymidine, AZT) is a class of Nucleoside reverse transcriptase inhibitor (NRTI), has been used for successive treatment for HIV/AIDS infection. It works by selectively inhibiting the viral reverse transcriptase, an enzyme, so that viral replication process inhibited and leads to patient clinical and immunological responses. This virustatic drug has lower bioavailability of 75% due to considerable first-pass metabolism and lower half-life of 0.5 to 3 hours, thus necessities administration of frequent dosing to maintain therapeutic level. However, major side effects like neutropenia and anemia are commonly associated with frequent administration [5]. In the present study an attempt has been made to develop and characterize floating microbeads of Zidovudine hydrochloride. On oral administration, it prolongs the gastric residence time, increases the drug bioavailability and sustained the action for a longer period of time.

MATERIALS AND METHODS

Zidovudine Hydrochloride (ZHL) was collected from Nicholas Piramal (India) Ltd. Sodium alginate, Hydroxy propyl methyl cellulose (HPMC-K100M), Calcium chloride, Barium chloride were obtained from S. D. Fine Chemicals (Mumbai, India)Ltd, Sodium bicarbonate and sodium citrate were obtained from Qualigens Fine Chemical (Mumbai, India) Ltd. Acetic acid was purchased from E-Merck (Mumbai, India) Ltd. All other chemicals and solvents having of high analytical gradation were used in the present study.

Preparation of floating microbeads

Floating microbeads containing Zidovudine Hydrochloride were prepared using ion-gelation method. The polymeric mixtures of sodium alginate and HPMC-K100M were kept constant at a ratio of 9:1(w/w). The drug and polymeric mixtures were added in gas forming agent $NaHCO_3$, ranging from 100 to 250 mg in all formulations. The resultant solutions poured dropwise using a 24G syringe needle separately in 1% (w/v) calcium chloride and 1 %(w/v) barium chloride solution containing 10% (v/v) acetic acid as shown in (**Table 1**). The solution containing suspended beads were stirred with a magnetic stirrer bar for 10mins to improve mechanical strength and allowed to complete the reaction to produce gas. The beads were collected, washed with ethanol and distilled water and subsequently dried at low temperature (0-4^{0}C) [6].

Table 1: Composition of different formulations

Sr. No.		Formulations				
	Composition	F1	F2	F3	F4	F5
1	Zidovudine hydrochloride (mg)	300	300	300	300	300
2	Sodium alginate (mg)	450	450	450	450	450
3	HPMC K100M (mg)	50	50	50	50	50
4	Sodium bicarbonate (mg)	100	150	200	250	-
5	*Cross linking agents (g)	1	1	1	1	1
6	Sodium citrate	20	20	20	20	20

*Symbolized as cross linking agent for both Calcium chloride and Barium chloride

Micromeritic property

The flow properties of microspheres were characterized in terms of bulk density, tapped density, percentage of compressib-ility and packing factor [7].

Particle size and morphology

Particle size of the prepared beads were determined in three set using an optical microscope (Model BH-2, Olympus, Japan) fitted with a stage and an ocular micrometer. The external and internal morphology of micro gel beads were studied by scanning electron microscopy. The micro beads were coated with gold palladium under an argon atmosphere using a gold sputter module in a high

vacuum evaporator. The coated samples were then observed with a scanning electron microscope [8].

In-vitro buoyancy

In-vitro evaluation of floating behaviour *studies* were performed by placing 50 mg particles in a USP 24 dissolution test apparatus II and subsequent addition of 900ml 0.1 N Hcl containing 0.02% w/v tween 20 (37^0C) to exclude floating due to non-wetted surfaces followed by horizontal shaking (37^0C, 50 rpm). The flasks were allowed to stand for 5 mins without agitation at predetermined time intervals of 2, 4, 6 and 8 hrs. The floated beads were recovered and dried. Then the percentage of floating particles were calculated [9, 10].

Determination of drug content and encapsulation efficiency.

Accurately weighed (50 mg) grounded powder of beads was soaked in 50 ml of methanol and allowed to disintegrates completely for 24 hrs. The resultant dispersion was sonicated using a probe sonicator (S-4000, Misonix, Farmingdale, N.Y.) for 20 minute then filtered through a 0.45 µm filter. The filtrate was diluted by methanol to maintain proper concentration and drug content was measured spectroph-otometricaly (Jasco V-570, Japan**)** at a wavelength of 266 nm [11]. The encapsulation efficiency was calculated by the following equation.

(Actual drug content) / (Total mass of microbead) X 100…… (1)

In-vitro drug release studies

In-vitro dissolution studies were performed for all the formulation beads using USP 24 dissolution test apparatus II with a paddle type (Veego scientific, Mumbai, India). The dissolution media was 900ml of 0.1N Hcl, of which temperature was maintained at 37 $\pm 0.5^0$ C and stirred at 100 rpm. Samples (1ml) were withdrawn at suitable interval of time and volume was adjusted. It was then assayed spectrophotometricaly (Jasco V-570, Japan) at 266 nm [12].

FT-IR spectroscopy:

The drug-excipient interaction were studied using FTIR (FTIR 8400S, Schimazu). IR spectra for drug and powdered micropaticles were recorded in a Fourier transform infrared spectrophotometer with KBr pellets [13]. The spectra were scanned over 4000-500 cm^{-1} range.

Differential scanning colorimetry (DSC) study:

The DSC analysis of pure drug and drug-loaded microparticles were carried out using Shimadzu DSC 60. The analysis was performed at a rate 10^0 C min-1 ranging from 20^0C to 300^0C temperature [14].

Statistical analysis

Experimental results were expressed as mean ± SD. Normality test and one-way analysis of variance (ANOVA) were applied to check significant differences in drug release from different formulations. Differences were considered to be statistically significant when $p < 0.05$ for ANOVA

RESULTS AND DISCUSSION:

Micromeritic property

All the formulations produced optimal flow properties as represented in terms of compressibility. (**Table 2**) shows micromeritic properties of prepared formulations. Among them, CF5 and BF5 exhibited highest packing factor indicating its high packing rate. The flow properties of barium chloride cross linked formulations showed the excellent flow ability as compared to calcium chloride cross linked formulations. This may be due to high rigidity of barium alginate with formation of more regular geometrical structure.

Particle size and morphology

The prepared formulations were subjected to analyse morphological characters. Figures showed the surface and cross sectional morphology of microbeads prepared with sodium bicarbonate ($NaHCO_3$). It was observed that microbeads with $NaHCO_3$ showed irregular surface with pores (**Fig 1a**). Spherical particles were unable to form due to produced of releasing CO_2 gas which burst the prior to sufficient hardening of the bead wall and made the surface rough. The cross sectional views of floating microbeads were also examined with SEM (**Fig 1b**). Many large crystals of drug were present in the alginate gel matrix. The no. of observed pores appears to be directly related to the amount of incorporated gas forming agent.

Table 2: Micromeritic properties of prepared formulations

	Micromeritic Properties			
Formulation	**Bulk density (g/cm3)**	**Tapped density (g/cm3)**	**Compressibility**	**Packing factor**
CF1	0.29 ±0.011)	0.39 ±0.026	25.64 0±.338	1.34 ±0.06
CF2	0.33 ±0.015	0.44 ±0.02	25 ±0.25	1.42 ±0.025
CF3	0.42 ±0.030	0.53± 0.041	20.75 ±0.52	1.26 ±0.014
CF4	0.48 ±0.023	0.57 ±0.03	15.78 ±0.261	1.18 ±0.035
CF5	0.51 ±0.026	0.73 ±0.37	30.13 ±0.486	1.43 ±0.040
BF1	0.34±0.12	0.41±0.015	17.07±0.636	1.2±0.152
BF2	0.33±0.01	0.39±0.032	15.38±0.811	1.18±0.037
BF3	0.31±0.02	0.36±0.026	13.88±0.454	1.16±0.026
BF4	0.26±0.023	0.32±0.02	18.75±0.411	1.23±0.015
BF5	0.29±0.014	0.34±0.051	14.07±0.442	1.17±0.025

Data are represented as mean ± standard deviation, n=3

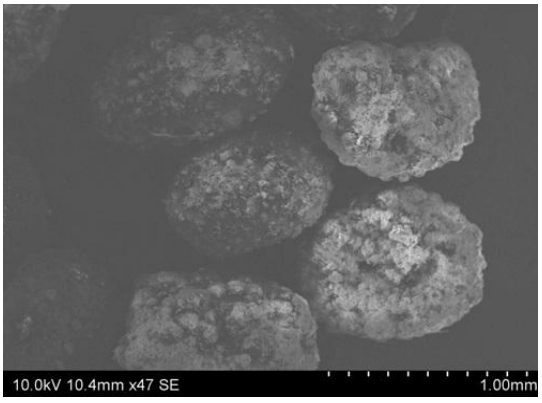

Fig. 1(a): SEM of whole Barium alginate bead containing Zidovudine hydrochloride showing external morphology (50.0×)

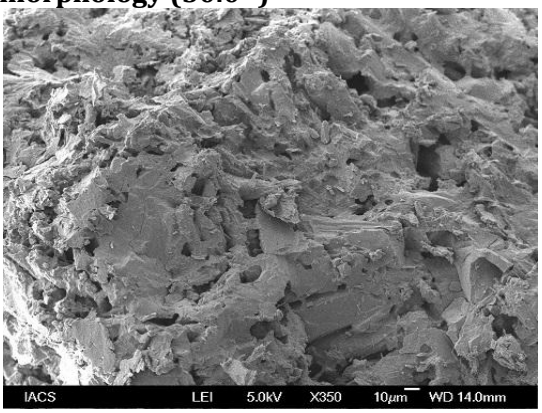

Fig. 1(b): SEM of cross section of Calcium alginate bead containing cefuroxime axetil showing internal morphology (350.0×)

In-vitro buoyancy

The microbeads were designed in such a manner that after contacting with gastric fluid the liberated carbon dioxide gets trapped under the hydrocolloid gel and causes drifting. The alginate floating microbeads were shown good floating ability as given in (**Table 3**).Result shows as the concentration of sodium bicarbonate increases there was an increased in the percentage of buoyancy. Initially, a minimum of 77 percentage microbeads were floated for 2 hrs with respect to all formulations except CF_5 and BF_5 because of absence of $NaHCO_3$.More than 60 percent of particles kept floating for 8 hr. Buoyancy percentage of the microbeads were in the range of 60% to 80 % at the end of 8 hrsfor both the formulation types. While comparing from the stand point of buoyancy, as shown in (**Figure 2 and 3**), the $NaHCO_3$ containing calcium chloride resulted better floating ability than barium chloride because the density of calcium chloride is less than that of Barium chloride.

Drug content and encapsulation efficiency

Drug content refers initial amount of the drug present in a formulation whereas encapsulation efficiency denotes the ratio between actual drug content and total mass of microbeads. Microbeads preparation is a means of encapsulation. In the present study, however, encapsulation efficiency gradually decreased with increasing the concentration of $NaHCO_3$, due to increased porosity. CF5 and BF5 formulations showed lowest percentage of drug contents such as 77.73±0.72 and 77.97±0.78 respectively whereas the encapsulation efficacy was found to be highest for both CF5 (82.88±0.098%) and BF5 (86.64±0.219%) as shown in (**Table-4**).

Table 3: *In-vitro* buoyancy study of prepared microbeads

Formulation code	Buoyancy (%)			
	2 hrs	4 hrs	6 hrs	8 hrs
CF1	88.64±1.023	80.33±3.122	72.13±1.782	61.33±2.106
CF2	92.66±2.015	86±1.362	78.02±1.112	68.23±2.184
CF3	96.62±1.007	89.66±2.492	82.04±1.403	73.32±1.009
CF4	99.33±2.256	93.72±2.187	85.66±1.856	80.43±2.001
CF5	0	0	0	0
BF1	77.60±1.007	70.66±1.895	64.38±2.014	58.46±2.003
BF2	80.66±1.284	73±1.006	65.42±2.048	58.26±1.049
BF3	82.02±1.14	74.12±1.427	67.76±1.204	61.43±1.182
BF4	86.33±2.003	74.17±1.049	71.19±1.182	67.37±1.002
BF5	0	0	0	0

Data are represented as mean ± standard deviation, n=3

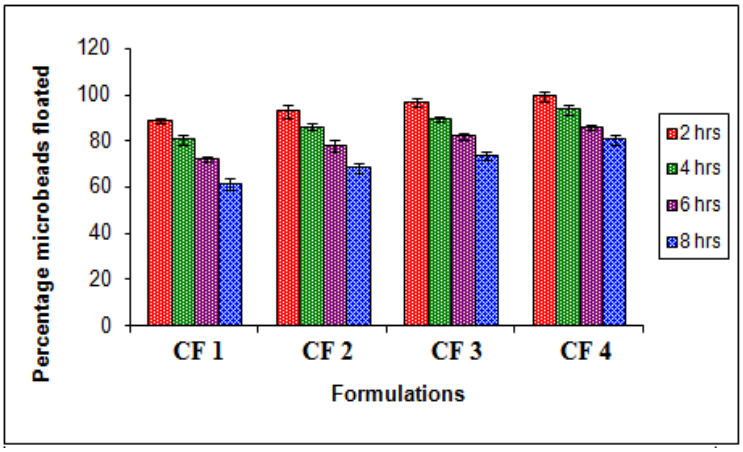

Fig. 2: *In-vitro* buoyancy studies for Calcium chloride cross- linked microbeads

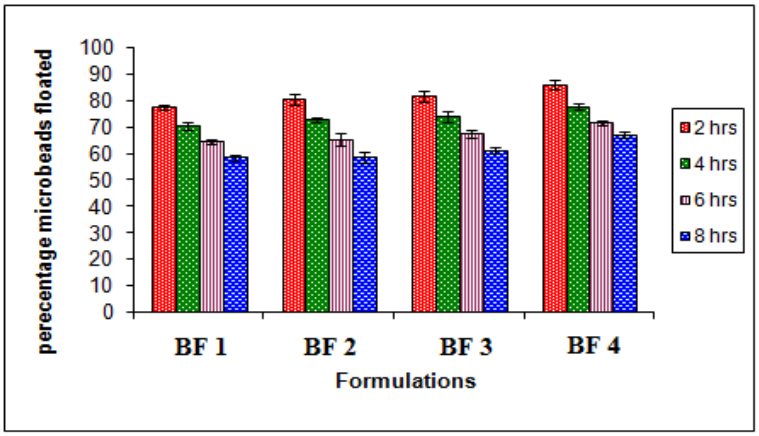

Fig. 3: *In-vitro* buoyancy studies for Barium chloride cross-linked microbeads

In-vitro drug release studies

The dissolution studies of different formulations were performed in 0.1N HCl taken as the dissolution medium. The drug releases from floating microbeads were found to be above 90% at the end of 12 hrs. Approximately 7 % of the drug was released initially. This could be due to the release of the drug lined to the surface of the microbeads. As the concentration of $NaHCO_3$ increase gradually the release of drug content from different formulations were increasing. Among formulation from CF1 to CF5, CF5 had slow release pattern

because of absence of $NaHCO_3$.Similar release pattern were observed in all formulations having barium chloride. Maximum rate of drug releases found in BF_2 formulation (Barium chloride cross linking formulation) and CF_3 (calcium chloride cross linking formulation) was about 98.67±0.116% and 98.65±0.112 % respectively. There is no variation in concentration of cross linking agents such as Calcium chloride and Barium chloride for both formulations as mentioned in the (**Table 1**). Only the concentration of sodium bicarbonate taking as gas forming agent is differed. Therefore, there was no such much variation in the release pattern. For the first 8 hrs CF_3 released 77.27±0.189 % and BF_2 released 73.36±0.142% of drug. But up to 12 hrsCF_3 and BF_2 sustained the drug release for 98.65±0.112 % and 98.67±0.116 % respectively. As we know that barium cations interacted with the alginate molecules to a greater extent than calcium cations. The varying effects of the salts on the properties of the microsphere beads were largely attributed to their ability to interact with the alginate molecules. Among the two batches, barium chloride cross linked had shown the slow release pattern of drug due to high rigidity of the barium alginate (**Figure-4**).

Table 4: Physiochemical characterization of prepared microbeads

Formulation code	Drug content (%)	Encapsulation efficiency (%)	Mean particle size(µm)
CF1	86.26±0.12	54.75±0.178	649.12 ?ı .56
CF2	92.72±0.85	47.53±0.209	665.85 ? 0.70
CF3	96.31±0.45	41.71±0.142	711.61 ? .96
CF4	98.98±0.39	35.71±0.165	763.64 ?ı˜ 03
CF5	77.73±0.72	82.88±0.098	537.85 ? .93
BF1	87.14±0.72	52.56±0.216	540.38?ı .30
BF2	93.26±0.54	50.21±0.164	551.86?ı .52
BF3	96.77±0.52	48.8±0.263	586.67? .89
BF4	99.37±0.25	42.8±0.215	612.67?ı .47
BF5	77.97±0.78	86.64±0.219	481.70?ı .49

Data are represented as mean ± standard deviation, n=3

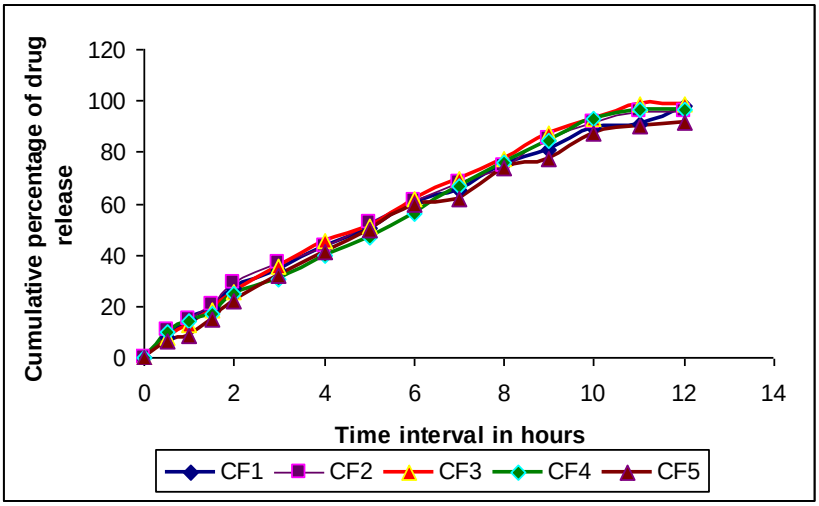

Fig. 4: *In-vitro* drug release profile for calcium chloride cross-linked formulations in gastric medium

The data obtained from *in-vitro* dissolution studies were fitted to zero-order, first-order and Korsemeyer-Peppas equations. The interpretation of data was based on the value of the resulting regression coefficients. The zero-order plots were found to be fairly linear as indicated by their high regression values. To confirm the exact mechanism of drug release, the data

were fitted according to Korsemeyer-Peppas equation.

$M_t/M_T = k\,t^n$ ..... (2)

Where M_t corresponds to the amount of drug release in time t, M_T is the total amount of drug released after an infinite time and k stands for constant related to the structural and geometric properties of the drug delivery system.

The value of '*n*' gives an indication of the release mechanism. Regression analysis was performed and regression values 'R^2' were 0.990 to 0.997 for different formulations and slope values ($0.5<n<1.0$) suggest that the release of Zidovudine hydrochloride from floating microbeads followed non-Fickian diffusion mechanism. Among all formulations, BF2 released the drug for a period of 12 hrs at a controlled manner (**Figure 5**).

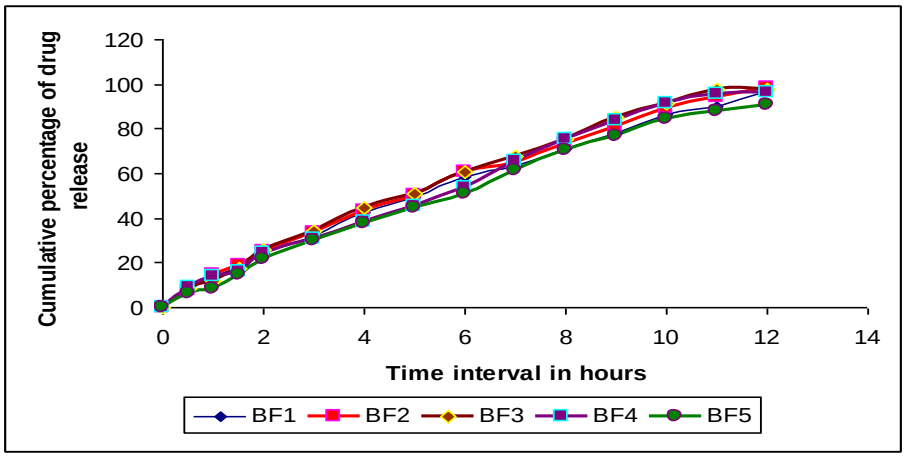

Fig. 5: *In-vitro* drug release profile for Barium chloride cross-linked formulations in gastric medium

FT-IR spectroscopy and DSC study

During the above mentioned study there were no such major interaction found between drug and polymer and excipients as cited in **Figure 6(a), 6(b)** and (**Figure 7**).

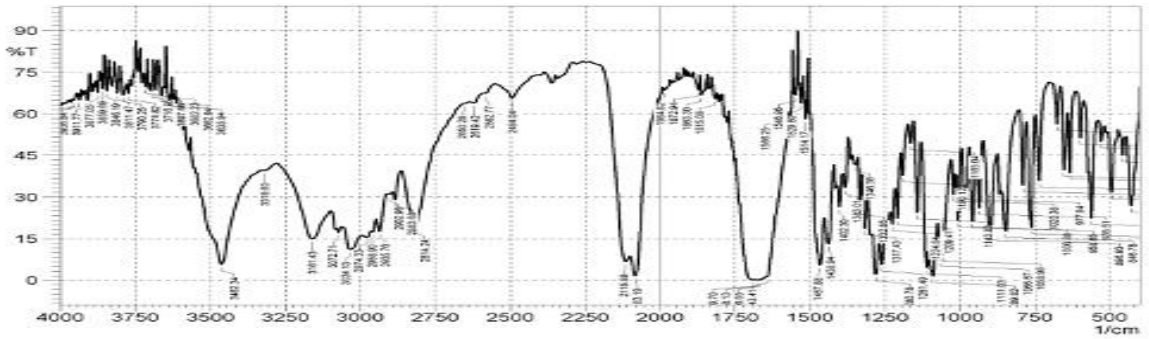

Fig.6(a): FT-IR spectrum of Zidovudine hydrochloride

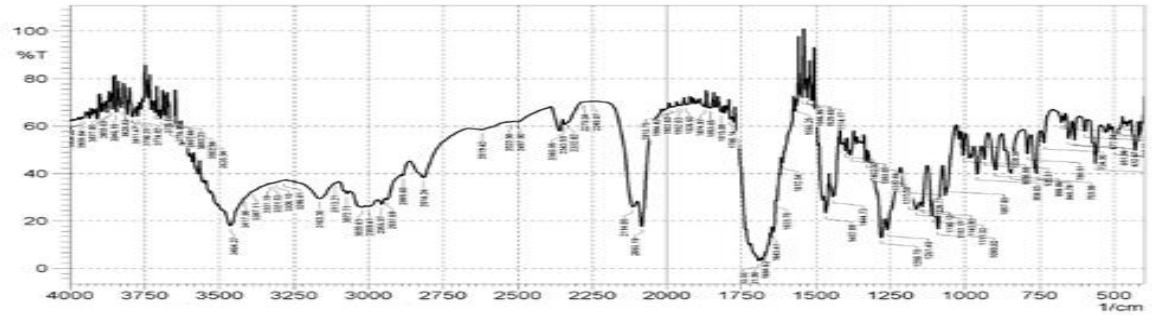

Fig.6(b): FT-IR spectrum of best formulation

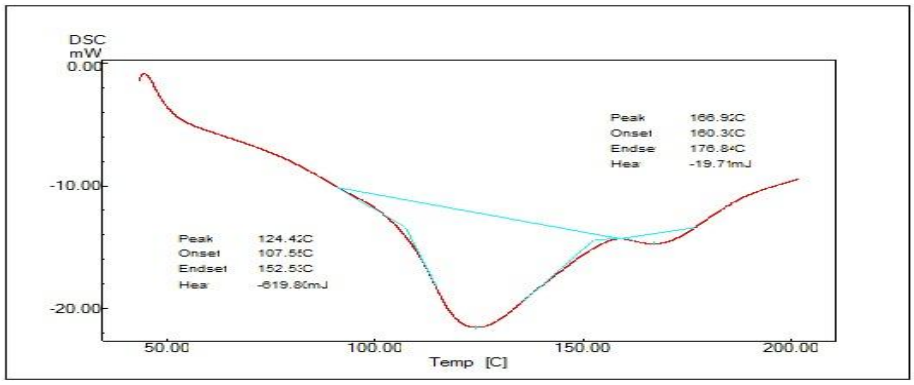

Fig.7: DSC study of best formulation

CONCLUSION

A successful attempt has been made to formulate floating alginatemicrobeads of Zidovudine Hydrochloride using gas forming agent as sodium bicarbonate and curing agents as a gastro retentive drug delivery system adopting ion-gelation method can alternatively be used to avoid multiple dosing, thereby reducing the chance of dose dumping.

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