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Controlled Release Microbeads of Zidovudine Incorporated with Natural Waxes: Design and Characterization

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ABSTRACT

Zidovudine (AZT) is an antiretroviral drug with activity against Human Immunodeficiency Virus (HIV) Type 1. However, only temporary and limited benefits are observed in HIV infected patients treated with zidovudine alone or in combination with other antiretroviral drugs. Upon oral administration, it exhibits dose dependent toxic effects such as hepatotoxicity, hyperglycemia, hyperlipidemia, lactic acidosis, lipodystropy, osteonecrosis. These side effects often require dosage reduction or even cessation of treatment. Hence, in the present work an attempt is being made to develop a stable drug delivery system for zidovudine in the form of controlled release microbeads incorporated with different types of waxes. Wax incorporated microbeads of Zidovudine were prepared by melt extrusion-ionotropic gellation method. A series of tests have been carried out to characterize the microbeads in vitro, including particle size distribution, SEM analysis, loading parameters, in vitro release studies and stability studies. Compatibility studies proved that there was no interaction between zidovudine and different waxes used. Zidovudine beads were roughly spherical in nature, which was confirmed by Scanning electron microscopy. Zidovudine loaded beads with normal frequency distribution were obtained. A maximum of 74.65% drug entrapment efficiency was obtained. The in-vitro performance of Zidovudine beads showed sustained release up to 24 hrs depending on the wax concentration. Finally it can be concluded that the formulated matrix type microbeads with bees wax were more feasible and effective than carnauba wax beads in encapsulating zidovudine and thereby increasing the effectiveness of the drug.

Keywords: Zidovudine (AZT), Controlled release, Alginate gel beads, Natural waxes.

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INTRODUCTION

For any drug therapy to be successful the drug must reach the target tissue or systemic circulation in optimum concentration which should be maintained for desired time. Therapeutic response of the drug also depends on the pharmacokinetics of the drug in an individual patient and frequency of dosing. However, for drugs with shorter half life, it is not possible to maintain the drug concentration within therapeutic range without frequent dosing. But frequent dosing may lead to patient noncompliance and drug toxicity. In such circumstances, the problem can be solved by developing new dosage forms like prolonged release dosage forms with similar therapeutics response as that of conventional dosage forms and longer duration of action.[1]

Microbeads are small, solid and free flowing particulate carriers containing dispersed drug particles either in solution or crystalline form that allow a sustained release or multiple release profiles of treatment with various active agents without major side effects. Additionally, the beads maintain functionality under physiological conditions, can incorporate drug to deliver locally at high concentration ensuring that therapeutic levels are reached at the target site while reducing the side effects by keeping systemic concentration low. In the pharmaceutical field, the use of a biocompatible polymer, preferably naturally occurring shows remarkable advantages compared with the normal synthetic polymers.[2-4]

Zidovudine (AZT) is a nucleoside analogue of thymidine, used in the treatment of AIDS in HIV infected patients. Following oral administration it is rapidly absorbed from stomach, with peak plasma concentrations occurring within 1 hour after dosing. The bioavailability and half life of zidovudine are 60% and 1 hour respectively after oral administration. Considering the bioavailability and half life, it requires frequent administration of drug at a high dose. However patients receiving zidovudine frequently develop side effects like anaemia and leucopoenia which are dose dependent and reduction of the total administered dose reduces the severity of the toxicity. The development of a sustained-release dosage form of zidovudine would be beneficial in comparison with the current intermittent dose regimens. To overcome inherent drawbacks associated with conventional dosage forms of zidovudine, an attempt is being made to develop an alternative drug delivery system in the form of microbeads incorporated with natural waxes.[5-8]

MATERIALS AND METHOD

Zidovudine was obtained as a gift sample from Strides Arco Labs Bangalore, Carnauba wax and white bees wax were obtained from Loba Chemie Pvt Ltd, Mumbai and all other solvents and chemicals used were of analytical grade and purchased from commercial sources.

Preparation of Wax Incorporated Microbeads:

For the present study, biodegradable polymer sodium alginate combined with different waxes is used with the active ingredient for preparation of microbeads (Table 1).

Zidovudine loaded microbeads were prepared by hot melt extrusion along with Ionotropic gellation method. Accurate quantity of polymer was dissolved in 50ml of distilled water and stirred to form dispersion. Drug was added to the above dispersion and again stirred for uniform distribution. In another beaker, various amounts of waxes (viz. white bees wax, carnauba wax) were melted in water bath at 60–85°C, depending on the melting range of the waxes used. The molten wax was added to the homogenized mixture of polymer and AZT which was already heated to same temperature and stirred until a homogenous mixture was obtained. The hot melted mixture was extruded through a 23G syringe needle into calcium chloride solution (4% w/v). The beads were allowed to remain in the same solution for 30 min to improve their mechanical strength. The formed beads were separated, washed with water and allowed to dry at room temperature overnight.[9-12]

Table 1: Formulation Design of Zidovudine Loaded Wax Microbeads

Sl.No	Ingredients	F_1(gm)	F_2(gm)	F_3(gm)	F_4(gm)	F_5(gm)	F_6(gm)
1	Zidovudine (AZT)	0.5	0.5	0.5	0.5	0.5	0.5
2	Sodium Alginate	2	2	2	2	2	2
3	White Bees Wax	1	2	3	-	-	-
4	Carnauba Wax	-	-	-	1	2	3

Evaluation of Drug Loaded Microbeads:

Drug Polymer Interaction (FTIR) Study:

Drug polymer interactions were studied by FT-IR spectroscopy. 1-2mg of AZT alone, mixture of drug and polymer, beads were weighed and mixed properly with potassium bromide uniformly. A small quantity of the powder was compressed into a thin semitransparent pellet by applying pressure. The IR- spectrum of the pellet from 500–4000 cm^{-1} was recorded taking air as the reference and compared to study any interference.[13-15]

Surface Morphology:

Scanning electron microscopy (SEM) has been used to determine particle size distribution, surface topography, texture, and to examine the morphology of fractured or sectioned surface. SEM studies were carried out by using JEOL JSM T-330A scanning microscope (Japan).[16,17]

Frequency Distribution Analysis:

The diameter of gel beads (100 beads) of each formulation was determined using optical microscopy. In order to define a frequency distribution or compare the characteristics of particles

with many different diameters, the frequency distribution can be broken down into different size ranges, which can be presented as histogram.[18,19]

Drug Content:

To determine the drug content and encapsulation efficiency of the beads, 100 mg beads were crushed and dispersed in suitable solvent (methanol). The dispersion was sonicated for 15 minutes, left overnight for 24 hrs and filtered. A 1 ml sample was taken and diluted with suitable solvent (methanol), and drug content assayed using a UV-visible spectrophotometer at λ_{max} of 266 nm. The drug content of each formulation was recorded as mg / 100 mg of microbeads.[20]

Drug Entrapment Efficiency:

The drug entrapment efficiency of prepared microbeads was determined by using the equation.[21]

EE (%) = Actual Drug Content / Theoretical Drug Content X 100

***In vitro* Dissolution Study:**

Drug loaded zidovudine microbeads equivalent to 100 mg of zidovudine was loaded into the basket of the dissolution apparatus. Dissolution study carried out for 24 hrs in 7.4 pH phosphate buffer. 1 ml of the sample was withdrawn from the dissolution media at suitable time intervals and diluted to 10 ml using phosphate buffer and the same amount was replaced with fresh dissolution medium. The absorbance was measured at 266 nm by using UV spectrophotometer, against a blank solution. Dissolution profiles of the formulations were analyzed by plotting cumulative percentage drug release versus time. The data obtained were also subjected to kinetic treatment to understand release mechanism.[22-27]

Stability Study:

The ability of microbeads to retain the drug was assessed by storing the wax incorporated microbeads at different storage conditions like 4±1ºC, 25±2ºC; 60±5%RH and 37±2ºC; 70±5%RH for one month. Samples were withdrawn periodically by solubilising it into appropriate solvent and the percentage drug leakage was calculated using UV spectrophotometric analysis at 266nm.[28]

RESULTS AND DISCUSSION

In the current research, wax incorporated microbeads loaded with zidovudine were formulated by a combination of two methods, hot melt extrusion and ionotropic gellation using sodium alginate as biodegradable polymer and bees wax and carnauba wax as rate controlling polymers. The prepared microbeads were characterized for their post formulation parameters.

FTIR Studies:

From the FTIR studies given in Figure 1-4, showed no chemical interaction between the drug molecule, polymers and waxes used.

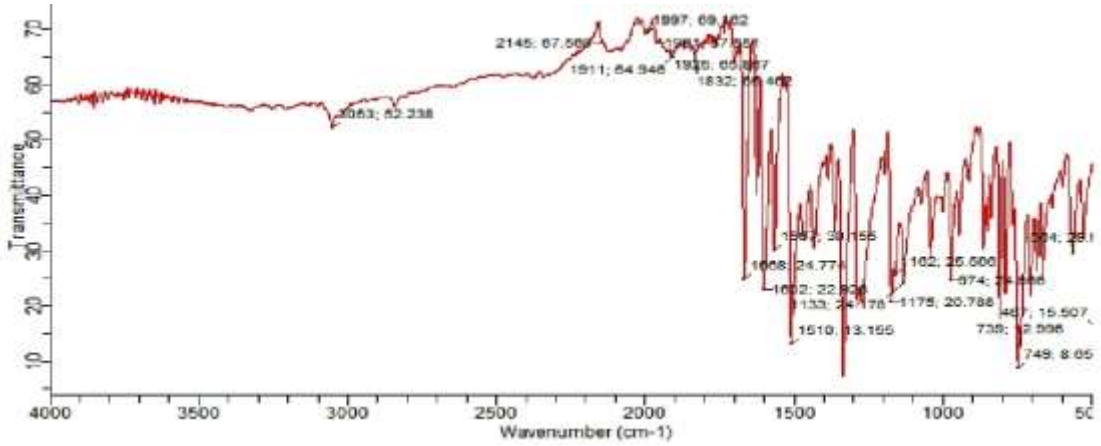

Figure 1: FTIR spectrum of pure drug (Zidovudine)

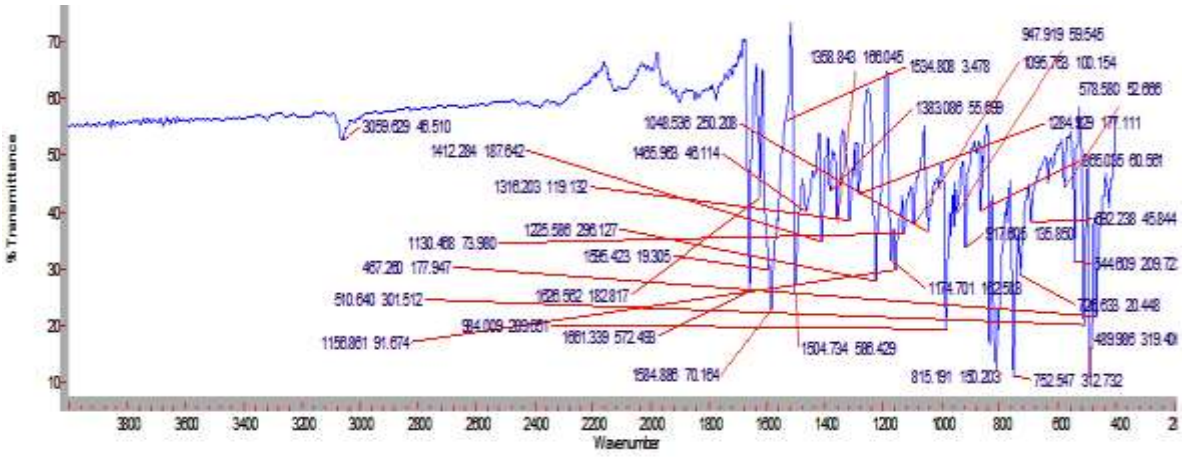

Figure 2: FTIR spectrum of blank microbeads (Bees wax + Carnauba wax)

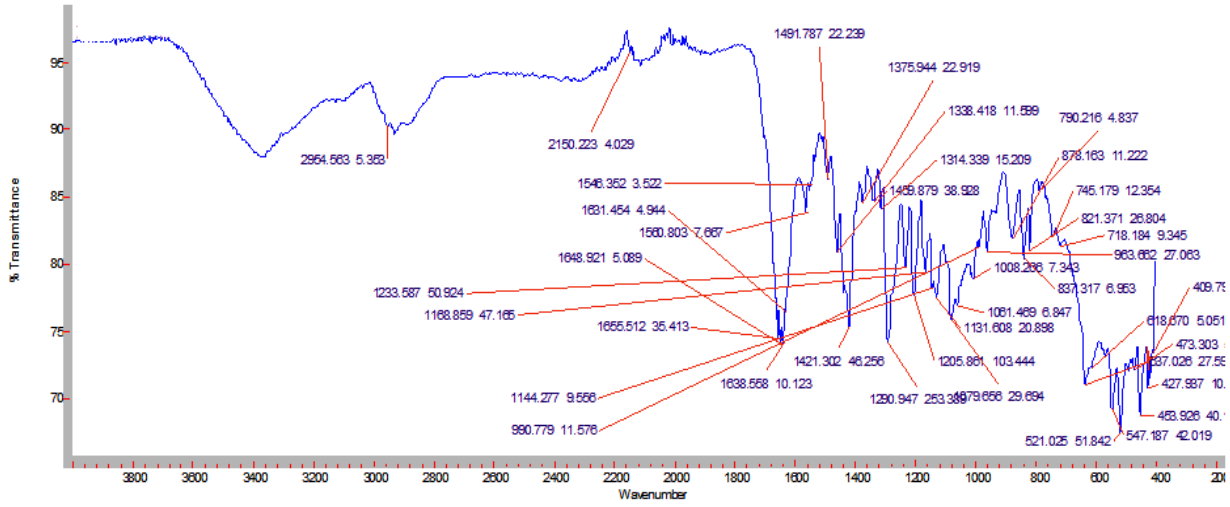

Figure 3: FTIR spectrum of zidovudine loaded bees wax microbeads

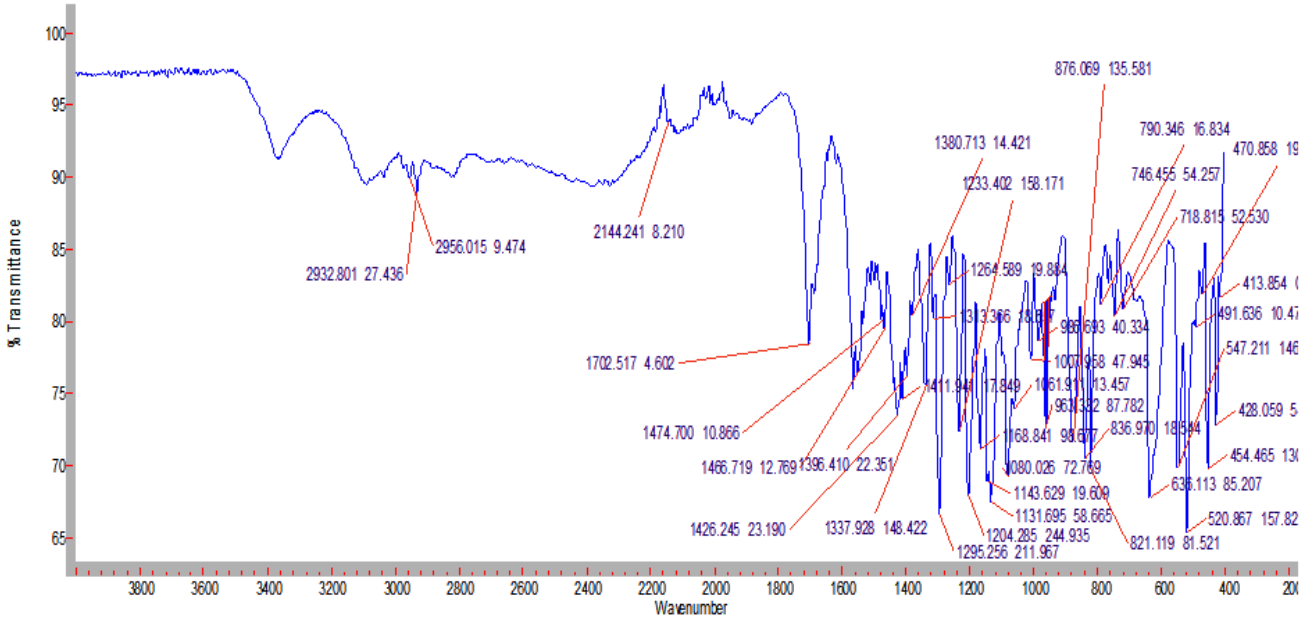

Figure 4: FTIR spectrum of zidovudine loaded carnauba wax microbeads

Particle Size:

The mean particle size of the drug loaded microbeads increased with increase in the concentration of the wax which can be attributed to the enhanced viscosity of the droplets. Furthermore, microbeads incorporated with carnauba wax showed greater particle sizes than beads with bees wax. Zidovudine loaded microbeads having a size range of 712µm to 976µm (Figure 5 & 6) with normal frequency distribution was obtained.

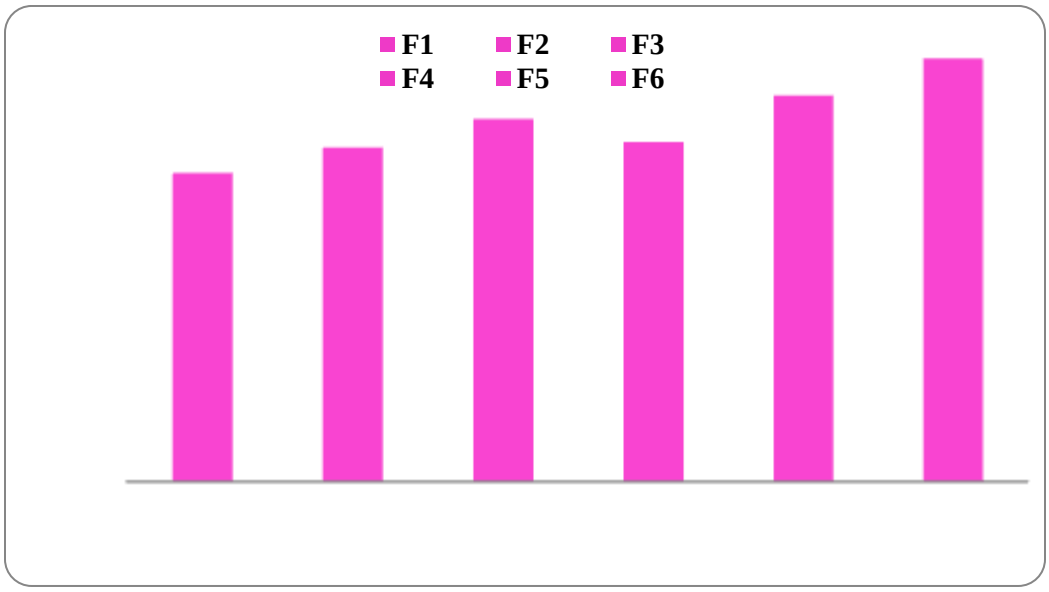

Figure 5: Average diameter of Zidovudine microbeads

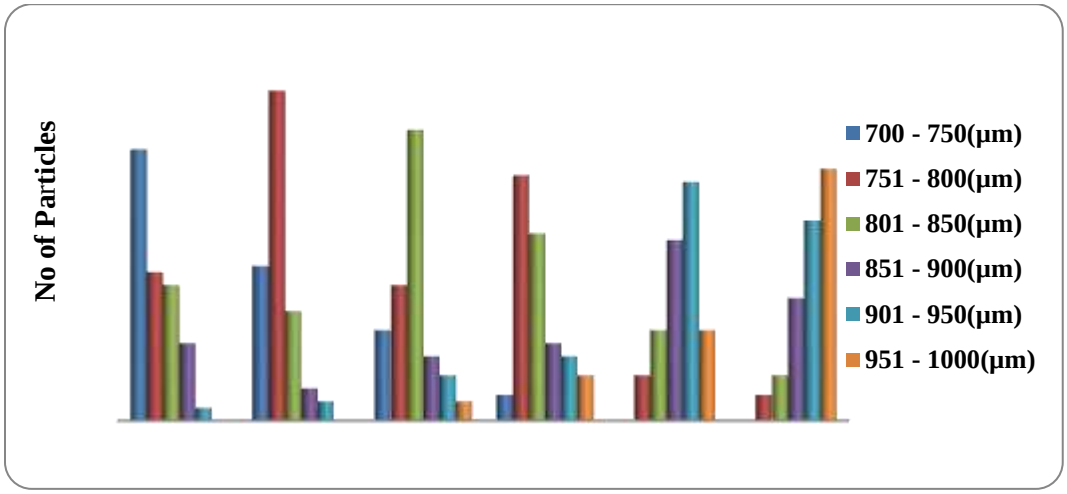

Figure 6: Frequency distribution of Zidovudine microbeads

Scanning Electron Microscopy (SEM):

All the batches of microbeads were spherical in shape with variable surface characteristic features based on the type of wax used for preparing them. Microbeads incorporated with carnauba wax showed rough surfaces whereas bees wax microbeads had smooth surfaces.

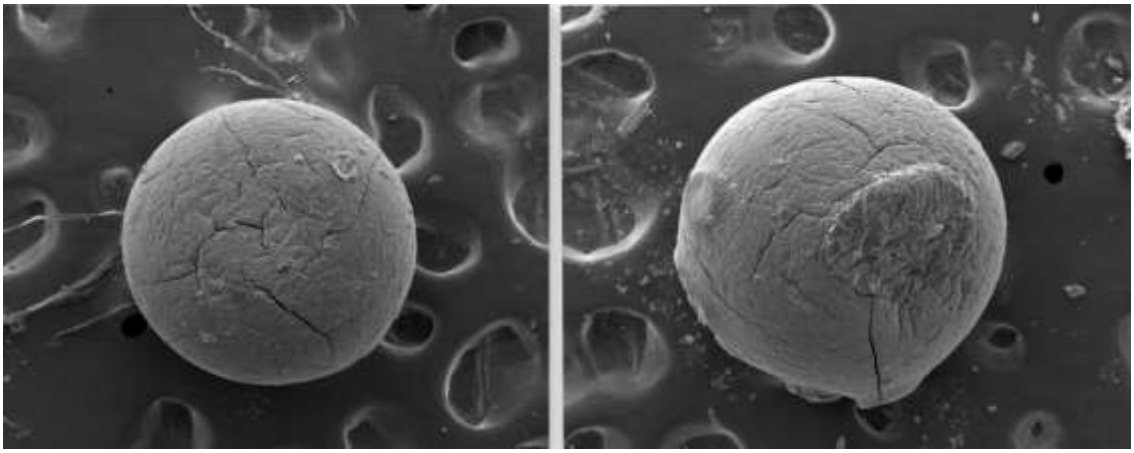

Figure 7: SEM of Zidovudine microbeads incorporated with bees wax

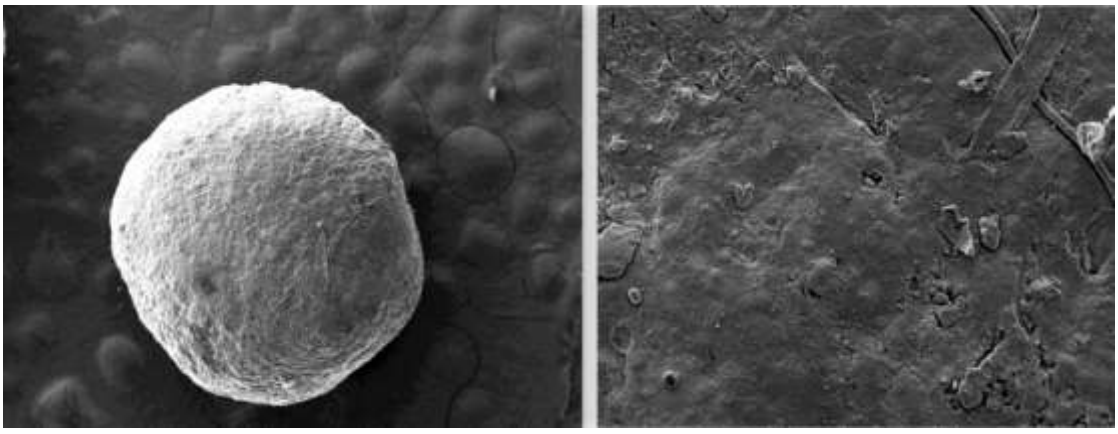

Figure 8: SEM of Zidovudine microbeads incorporated with carnauba wax

Percentage Drug Entrapment Efficiency:

The percent of drug content in the formulations was proportional to the size of the microbeads and was found to be in the range of 23.54% to 39.17%. Percentage drug entrapment of the microbeads was found to be within the range of 51.92% to 74.65% which increased with increase in the wax concentration. Moderate entrapment of zidovudine in the microbeads could be attributed to its hydrophilic nature. Furthermore it was found that the entrapment efficiency of bees wax microbeads was better than carnauba wax microbeads (Figure 9).

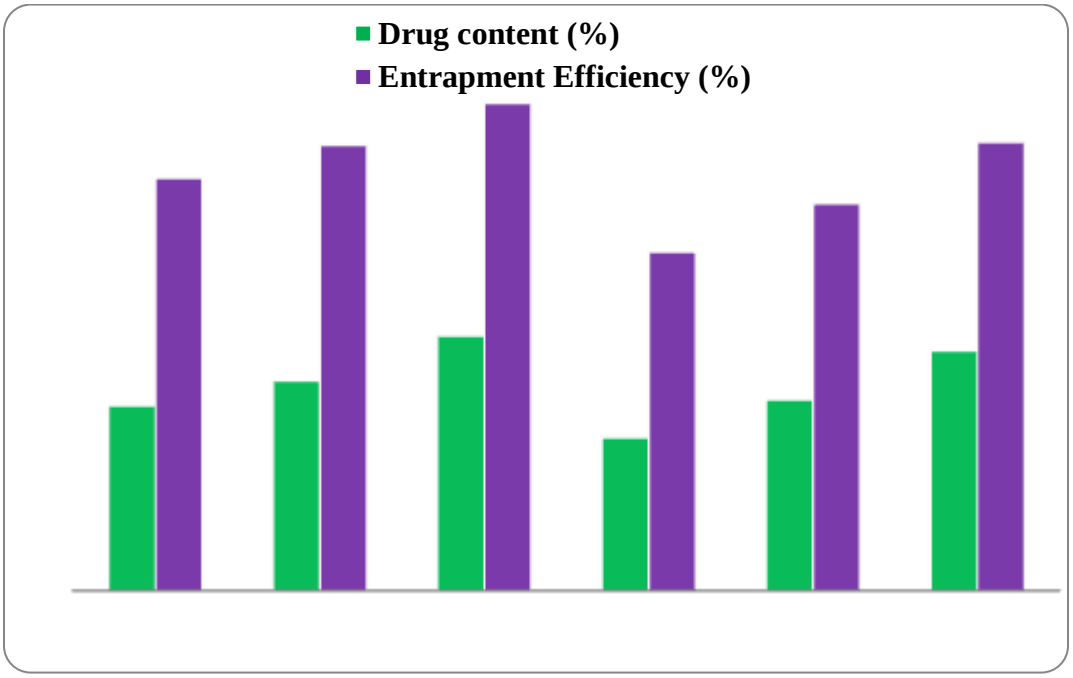

Figure 9: Percentage Drug content & Entrapment Efficiency of AZT microbeads

***In vitro* Release Studies:**

The *in vitro* performance of zidovudine loaded wax microbeads showed prolonged and controlled release for a period of 24 hours. The release showed a biphasic release with an initial burst effect. At the end of first hour, drug release was 23.21%, 20.43%, 18.23%, 21.12%, 18.43 and 17.54% for F_1 to F_6 respectively which can be attributed to the loading of drug onto the surface of microbeads (Table 2 & Figure 10). Percentage drug release was found to decrease with increase in concentration of the wax in the microbeads. Microbeads incorporated with carnauba wax retarded the drug release at higher rates when compared with microbeads incorporated with bees wax. However, bees wax incorporated microbeads had an optimum release at the end of 24^{th} hour with all the other parameters within the specified limits.

Table 2: *In vitro* Release Profile of Formulations F_1-F_6

Time (hrs)	Percentage Drug Release					
	F_1	F_2	F_3	F_4	F_5	F_6
0.5	11.34	9.32	6.01	8.45	6.24	6.55
1	23.21	20.43	18.23	21.12	18.43	17.54
2	26.35	23.12	21.32	24.65	21.61	20.35
3	29.82	26.54	24.51	27.51	25.52	23.93
4	33.61	30.62	28.81	31.9	29.3	27.22
6	41.24	39.71	36.14	40.13	38.16	35.41
8	49.85	48.87	45.15	48.77	45.23	44.63
12	63.09	61.64	60.14	61.78	59.56	58.12
16	75.15	73.34	71.12	72.57	70.25	69.97
20	86.34	84.23	82.37	83.98	82.11	80.99
24	97.23	95.54	94.87	94.87	93.43	90.29

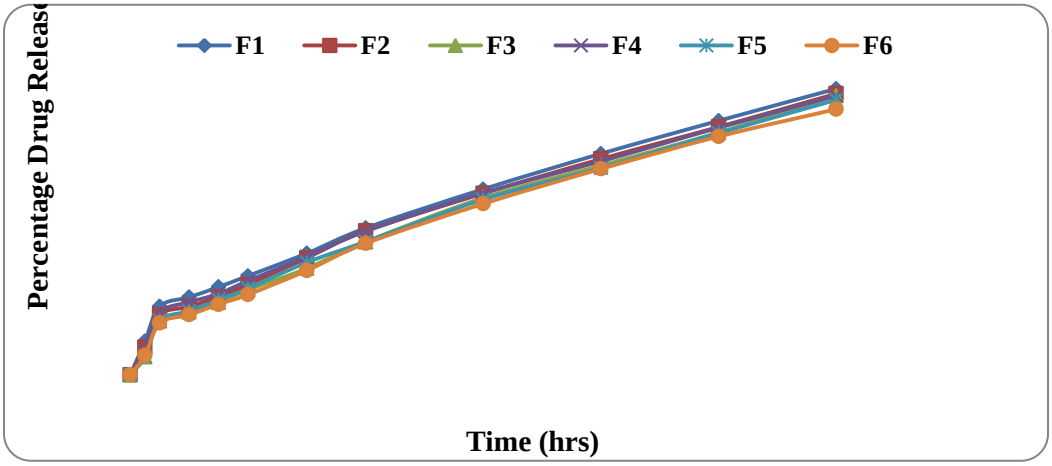

Figure 10: *In vitro* dissolution profiles of drug loaded microbeads (F_1-F_6)

Stability Studies:

The selected batch of zidovudine loaded microbeads was evaluated for physical and chemical stability by storing the formulation at 3 different temperatures as previously described for a period of one month and evaluated for any changes in the percentage drug entrapment. There are no significant changes in the percentage drug entrapment for the formulations stored at 4 ºC. But it was found that there was a considerable reduction in percentage drug entrapment of the microbeads stored at 25ºC±2ºC; 60±5%RH as well as 37ºC±2ºC; 70±5%RH (Table 3 & Figure 11).

Table 3: Percent drug leakage from microbeads (F_3) at different storage conditions

Storage Condition	Percentage drug leakage		
	10 days	20 days	30 days
4ºC±1ºC	0.86	1.82	2.43
25ºC±2ºC; 60±5%RH	5.73	9.54	14.09
37ºC±2ºC; 70±5%RH	7.41	10.99	16.31

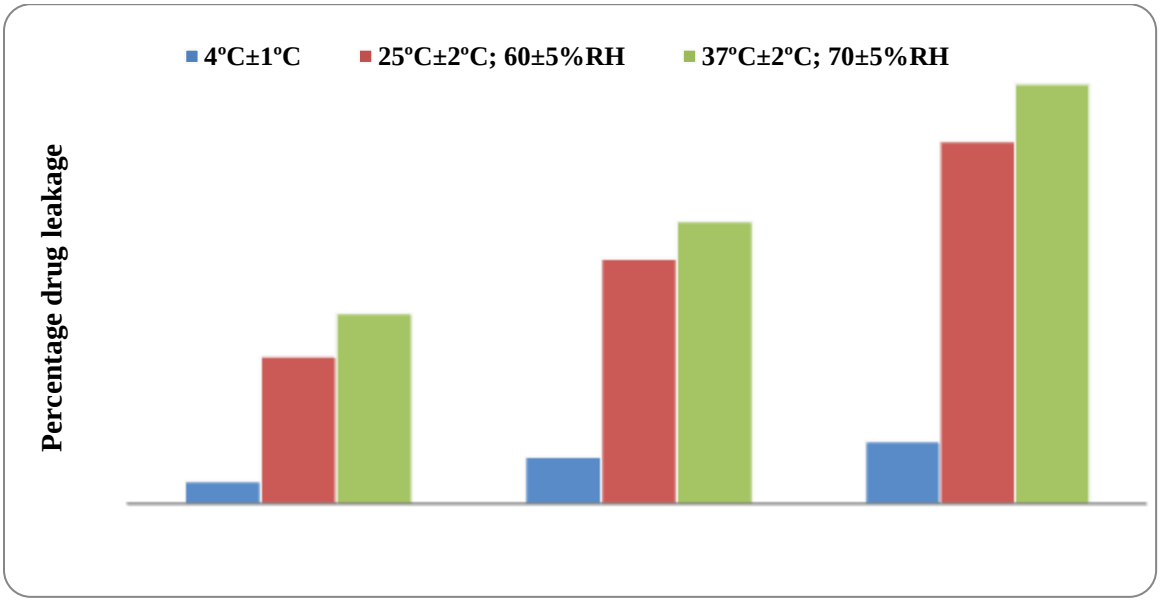

Figure 11: Percent drug leakage from microbeads (F_3) at different storage conditions

CONCLUSION

After observing all the experimental results it was conclusively demonstrated that wax incorporated microbeads loaded with zidovudine were formulated by hot melt extrusion along with ionotropic gellation method using sodium alginate as biodegradable polymer and bees wax and carnauba wax as rate controlling polymers. *In vitro* dissolution profiles showed that the release was sustained for a period of 24 hrs. The stability studies showed that the formulations should be stored at 4±1°C. Formulation with higher concentration of wax showed optimum results within all the evaluated parameters and hence considered as the ideal formulations. However, a more promising controlled release was observed in formulation with highest amount of bees wax which showed the best results with other parameters within the specified limits. Future research can be directed towards *in vivo* studies. Finally, this microparticulate drug delivery technology can be further explored for the drugs exhibiting less half life and narrow therapeutic indices.

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