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Research Article

FORMULATION AND EVALUATION OF ZIDOVUDINE LOADED OLIBANUM RESIN MICROCAPSULES: EXPLORING THE USE OF NATURAL RESINS AS BIODEGRADABLE POLYMERIC MATERIALS FOR CONTROLLED RELEASE

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ABSTRACT

The aim of the present study is to develop and evaluate natural biodegradable microcapsules of zidovudine (AZT) by using olibanum resin as microencapsulating agent, which after oral administration could improve the bioavailability of the drug, in order to provide the sustained release to minimize the dose dependent side effects as well as to improve patient compliance. The proposed system was evaluated *in vitro* for particle morphology, microencapsulation efficiency, production yield, micromeritic properties, release profile and release kinetics etc. Physico-chemical characteristics of AZT and AZT loaded microcapsules were evaluated by differential scanning calorimetry (DSC), X-ray diffraction (XRD), and infrared spectroscopy (FTIR). The resin coated microcapsules were found to be spherical, discrete and free flowing. Microencapsulation efficiency was in a narrow range (81-88%) suggesting an identical distribution of drug in different batches. DSC and XRD results showed a partial modification in AZT's solid state. Zidovudine release from optimized batches of resin coated microcapsules was slow and over 24 hours depending on the core: coat ratio. Drug release was found to be following Fickian diffusion mechanism. The resin coated microcapsules exhibited good controlled release characteristics and were found to be suitable for once a day oral controlled release product.

Keywords: AZT, Olibanum, Biodegradable, Microcapsules, Resin, Controlled release

INTRODUCTION

Drug discovery alone is insufficient in treating diseases; often correct dosing and targeting are equally important for clinical success. Researchers in the area of controlled or sustained drug delivery systems specifically concentrate in to these areas to enhance the efficacy of therapeutics for specific treatment regimens. Controlled drug delivery systems are aimed at controlling the release of the drug at a therapeutically effective rate, prolonging the duration of drug delivery & therapeutic response and targeting the delivery of the drug to a tissue. [1, 2, 3, 4]

One of the very common types of orally administered controlled release system is microparticles which includes microcapsules and microspheres, produced by a process known as microencapsulation. Moreover these are multiunit systems that spread over a large surface area of absorbing mucosa and prevent exposure to a high drug concentration, when compared to single unit dosage form on chronic dosing. They release the drug more uniformly instead of vagaries of gastric emptying and different transit rates through the gastrointestinal tract. [5, 6, 7] Microencapsulation by various polymer and their applications are described in standard text books. [8,9,10]

Although a variety of polymeric materials are available to serve as a release retarding microencapsulating agent but use of natural biodegradable polymers to prolong the delivery of the drugs is always an area of active research despite the advent of synthetic biodegradable polymers. Natural biodegradable polymers remains attractive primarily because they are readily available in the nature, relatively inexpensive, products of living organisms, readily undergoes in-vivo degradation, non-toxic and capable of chemical modifications. [11] In the present study olibanum resin was used as a natural biodegradable microencapsulating agent to retard the release of the drug. Olibanum is obtained from the oleo gum resin of incised trunk of the tree *Boswellia serrata* belonging to the family Burseraceae, commonly known as Sallaki guggul, Salai gum and Indian Olibanum. 10 species of *Boswellia* occur in tropical parts of Asia and Africa. In India *B. serrata* species found in dry and hilly area of Bihar, Madhya Pradesh and Gujarat. Olibanum consist of mainly an acid resin (56-60%), gum (30-36%) and volatile oil (3- 8%). Gum is mainly composed of arabinose with small amount of xylose and galactose. [12, 13]

One of the most popular methods for the formulation of biodegradable microparticles is solvent evaporation technique, where the drug is dissolved or dispersed in to an organic polymer solution, which is then emulsified in to a continuous aqueous or oil phase. The microparticles are formed after removing the solvent. [14, 15]

Aquired immunodeficiency syndrome (AIDS), caused by Human Immunodeficiency Virus (HIV) is an immuno suppressive disease results in life-threatening opportunistic infections and malignancies. [16, 17] Since its first identification in California about three decades ago in 1981, more than 25 million people all over the world has been killed by this dreaded killer. [18] UNAIDS 2012 report on global AIDS epidemic showed 34 million people were living with HIV at the end of 2011 and around 1.7 million people died from AIDS related causes worldwide in 2011. [19] To date there are approximately 30 antiretroviral products, formulated singly or in combination to treat patient with HIV. [20]

Zidovudine (3'-azido-3'-deoxythymidine or AZT) originally synthesized in 1964 as a potential anticancer agent, was approved as first antiretroviral agent ever in 1987 for the treatment of AIDS [21, 22]. However along with its therapeutic effectiveness, AZT is also associated with certain limitations like poor-bioavailability, dose-dependent hematological toxicity, short biological half life, low therapeutic index etc. But administration of antiviral agents like AZT is required chronically or possibly for the life time of the patient. In case of oral route the dose of AZT ranges from 3mg/kg to 10mg/kg body weight at every four hours interval to maintain the constant therapeutic blood levels. These frequent dosing intervals are

undesirable in terms of patient compliance and generating toxicity (associated with excessive plasma levels) immediately after oral or

intravenous administrations. In order to succeed in an effective therapy for AIDS, it is crucial to maintain the systemic drug concentration consistently above their target antiretroviral concentration throughout the course of their treatment without much oscillation in its plasma levels, which can be done by formulating controlled or sustained release dosage forms of AZT. Therefore, AZT is an ideal candidate for sustained release microsphere formulation, resulting in more reproducible drug absorption and reducing the dosing frequency, thereby improving patient compliance as compared to immediate release dosage forms. The objective of the present study was to formulate and evaluate natural biodegradable sustained release microcapsules of AZT using olibanum resin as release retardant .[23, 24, 25, 26, 27, 28, 29]

MATERIALS AND METHODS

Zidovudine was obtained as gift sample from HETERO DRUGS Ltd. (Hyderabad, India). Olibanum resin was obtained as a gift sample from Girijan Corporation, (Viasakhapatnum, India). Acetone (Merck), diethyl ether (Qualigens), Light liquid Paraffin (Qualigens), span 80 (Finnar chemicals) etc are used. All reagents were of pharmaceutical grade and were used as received.

Preparation of microcapsules

Zidovudine-loaded microcapsules were prepared by an industrially feasible emulsion solvent evaporation technique. Acetone was used as the polymer solvent, light liquid paraffin as oil phase, Span 80 as emulsifying agent and n-hexane to wash away the paraffin oil. To prepare microcapsules with various drug to polymer ratios (w/w), accurately weighed amount of Zidovudine was dissolved in acetone solution (w/v) of olibanum resin. The drug to polymer ratio was varied keeping the amount of drug and solvent constant in all cases, but changing the amount of polymer. The oil phase was prepared by dispersing Span 80 as the emulsifying agent in liquid paraffin, with a constant composition in all cases. The organic phase was poured into the oil phase under constant stirring rate at 1200 rpm contained in a 500 ml beaker to emulsify the added dispersion as fine droplets. A Remi medium duty stirrer with a digital speed meter (Model RQT 124) was used for stirring. After the emulsion formation, acetone was completely removed by evaporation at room temperature during an approximately 3 h stirring period. The light mineral oil was decanted and the microcapsules were collected, washed three times with 100 ml of n-hexane at room temperature, afterwards the microcapsules were separated by vacuum filtration and air dried for 12 h to obtain discrete microcapsules. [30]

Estimation of Zidovudine

Accurately weighed microcapsules equivalent to 100 mg of drug was crushed and suspended in 100 ml of pH 7.4 phosphate buffer. The resulting mixture was stirred at 1000 rpm for 2 hrs and kept overnight. Then the solution was filtered, diluted suitably and analyzed for drug content at 264.99 nm using UV-visible spectrophotometer (Carry 60, Agilent, Australia). [31]

Production Yield and Microencapsulation efficiency [32]

The yield of the microcapsules was expressed as percentage of the weight of the dried microcapsules at room temperature compared to the theoretical amount. Production yield or percentage yield is calculated by using the following Equation.

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of microcapsules obtained}}{\text{Weight of raw materials}} \times 100$$

Microencapsulation efficiency [33] was calculated using following formula:

$$\text{Microencapsulation efficiency (\%)} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Micromeritic properties

Micromeritic properties, such as angle of repose, tapped density and bulk density were measured. The angle of repose [34] was calculated by static method using Funnel. The experiments were carried out in triplicate.

Determination of particle size distribution by sieve analysis

Separation of the microcapsules into various size fractions was carried out using a mechanical sieve shaker. A series of five standard stainless steel sieves (Geologists Syndicate Pvt. Ltd, India) having mesh size of #10, #20, #30, #50 and #80 were arranged in an order of decreasing aperture size. About 10 g of drug loaded microcapsules were placed on the uppermost sieve. The sieves were shaken for a period of 10 min, and then the particles on each screen were weighed. [35] The procedure was carried out three times for each product.

Characterization of AZT microcapsules

FT-IR studies

Drug-polymer interactions were studied by FT-IR spectroscopy using the instrument Shimadzu, Japan, FTIR-8400S. The spectra were recorded for pure drug Zidovudine and microcapsules containing drug. Samples were prepared in KBr discs (2 mg sample in 200 mg KBr) with a hydrostatic press at a force of 5.2 N/m^2 for 3 min. The scanning range was 400–4000 cm-1 and the resolution was 4 cm-1. [32]

Surface scanning electron microscopy (SEM)

The surface morphology of the microcapsules was observed by using scanning electron microscope (LEO 440i, England). The samples were mounted on an aluminum sample stub using adhesive carbon tape and placed in a low humidity chamber for 12 h prior to analysis. Samples were coated with gold-palladium for 60 sec under an argon atmosphere using ion sputter coater in a high vacuum evaporator equipped with a rotary stage tray. Images were taken at an acceleration voltage of 20 kV. [32]

Differential scanning calorimetry

The thermal behavior of the microcapsules was investigated using differential scanning calorimeter (DSC 60, Shimadzu, Japan). Samples of about 5 mg were placed in 50 μm perforated aluminium pans and sealed. All samples were run at a heating rate of 10˚/min over a temperature range of 5–300˚C in atmosphere of nitrogen as purging gas at a flow rate of 25 ml/min. [32]

X-ray diffraction analysis

Microcapsules were subjected to X-ray diffraction analysis, using Philips PW 170 system (Philips USA) with Cu-Kα radiation (400 kV, 30 mA, and scan speed 1˚/min) to investigate the physical state of zidovudine entrapped in the microcapsules. [32]

In-vitro drug release studies

The *in-vitro* release rate study of AZT from resin-coated microcapsules were carried out for 24 hours using paddle type dissolution apparatus (USP-XXIII, ETC-11L, Electrolab, Mumbai) containing 900 ml of dissolution medium maintained at 37±0.5ºC and speed of agitation at 100 rpm. [36] An accurately weighed quantity of microcapsules containing around 100mg of drug were suspended in dissolution medium consisting 900 ml of phosphate buffer pH 7.4, and the process was continued up to 24 hours. The system was adjusted to ensure sink conditions. Aliquots (5 ml) of the dissolution medium were withdrawn at predetermined time intervals, filtered by using Whatman No. 42 filter and were replenished immediately with the same volume of fresh medium. Withdrawn samples were assayed spectrophotometrically at 264.99 nm, the detected wavelength of maximum absorbance of zidovudine in pH 7.4 phosphate buffer (Cary 60, Agilent Technologies). Olibanum resin did not interfere with Zidovudine absorption in pH 7.4 phosphate buffer at this wavelength. The analysis was carried out in triplicate.

Kinetic models and the analysis of the release profiles

The *in vitro* release profiles were fitted on various kinetic models like Higuchi, first-order, Peppas and zero-order equations in order to find out the mechanism of drug release. The rate constants were calculated from the slope of the respective plots. The data obtained were also put in Korsemeyer-Peppas model in order to find out *n* value, which describes the drug release mechanism. The mechanism of drug release from spherical polymeric devices may be Fickian diffusion when the value of $n = 0.43$ or less, anomalous (non-Fickian) transport when the value of *n* lies between 0.43 and 0.85, and case II transport when $n = 0.85$. An exponent value of *n* greater than 0.85 signifies super case II transport mechanism. [37]

RESULTS AND DISCUSSION

Preparation of microcapsules, production yield (%), Estimation of drug content and microencapsulation efficiency (%)

In an attempt to modify the release of zidovudine from the microcapsules, different batches of formulations were prepared in which the increasing amounts of olibanum resin were added to the fixed weight of zidovudine. When hydrophilic drugs like AZT are encapsulated using an aqueous phase as the processing medium, preferentially they partition out in to the aqueous medium leading to low encapsulation efficiency. [38] It has been reported that as much as 80% AZT can partition out in to the outer aqueous processing medium depending on the processing conditions. [28] In the present study an attempt was made to encapsulate AZT with sufficiently high encapsulation efficiency employing a natural biodegradable resin like olibanum and using a non-aqueous processing medium (liquid paraffin). Span 80, a non-ionic surface active agent having HLB value 4.3 was used to stabilize the emulsification process by reducing the interfacial tension. The highest product yield and encapsulation efficiency was achieved by increasing the drug-polymer ratio (Table 1). It was observed that the encapsulation efficiencies were within a narrow range suggesting an identical distribution of drug in different batches.

Table 1: Data showing core: coat ratio, production yield and microencapsulation efficiency

Formulation Codes	Core: Coat ratio	% Yield	Microencapsulation Efficiency
ZO1	1: 0.1	46.27	81.615
ZO2	1: 0.25	48.59	84.474
ZO3	1: 0.5	56.36	85.806
ZO4	1: 0.7	58.73	86.616
ZO5	1: 0.8	61.98	87.191
ZO6	1: 0.9	62.42	88.592

SEM and micromeritic studies

The SEM photomicrographs of the optimized formulation of AZT indicated that the microcapsules were discrete, spherical, free flowing, multinucleate, and uniform in shape (Figure 1). Surface of the microcapsules appear to be rough, may be due to the presence of drug. The different batches of AZT loaded microcapsules were assessed for parameters like angle of repose, bulk density, tapped density, Carr's index and Hausner's ratio. The results were given in the table 2. The flow properties of different batches of microcapsules were excellent as the angle of repose values were found to be less than 25, compressibility index less than 15% and Hausner's ratio less than 1.25 in case of all the batches. It suggests that microcapsules don't require a glidant.

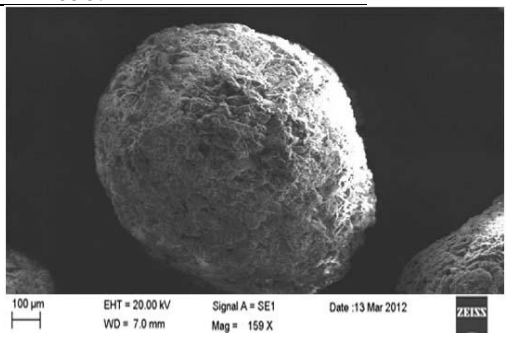

Fig. 1: Scanning electron micrographs of AZT loaded microcapsules (ZO4)

Table 2: Flow properties of microcapsules

Formulation Codes	Angle of Repose ±S.D.	Loose Bulk Density (g/cm³) ±S.D.	Tapped Bulk Density (g/cm³) ±S.D.	Carr's Index (%)	Hausner's Ratio
ZO1	22.4±0.043	0.376±0.004	0.435±0.11	13.56	1.15
ZO2	23.38±0.07	0.402±0.007	0.45±0.017	10.66	1.11
ZO3	21.75±0.117	0.42±0.004	0.476±0.004	11.76	1.13
ZO4	20.36±0.026	0.446±0.006	0.516±0.016	13.97	1.16
ZO5	24.26±0.091	0.463±0.012	0.526±0.014	11.97	1.13
ZO6	22.3±0.062	0.499±0.008	0.553±0.025	9.76	1.1

S.D.: Standard deviation; n=6

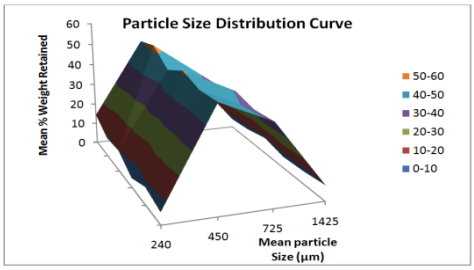

Figure 2: Particle size distribution curve of different batches of microcapsules

FT-IR studies

The results of FTIR spectral studies showed that there was no significant interaction between the drug and polymer. It was observed that there are no major degenerative interactions and hence the polymers could be used safely to formulate the microcapsules. Pure drug showed sharp characteristic peaks of carbonyl group in 1,678 cm^{-1} and of azide group in 2.085 cm^{-1}. One band in 1378 cm^{-1} is assigned to CH_2 and one band in 1285 cm^{-1} is assigned to C-O-C and the C-OH grouping. All the above characteristic peaks appeared in the spectrum of microcapsules too indicating there was no modification or interaction between drug and resin. This is also supported by the fact there was no appearance and disappearance of new or existing peaks.

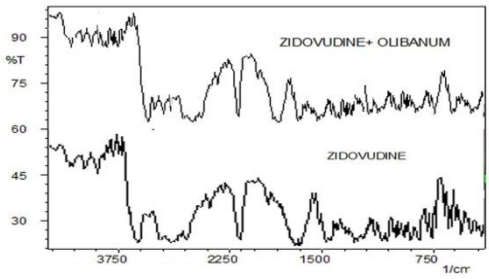

Figure 3: FTIR spectra of pure AZT and AZT loaded microcapsules (ZO4)

Differential scanning calorimetry

The compatibility of AZT in olibanum microcapsules was evaluated through DSC analysis. The DSC thermograms of pure AZT and AZT-loaded olibanum microcapsules are presented in Figure 4. It was evident from the DSC profile that AZT exhibited a sharp endothermic peak associated with crystal melting at a temperature of 126.79^{0}C, which corresponds to the reported melting temperature of the drug. A similar DSC profile (Figure 4) of the drug appeared at the temperature corresponding to its melting point in the AZT-loaded olibanum microcapsules but with a slight change in its sharp appearance. It appears that there is a minor reduction of drug crystallinity in the microcapsules. The DSC study apparently revealed that the drug was compatible with the polymer and neither drug decomposition nor drug-polymer interactions occurred in the freshly prepared microcapsules.

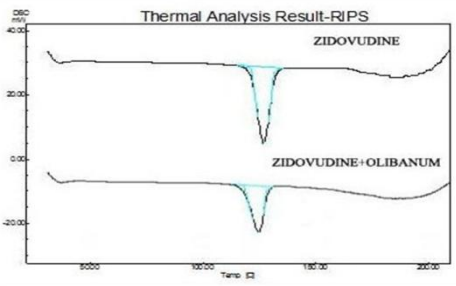

Figure 4: DSC curves of pure AZT and AZT loaded microcapsules (ZO4)

X-ray diffraction analysis

The thermal behavior coupled with the X-ray crystallographic data suggested that the diffractogram of pure AZT indicates the crystalline structure of the drug. The diffractogram of AZT-loaded olibanum microcapsules shows a similar pattern with a slight decrease in the intensity of the peaks, which suggests that the drug was able to disperse almost homogenously in the microcapsules. This result confirms a partial change in the solid state of AZT from crystalline to amorphous. Similar results reported for other sustained release microsphere studies had the same interpretation for zidovudine, famotidine etc. [38, 39]

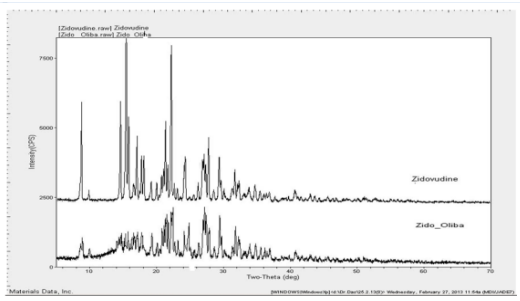

Figure 5: X-ray diffractograms of pure AZT and AZT loaded microcapsules (ZO4)

***InVitro* drug release behavior**

The *in vitro* drug release study of different batches of microcapsules was carried out in pH 7.4 phosphate buffer. In order to keep the total surface area of the microcapsules constant and thus to get comparable results, the release studies were carried out using the same size fractions (450µm) of microcapsules containing equivalent amount of AZT from different batches of microcapsules. The AZT release from different batches of microcapsules exhibited a biphasic kinetics mechanism; an initial burst release (23-40%), which was due to the presence of drug particles on the surface of the microcapsules followed by a much slower release. The initial burst effect may be attributed as a desired effect to ensure minimum therapeutic plasma drug concentration. The release profiles are illustrated in Figure 6. Drug release rates decreased with increasing amounts of resin in the formulation. Lower levels of resin corresponding to the drug in the formulations resulted in an increase in the drug release rate.

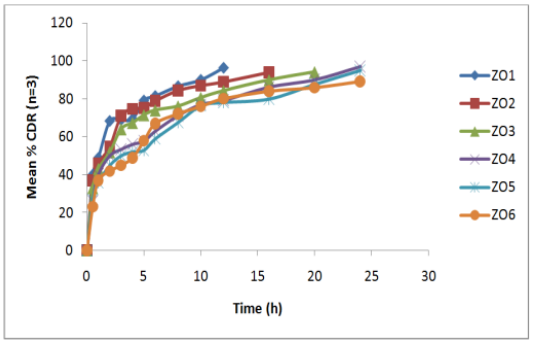

Figure 6: In Vitro release profile (Zero order) of AZT loaded microcapsules from different batches

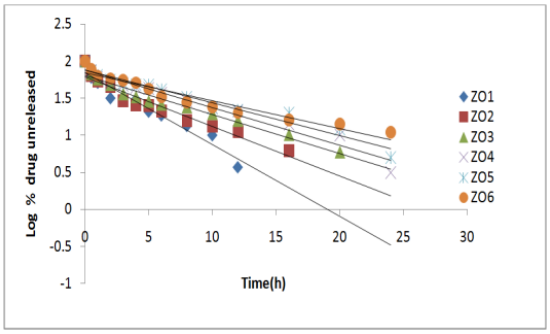

Figure 7: *In Vitro* release profile (First order) of AZT loaded microcapsules from different batches

Release Kinetics

The *in vitro* drug release profiles of AZT were applied on various kinetic models in order to evaluate the mechanism of drug release. The different kinetic models evaluated were zero order, first order and Higuchi. After linearization of the results obtained in the dissolution test, the best fit with higher correlation coefficients (R^2) was shown in first order, Higuchi and followed by zero order equations as given in the table 3. High correlation was observed in the first-order rather than Higuchi and zero-order models, indicating that the drug release from resin coated microcapsules was diffusion controlled. The data obtained were also put in Korsemeyer-Peppas model in order to find out *n* value, which describes the drug release mechanism. The *n* values of microcapsules of different drug to polymer ratio were ranged between 0.266-0.342 (< 0.43), indicating that the mechanism of the drug release was diffusion controlled based on Fick's law.

Table 3: *In vitro* release kinetic parameters of AZT-loaded olibanum microcapsules

FORMULATIONS	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORSEMEYER PEPPAS MODEL
	R^2	K_0 (%/h)	R^2	K (h^{-1})	R^2	K_h(%/$h^{1/2}$)	*n*
ZO1	0.682	5.752	**0.96**	0.246	0.894	24.5	**0.266**
ZO2	0.637	4.34	**0.928**	0.152	0.874	21.47	**0.274**
ZO3	0.658	3.406	**0.948**	0.119	0.885	18.74	**0.277**
ZO4	0.757	3.037	**0.957**	0.115	0.937	17.68	**0.311**
ZO5	0.784	3.024	**0.957**	0.101	0.95	17.42	**0.315**
ZO6	0.732	2.984	**0.931**	0.085	0.926	17.56	**0.342**

CONCLUSIONS

In conclusion, the attempt to prepare controlled release biodegradable microcapsules of zidovudine using olibanum resin as microencapsulating agent was successful. The method employed was an industrially feasible one, as it involves emulsification and removal of solvent which can be controlled precisely. Since the resin is from natural origin, it is non-toxic, biodegradable and comparatively cheaper than other synthetic biodegradable polymers. Further studies in the area of novel drug delivery systems can be carried out by taking this resin as a natural biodegradable polymer in future.

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