DESIGN AND EVALUATION OF ZIDOVUDINE LOADED NATURAL BIODEGRADABLE MICROCAPSULES EMPLOYING COLOPHONY RESIN AS MICROENCAPSULATING AGENT

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ABSTRACT

The aim of the present study is to develop and evaluate natural biodegradable microcapsules of zidovudine (AZT) by using colophony 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. An industrially feasible emulsification-solvent evaporation method was used for preparation of different batches of microcapsules. 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 (91-97%) 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, Colophony, 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 therapeutic 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 and independent 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 is more 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 colophony (popularly known as resin) which is an oleoresin obtained from the plant Pinus palustris and other species of Pinus belonging to the family Pinaceae is used as the release retarding agent. Colophony contains mainly resin acids which are unsaturated, about 90% of these are abietic acid. It also contains esters of oleic acid, resin acid, around 0.5 % volatile oil, 5 to 6 % resenes etc. Other acids in the colophony are sapinic acid and pinmaric acid [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 microcapsule 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 colophony resin as release retarding agent [23, 24, 25, 26, 27, 28, 29].
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 hydronstic press at a force of 52 N/m² 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 µl perforated aluminium pans and sealed. All samples were run at a heating rate of 10°C/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 colophony 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±0.5°C and speed of agitation at 100 rpm [35]. 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 [Carly 60, Agilent Technologies]. Colophony 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 [36].

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 colophony 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 [37]. It has been reported that as much as 80% AZT can partition out into 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 colophony 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

<table>
<thead>
<tr>
<th>Formulation Codes</th>
<th>Core: Coat ratio</th>
<th>% Yield</th>
<th>Microencapsulation Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZR1</td>
<td>1: 0.05</td>
<td>50.03</td>
<td>91.205</td>
</tr>
<tr>
<td>ZR2</td>
<td>1: 0.1</td>
<td>52.71</td>
<td>92.26</td>
</tr>
<tr>
<td>ZR3</td>
<td>1: 0.25</td>
<td>56.15</td>
<td>93.371</td>
</tr>
<tr>
<td>ZR4</td>
<td>1: 0.5</td>
<td>64.87</td>
<td>95.729</td>
</tr>
<tr>
<td>ZR5</td>
<td>1: 0.6</td>
<td>66.23</td>
<td>96.5</td>
</tr>
<tr>
<td>ZR6</td>
<td>1: 0.7</td>
<td>69.49</td>
<td>97.773</td>
</tr>
</tbody>
</table>

**SEM and micromeritic studies**

The SEM photomicrographs of the optimized formulation of AZT indicated that the microcapsules were discrete, spherical, free flowing 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 near or 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.

**Sieve analysis results of microcapsules**

Microcapsules from different batches presented a narrow particle size distribution and most of them fallen into 450-725 μm range. The sieve analysis of different microcapsules showed that a large proportion of microcapsules (40-60%) in a batch were in the size range of ~30 +50 (450 μm) mesh.

![Fig. 1: Scanning electron micrographs of AZT loaded microcapsules (ZR4)](image-url)

Table 2: Flow properties of microcapsules

<table>
<thead>
<tr>
<th>Formulation Codes</th>
<th>Angle of Repose ±S.D.</th>
<th>Loose Bulk Density (g/cm³) ±S.D.</th>
<th>Tapped Bulk Density (g/cm³) ±S.D.</th>
<th>Carr's Index (%)</th>
<th>Hausner's Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZR1</td>
<td>26.7 ±0.022</td>
<td>0.341±0.04</td>
<td>0.389±0.01</td>
<td>12.33</td>
<td>1.14</td>
</tr>
<tr>
<td>ZR2</td>
<td>26.21±0.032</td>
<td>0.269±0.021</td>
<td>0.311±0.02</td>
<td>13.5</td>
<td>1.15</td>
</tr>
<tr>
<td>ZR3</td>
<td>24.89±0.024</td>
<td>0.338±0.062</td>
<td>0.397±0.07</td>
<td>14.86</td>
<td>1.17</td>
</tr>
<tr>
<td>ZR4</td>
<td>21.56±0.053</td>
<td>0.334±0.073</td>
<td>0.372±0.23</td>
<td>10.21</td>
<td>1.11</td>
</tr>
<tr>
<td>ZR5</td>
<td>22.47±0.042</td>
<td>0.321±0.054</td>
<td>0.359±0.051</td>
<td>10.58</td>
<td>1.11</td>
</tr>
<tr>
<td>ZR6</td>
<td>20.07±0.035</td>
<td>0.324±0.085</td>
<td>0.368±0.052</td>
<td>11.95</td>
<td>1.13</td>
</tr>
</tbody>
</table>

S.D.: Standard deviation; n=6
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.

Differential scanning calorimetry

The compatibility of AZT in colophony microcapsules was evaluated through DSC analysis. The DSC thermograms of pure AZT and AZT-loaded colophony 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°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 colophony 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.

Fig. 2: Particle size distribution curve of different batches of microcapsules

Fig. 3: FTIR spectra of pure AZT and AZT loaded microcapsules (ZR4)
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 colophony 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]
In Vitro 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 effect (20-40%), 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.

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

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Zero Order K&lt;sub&gt;r&lt;/sub&gt; (%/h)</th>
<th>First Order K&lt;sub&gt;1&lt;/sub&gt; (h&lt;sup&gt;−1&lt;/sup&gt;)</th>
<th>Higuchi Model R&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Korsmeyer Peppas Model R&lt;sup&gt;2&lt;/sup&gt;</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZR1</td>
<td>0.860</td>
<td>6.131</td>
<td>0.911</td>
<td>0.204</td>
<td>0.946</td>
</tr>
<tr>
<td>ZR2</td>
<td>0.873</td>
<td>5.149</td>
<td>0.919</td>
<td>0.216</td>
<td>0.962</td>
</tr>
<tr>
<td>ZR3</td>
<td>0.887</td>
<td>3.82</td>
<td>0.931</td>
<td>0.115</td>
<td>0.948</td>
</tr>
<tr>
<td>ZR4</td>
<td>0.929</td>
<td>3.08</td>
<td>0.944</td>
<td>0.096</td>
<td>0.973</td>
</tr>
<tr>
<td>ZR5</td>
<td>0.949</td>
<td>3.279</td>
<td>0.949</td>
<td>0.085</td>
<td>0.953</td>
</tr>
<tr>
<td>ZR6</td>
<td>0.949</td>
<td>3.279</td>
<td>0.946</td>
<td>0.078</td>
<td>0.953</td>
</tr>
</tbody>
</table>

CONCLUSIONS

In conclusion, the attempt to prepare controlled release biodegradable microcapsules of zidovudine using colophony resin as a 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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