FORMULATION AND EVALUATION OF ZIDOVUDINE LOADED CHITOSAN NANOPARTICLES FOR ANTIVIRAL THERAPY

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ABSTRACT
The goal of the present investigation was to formulate and evaluate chitosan Nanoparticles of Zidovudine for antiviral therapy. Nanoparticles of Zidovudine were prepared using chitosan, liquid paraffin and Tween-20 using Emulsion droplet coalescence method. The concentration of the polymer Chitosan was selected based on the results on preliminary screening. The nanoparticles prepared were evaluated for morphology, loading efficiency and in vitro release. The particle shape and morphology of the prepared Zidovudine nanoparticles were determined by SEM analysis. The amount of Zidovudine entrapment in the nanoparticles was calculated by the difference between the total amount of drug added to the nanoparticle and the amount of non-entrapped drug remaining in the aqueous supernatant. A Franz diffusion cell was used to monitor Zidovudine release from the nanoparticles. The formulations CF1 and CF2 showed good drug release from the polymer. The percentage cumulative drug release after 12 hours was 75.54 and 75.89 respectively. However about 15% initial burst release was found at 1 hour in all formulations. CF2 released 75.89% of Zidovudine in 12 hours with a burst drug release nearly 14.86% of drug within the initial 1 hour. Formulations 4 out of 2 showed good drug release from the polymer, the percentage cumulative drug release after 12 hours were in the range of 72-75%. Among the four formulations CF2 (1% Chitosan) showed maximum drug release in 12 hours diffusion study and good entrapment efficiency. In-vitro antiviral study revealed that the formulated nanoparticles were found to have good viral activity.

Key words: Emulsion droplet coalescence, Nanoparticles, Chitosan, Zidovudine.

INTRODUCTION
Novel drug delivery systems present an opportunity for formulation scientists to overcome the many challenges associate with antiretroviral (ARV) drug therapy. The currently available anti–HIV drugs classified into the nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors and recently fusion, integration inhibitors. Most of these drugs bear some significant drawbacks such as relatively short half-life, low bioavailability, poor permeability and undesirable side effects. So the efforts have been made to design drug delivery systems for antiretroviral therapy as reducing dosing frequency, increase bioavailability, decrease degradation/metabolism in GIT, improve CNS penetration and inhibit CNS efflux, delivery them to target cells and selectively minimal side effects (Arvind S et al., 2010).

Antiviral drugs are a class of medication used specifically for treating viral infections (Anonymous 1). Like antibiotics for bacteria, specific antivirals are used for specific viruses. Unlike most antibiotics, antiviral drugs do not destroy their target pathogen; instead they inhibit their development.

In this trend nanoparticles show a fastest development from out of total novel drug delivery systems. Nanoparticles are stable, solid colloidal particles consisting of macromolecules materials and ranging in
size from 10 to 1000nm. Drugs can be absorbed on the particle surface or can be entrapped or dissolved in the particle matrix. Nanoparticles are known to accumulate in the tissue because of phagocytosis by MO/Mac. Thus using nanotechnology, engineering researchers have developed a small but powerful device capable of enhancing the targeted delivery of drugs to treat life-threatening illnesses (Yung-chih K, 2005).

Zidovudine is a thymidine analogue. It is phosphorylated in the body to its active form zidovudine triphosphate which interferes in DNA synthesis of retroviruses by inhibiting DNA replication. Zidovudine inhibits the key enzyme reverse transcriptase. Human DNA polymerase is inhibited only at a conc. 100 times more than that required to inhibit viral reverse transcriptase. The bioavailability of Zidovudine is 60-70%.

MATERIALS AND METHODS

Zidovudine was obtained as a gift sample from Micro Labs, Bengaluru. Chitosan(viscosity 100 cps) was purchased from Para’s Pharma Chem suppliers (Pune). Liquid paraffin, tween 20, sodium chloride was obtained from Spectrum Chemicals and Reagents, Cochin, India. Sodium hydroxide, Sodium dihydrogen phosphate and disodium hydrogen phosphate was procured from Burugoyne Uribiges & Co, Mumbai, India.

Preparation of drug loaded nanoparticles
Method: emulsion-droplet coalescence method

Chitosan was dissolved in 1% acetic acid and 50 mg of Zidovudine in phosphate buffered saline. This solution was added to 10 ml of liquid paraffin containing 5% v/v tween 20. This mixture was stirred using a homogeniser 3 minutes to form water in oil (w/o) emulsion (Bodmeier R et al., 1989).

The resultant Zidovudine nanoparticles were centrifuged at 3000 rpm for 60 mts (REMI, India) and washed using ethanol and water, consecutively to remove the remaining surfactant and liquid paraffin. Later they were dried in air for 3 hour followed by hot air oven at 50° for 4 hour and stored in a dessicator (Conti B et al., 1998). (Table 1)

Evaluation of Nanoparticles
Detection of shape and morphology

The particle shape and morphology of the prepared Zidovudine nanoparticles were determined by SEM analysis. The nanoparticles were viewed using a Jeol-5610 L V (Tokyo, Japan) for morphological examination. Powder samples of dried nanoparticles were mounted onto aluminium stubs using double side adhesive tape and then sputter coated with a thin layer of gold at 10 Torre for vacuum before examination. The specimens were scanned with an electron beam of 1.2kv acceleration potential and images were corrected in secondary electron mode.

Determination of loading/entrapment efficiency

The amount of Zidovudine entrapment in the nanoparticles was calculated by the difference between the total amount of drug added to the nanoparticles and the amount of non-entrapped drug remaining in the aqueous supernatant. The latter was determined following the separation of drug loaded nanoparticles from the aqueous medium by centrifugation at 5000 rpm for 30 min. The supernatant was collected and the particles were washed with water and then subjected to another cycle of centrifugation. The amount of free Zidovudine in the supernatant was determined by UV-Visible spectrophotometer (UV1 v7.07 Thermo Scientific, Germany (Gaumet M et al., 2008).

In-vitro release study

A Franz diffusion cell was used to monitor Zidovudine release from the nanoparticles (Haliza K et al., 2009). The receptor phase was phosphate buffered saline (PBS, pH 7.4) thermostatically maintained at 37°C, with each release experiment run in triplicate. Dialysis membrane (Hi Media, Mumbai, India) with molecular weight cut off 12,000 to 14000 Daltons was used to separate receptor and donor phases. The latter consisted of a 2ml suspension of nanoparticles containing 10 mg of Zidovudine, mixed for 5 seconds to aid re-suspension, in a 1% w/v Tween 80 solution in PBS. Samples (1ml) from the receptor phase were taken at time intervals and an equivalent volume of PBS replaced into the receiver compartment. Diffusion of Zidovudine into the receptor phase was evaluated spectrophotometrically (Fraser IS et al., 1998).

RESULTS AND DISCUSSION

In total four formulations of Zidovudine loaded nanoparticles were prepared and evaluated for various parameters such as particle size, morphology, drug entrapment efficiency and in-vitro release.

Preliminary screening for encapsulation efficiency

The nanoparticle drug delivery system is prone for the delivery of drugs to the targeted site. In early stages of formula optimization studies, the w/o emulsion formation was the problem. It was overcome by replacing surfactant; the surfactant selected was tween 20, which had high encapsulation efficiency.

Preparation of nanoparticles

Nanoparticles were prepared by emulsion droplet coalescence method. It is a laboratory method proved for the preparation of nanoparticles. The concentrations of the polymer Chitosan were selected based on the results on preliminary screening. The
The surfactant used for the preparation was tween 20. The time taken to complete preparation was around 2 hours.

**DSC studies**

As DSC is useful tool to monitor the effect of additives on the thermal behavior of materials, these techniques were used to derive qualitative information about the physicochemical status of drug in particles. The peak for Zidovudine pure sample was obtained in 152.04°C. The peak in physical mixture and nanoparticles were 154.68°C and 157.51°C respectively.

**Entrapment efficiency and loading capacity**

The data of drug entrapment efficiency and drug loading capacity for drug loaded nanoparticles were as shown in the Table 2. The formulation CF1 showed around 32% of drug loading.

**In-vitro diffusion study**

The drug release profile from the nanoparticles were as shown in the graphs (Graph 1). The formulations CF1 and CF2 showed good drug release from the polymer. The percentage cumulative drug release after 12 hours was 75.54 and 75.89 respectively. However about 15% initial burst release was found at 1 hour in all formulations. CF2 released 75.89% of Zidovudine in 12 hours with a burst drug release nearly 14.86% of drug within the initial 1 hour.

**Table1. Formulation of Zidovudine nanoparticles**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Conc. Of Chitosan</th>
<th>Amount of drug (Zidovudine)</th>
<th>Conc. of Tween 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF1</td>
<td>0.5%</td>
<td>50mg</td>
<td>0.5%</td>
</tr>
<tr>
<td>CF2</td>
<td>1%</td>
<td>50mg</td>
<td>0.5%</td>
</tr>
<tr>
<td>CF3</td>
<td>1.5%</td>
<td>50mg</td>
<td>0.5%</td>
</tr>
<tr>
<td>CF4</td>
<td>2%</td>
<td>50mg</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

**Table 2. Summary of evaluation of nanoparticles**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CF1</th>
<th>CF2</th>
<th>CF3</th>
<th>CF4</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.2</td>
<td>7.5</td>
<td>7.7</td>
<td>7.8</td>
</tr>
<tr>
<td>Practical yield</td>
<td>128</td>
<td>160</td>
<td>130</td>
<td>275</td>
</tr>
<tr>
<td>Efficiency of practical recovery (mg)</td>
<td>85.32</td>
<td>87.49</td>
<td>85.46</td>
<td>89.36</td>
</tr>
<tr>
<td>Un encapsulated drug (mg out of 50mg)</td>
<td>8.4</td>
<td>10.6</td>
<td>11.3</td>
<td>13.5</td>
</tr>
<tr>
<td>Entrapment efficiency (%)</td>
<td>82.09</td>
<td>77.56</td>
<td>76.80</td>
<td>74.86</td>
</tr>
<tr>
<td>Loading capacity (%)</td>
<td>32.01</td>
<td>22.70</td>
<td>28.48</td>
<td>13.86</td>
</tr>
</tbody>
</table>

**CONCLUSION**

On preliminary screening different formulations were developed with various ratios of polymers and different surfactants. It revealed that formulations with the polymer concentration (1.0-2.0%) and surfactant (tween-20) had better drug release and entrapment efficiency. So the formulations were designed with that polymer concentration and surfactant. Four formulations were evaluated and among them CF1 and CF2 were found to have good results. Among the two formulations CF2 (1% Chitosan) showed maximum drug release in 12 hours diffusion study and good entrapment efficiency.

The work on formulation development of Zidovudine nanoparticle was very much advantageous than the existing dosage forms as the drug is targeting to the viral cells, hence better action.

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