INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) was first defined after a patient suffering an absence of immunity in New York about three decade ago. The pathogen inducing AIDS is human immunodeficiency virus (HIV). Individuals infected by HIV may cause serious disorders, such as AIDS dementia complex and HIV encephalopathy, in the central nervous system (CNS). Therefore, the inhibition to the growth of HIV in the CNS becomes one of the central issues in AIDS therapy [1]. ARV delivery systems that have been developed by achieving designed drug release kinetics specifically targeting drugs to macrophages, brain, and gastric mucosa and for addressing formulation difficulties such as solubility, stability and drug entrapment [2]. Nanoparticles (NPs) were capable of transport across the blood–brain barrier and could be qualified carriers for delivering antiretroviral reagents into the CNS. The NPs delivery system could reduce the side effects of active pharmaceutical ingredient with high biocompatibility to normal tissue. In addition to the merits of NPs were easy to scale up for fabrication, possessed stable properties, and could imbed and preserve light-avoiding pharmaceuticals in their lipid core. The drug-loaded NPs could significantly alter the body distribution, targeting, absorption, metabolism, and elimination of a drug [3].

Chitosan exhibits many advantages in developing nanoparticles, including biocompatibility, biodegradability, and low-immunogenicity. The high positive charge density also confers its mucoadhesive properties and makes it an ideal candidate for the delivery of drugs to mucosal tissues. Chitosan also has a very low toxicity. Its LD_{50} in laboratory mice is 16 g/kg body weight, which is close to sugar and salt [4].

Stavudine (D4T), a pyrimidine nucleoside analog, was approved by the U.S. Food and Drug Administration (FDA) in 1994 and could replace the clinical use of Zidovudine. D4T is activated by thymidine in T-lymph cells to yield stavudine-5'-triphosphate and to compete the association with pyrimidine-5'-triphosphate. Thus, the duplication of HIV is inhibited. However, the residence time of D4T
in plasma and the elimination half life of D4T after tissue binding are only 0.5-0.75 hr and 0.9-1.2 hr, respectively [5]. The present work is aimed to develop and optimize nanoparticle of stavudine drug to ensure satisfactory drug release with the help of polymers, to prevent drug toxicity due to accumulation, and prolong duration of action.

MATERIALS AND METHODS

Materials

Stavudine was obtained as a gift sample from Zydus cadila Mumbai. Chitosan (viscosity 35 cps, deacetylation degree 91.5%) was purchased from Otto chemicals, Mumbai. The chemicals used for synthesis of nanoparticles include Sodium alginate, acetic acid and calcium chloride from SD fine chemicals, Mumbai.

Methods

Drug polymer compatibility studies

Compatibility of drug with excipients was determined by carrying out FTIR studies. Infrared spectrum of Stavudine, Chitosan, Sodium alginate and physical mixture of drug and polymer was determined on Fourier Transform Infrared spectrophotometer (8400 S Shimadzu) using KBr dispersion method.

Formulation of Stavudine Nanoparticles

Preparation of Chitosan Gel

100 mg of chitosan was dispersed in 0.2 mg/ml of glacial acetic acid solution and stirred for 2 hrs continuously to obtain 0.1% chitosan gel solution. Then it was stabilized overnight to obtain clear chitosan gel. Like this, different concentration of chitosan gel was prepared [6].

Ionotropic Pre-Gelation Method

Stavudine nanoparticles (SNP) were prepared in a two step procedure based on ionotropic pre-gelation of polyanion with calcium chloride followed by polycationic crosslinking through a protocol described in Table 1. Thus 7.5 ml of 18mM calcium chloride solution was dropped for 60 min under 800 rpm into a beaker containing sodium alginate solution (0.063 %w/v). An amount of stavudine was mixed with the alginate solution before calcium chloride addition to provide an alginate pre-gel. Then, 25 ml of different concentrations of chitosan solution was added drop wise into the pre-gel over 120 min [7].

Characterization and evaluation of Nanoparticles

Particle Size Analysis

The particle size distribution of the drug-entrapping nanoparticles was analyzed by a zetasizer 3000 HSA with a photo correlation spectroscope (Malvern, Worcestershire, UK) at 25°C. This analysis assumed sphere-like SNPs without multiple scattering. The SNPs were immersed in 0.1 M tris buffer with a particle concentration of 2 mg/ mL. The suspension was gradually injected into a quartz tube to avoid the formation and interference of bubbles. The duration of the detection was 120 sec [8].

Surface Morphology

Shape and surface morphology of nanoparticles was studied using high-resolution scanning electron microscopy (SEM). The samples on conductive carbon paint were placed in a specimen holder, vacuum-dried, and sputter-coated with platinum using accelerating voltage of 2 kV for 90 sec.

Drug Entrapment efficiency

The encapsulation efficiency of nanoparticles was determined by centrifuging the nanoparticles using ultracentrifuge at 10000 rpm for 30 min. The amount of free Stavudine in the supernatant was measured by UV spectrophotometer at 265nm. The Stavudine entrapped in the nanoparticles was calculated as

\[
\text{Entrapment efficiency (\%) = \frac{(\text{Tp} - \text{Tf})}{\text{Tp}}} \times 100
\]

Where, Tp is the total Stavudine used to prepare the nanoparticles and Tf is the total free Stavudine in the supernatant [9].

In vitro Release studies

Drug release from nanoparticles in-vitro was carried out by dialysis method ( Dialysis membrane-60 HI MEDIA, Mumbai). The donor chamber filled with 5ml of nanoparticles suspension, whereas reservoir chamber containing the phosphate buffer pH 7.2. This total setup was placed on a rotary shaker rotating at 50 rpm at 37°C ± 1°C. In pre determined time intervals the content of receiver chamber was withdrawn and replaced with equal volume of fresh phosphate buffer, the amount of stavudine that diffused into the receiver chamber was quantified by UV- spectrophotometer at 265 nm [10].
Kinetic modeling of stavudine nanoparticles

Different mathematical functions have been used to model the observed data. Both the linear and non-linear models are being used in practice for dissolution modelling. Linear models include Zero order, Higuchi, whereas the non-linear models include First order, KorsMeyer-Peppas [11, 12].

RESULTS AND DISCUSSION

Drug polymer compatibility studies

FTIR studies were recorded for pure stavudine (figure 1), chitosan (figure 2), sodium alginate (figure 3), and stavudine with polymer nanoparticles (figure 4). In FT-IR studies, the characteristic stavudine Asymmetric stretching of methyl group around 2874 cm⁻¹ and symmetric stretching of aldehyde group at 1689 cm⁻¹ were observed in the stavudine loaded formulation and additionally, asymmetric stretching of C-O-C of stavudine at 1278 cm⁻¹ was also observed unchanged in formulation SNP5, suggesting no drug-polymer interactions in loaded nanoparticles.

Particle Size Analysis

The particle size analysis was carried out using Malvern Zetasizer ZS. The particle size was found to be in the nanometer range of 260nm-632nm (figure 5). The characteristics of the SNP prepared with different concentrations of chitosan were studied. The results indicated that the particle size increased with increasing the concentration of CS. The formation of nanoparticles was possible for some specific concentrations of CS. This fact was also verified in our study that in order to avoid the formation of any micro-particles, the concentration of chitosan needed to be below 1.0 mg/mL. In these concentration ranges, it seemed that the concentration of chitosan had little effect on the monodispersity of the nanoparticles. It is known that under acidic conditions, there is electrostatic repulsion between CS molecules due to the protonated amino groups of CS; meanwhile, there also exist interchain hydrogen bonding interactions between CS molecules. In low concentration of chitosan the intermolecular hydrogen bonding attraction and the intermolecular electrostatic repulsion are in equilibrium. Therefore chitosan concentration increases (0.05 - 0.7 mg/ml), chitosan molecules approach each other with a limit, leading to a limited increase in intermolecular cross-linking and thus larger but still nanoscale particles are formed. Above this concentration, microparticles are easily formed probably due to the stronger hydrogen bonding interactions leading to plenty of chitosan molecules involved in the cross-linking of a single particle. The formation of micro-particles usually leads to a flocculent precipitate due to the electrostatic repulsion between particles are not sufficient to maintain the stability of these large particles.

Surface Morphology

The typical Scanning electron microscope image of optimized formulation SNP5 showed in (figure 6). As revealed in this figure, SNP exhibited discrete, uniform smooth surface and spherical feature. This spherical configuration suggested that the application of the Stokes–Einstein equation (linking the hydrodynamic and thermodynamic views on the diffusion of microspheres) could be reasonable to estimate the particle size of SNP. In addition, this drug carrier showed bright periphery, representing the coating of surfactants in the external layer. The polymer stabilized the nano-sized structure and prevented SNPs from coagulation. Their average diameter of SNP5 was found to be 312 nm.

Entrapment Efficiency

The entrapment efficiency (figure 7) of SNP gradually increased from SNP1 (61 ± 0.3%) to SNP5 (83 ± 0.2%), and decreased with formulations SNP6, SNP7 and SNP8. This results suggested that as the polymer concentration increases the entrapment efficiency of drug and after certain concentration it was decreased due to saturation capacity of the polymer.

Invitro Dissolution study of Nanoparticle

From the above drug release studies it was observed that as the concentration increases there was decrease in the drug release and an initial burst of release may be attributed to the drug that is adsorbed on to the surface of nanoparticles. The stavudine containing nanoparticle was constantly agitated during the dissolution test, collisions among SNP could first disintegrate the structure of the polymer layer. The subsequent fluidic shear devastated the particulate surfaces and caused the release of a drug. As indicated in (Table 2) the cumulative percentage of stavudine released in the initial 2-4 hr was minor. This suggested that the current formulation could prevent the drugs from the initial burst release. As revealed in (figure 8), the rate of a drug released from SNP was pretty smooth. This
suggested that the spatial distribution of the drugs confined could be quite homogeneous.

**Release Kinetics**

In case of zero order \((Q = Q_0 - k_0 t)\) the graph was plotted in cumulative percent of drug release Vs time, and in First order release kinetics \((\ln Q = Q_0 - K_1 t)\). The graph was plotted in log cumulative percent of drug retained Vs time. For Higuchi model kinetics \((Q = K_2 t^{1/2})\) the graph was plotted in cumulative percent drug released Vs square root of time. Korsmeyer-Peppas model \((Q/Q_0 = K t^n)\) the graph was plotted in log cumulative percent of drug released Vs log time.

**Zero order kinetic model**

Here the graph is plotted between cumulative percent drug remaining Vs time. The regression coefficient value of zero order kinetic plot was found to be 0.989 and the slope was found to be 3.0645 (figure 9).

**First order kinetics plot**

Here the graph is plotted between log cumulative percent drug remaining Vs time. Regression coefficient is calculated and interpreted. The regression coefficient value of zero order kinetic plot was found to be 0.9717 and the slope was found to be 0.0255 (figure 10).

**Higuchi model**

In this model, graph is plotted between cumulative percent drug released Vs square root of time. Regression coefficient and slope values are calculated and interpreted. The regression coefficient value of zero order kinetic plot was found to be 0.8336 and the slope was found to be 0.3182 (figure 11).

**Koresmeyer pepps model**

In this model, graph is plotted between log cumulative percent drug released Vs log time. Regression coefficient and slope values are calculated and interpreted. The regression coefficient value of zero order kinetic plot was found to be 0.7565 and the slope was found to be 0.9334 (figure 12).

**Table 1: Formulations of Stavudine Nanoparticles**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>SNP1</th>
<th>SNP2</th>
<th>SNP3</th>
<th>SNP4</th>
<th>SNP5</th>
<th>SNP6</th>
<th>SNP7</th>
<th>SNP8</th>
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<td>Stavudine (mg)</td>
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<td>Chitosan (%w/v)</td>
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<td>Sodium alginate (%w/v)</td>
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<td>Calcium chloride (ml)</td>
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**Figure 1: IR Spectra of Stavudine**
Figure 2: IR Spectra of Chitosan

Figure 3: IR Spectra of sodium alginate

Figure 4: IR spectra of formulation SNP5
Figure 5: Particle size distribution of SNP

![Particle size distribution](image)

Figure 6: Typical SEM image of Optimized Formulation SNP5

![Typical SEM image](image)

Figure 7: Entrapment Efficiency of Nanoparticle

![Entrapment Efficiency](image)
Table 2: Dissolution Profile of Nanoparticle

<table>
<thead>
<tr>
<th>TIME(hr)</th>
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Figure 8: *In vitro* Dissolution Profile of Nanoparticle

![In vitro Dissolution profile of SNP](image1)

Figure 9: Zero Order Kinetic Plot of SNP5

![Data For Zero Order Kinetic Plot of PNP5](image2)
CONCLUSION

Stavudine is one of the most widely used anti-HIV drug. Designing a controlled release formulation for the drug Stavudine may prolong therapeutic concentration of drug in the blood and decrease frequency of dosing and also improve the efficacy of drug and patient compliance. Hence, an attempt was made to formulate a controlled release nanoparticle for the anti-HIV drug. The FTIR spectra revealed that there was no interaction between polymers and drug, hence they are compatible. The particle size analysis revealed that the optimized formulations gave particles in the range of 260-632 nm respectively. SEM analysis of the nanoparticles revealed that the formulations were smooth and spherical with ideal surface morphology. The percentage entrapment efficiency was higher in SNP5 formulation. As the concentration of polymer and crosslinking agent increased, the drug release decreased proportionally. For the mechanism of drug release, optimized formulation showed zero order release, while Korsmeyer peppas model with non-fickian release. From the above study the formula used for SNP5 formulation was concluded as an optimized formulation due to its better entrapment efficiency and superior cumulative percentage of drug release when compared with other formulations. In the present work, it can be concluded that the Nanoparticulate formulation can be an innovative and promising approach for the delivery of Stavudine for the treatment of HIV. Thus, our study confirmed that the Nanoparticulate formulation can be used as a possible alternative to traditional oral formulations of Stavudine to improve its therapeutic efficacy, reduce toxicity by reducing the possible accumulation of drug and also provide better prolonged drug release.

REFERENCE: