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Original Article

Preparation and evaluation of stavudine loaded chitosan nanoparticles

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ABSTRACT

Aim: The aim of the present study is to prepare and evaluate nanoparticles containing stavudine using chitosan as the polymer.

Methods: The stavudine loaded nanoparticles were prepared by ionic gelation of chitosan with tripolyphosphate anions. Nanoparticles of different core: coat ratio were formulated and evaluated for process yield, loading efficiency, particle size, zeta potential, *in vitro* drug release, kinetic studies and stability studies.

Results: The prepared nanoparticles were white, free flowing and spherical in shape. The infrared spectra and differential scanning calorimetry thermographs showed stable character of stavudine in the drug-loaded nanoparticles and revealed the absence of drug–polymer interactions. The chitosan nanoparticles have a particle diameter ranging approximately 212–342 nm and a zeta potential −24.8 to −33.54 mV. The formulation with the initial stavudine concentration of 0.5 mg/ml provided the highest loading capacity. The *in vitro* release behavior from all the drug loaded batches were found to follow first order and provided sustained release over a period of 24 h. No appreciable difference was observed in the extent of degradation of product during 90 days in which nanoparticles were stored at various temperatures.

Conclusion: The best-fit release kinetics was achieved with First order followed by Higuchi plot. The release of stavudine was influenced by the drug to polymer ratio and particle size & was found to be diffusion controlled. According to the data obtained, this chitosan-based nanotechnology opens new and interesting perspectives as drug carriers for treating the AIDS.

1. Introduction

Human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS) commonly referred to as HIV & AIDS have emerged as being amongst the most serious and challenging public health problems in the world. There are two species of HIV, namely, HIV 1 and HIV 2 with their respective subspecies. HIV 1 is the global common infection whereas the latter is restricted to mainly West Africa. HIV infection in the human body results mainly from the

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integration of the viral genome into the host cell for the purpose of cell replication.[1]

The current clinical therapy, known as highly active antiretroviral treatment (HAART), is considered as one of the most significant advances in the field of HIV therapy. HAART is a lifelong necessity and any non-compliance leads to a rapid increase in the viral load. The reason for this relapse is related to the poor targeting ability of the antiretroviral agent to the latent sites of infection. The two main objectives of the antiretroviral therapy are virological control and restoration of immunity. Once these two objectives are achieved, it is possible to delay the progression of the disease, minimize opportunistic infections, malignancies and prolong the survival of the patient.

Currently the five different classes of antiretroviral drugs available are Nucleoside Reverse Transcriptase Inhibitors (NRTI's), Nucleotide Reverse Transcriptase Inhibitors (NtRTI), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI), Protease Inhibitors (PIs), and more recently, fusion and integrase inhibitors. NRTI's are among the first agents to be used for the treatment of HIV/AIDS. These agents inhibit the reverse transcriptase enzyme responsible for the conversion of viral RNA to DNA within the host cell. These agents require intracellular metabolism to their triphosphate form, before activation. The approved NRTI's include zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir and most recently, emtricitabine.[2] Furthermore several antiretroviral drugs suffer from low bioavailability due to extensive first-pass effects and gastrointestinal degradation. In addition, for most drugs the half-life is short, thus necessitating frequent administration of doses thereby decreasing patient compliance and increasing side effects due to peak-trough fluctuations.

Stavudine is the FDA-approved drug for clinical use for the treatment of HIV infection, AIDS and AIDS-related conditions either alone or in combination with other antiviral agents. Stavudine, a nucleoside analog of thymidine, is phosphorylated using cellular kinases to the active metabolite stavudine triphosphate. Stavudine triphosphate inhibits the activity of HIV 1 reverse transcriptase by competing with the natural substrate thymidine triphosphate and by causing DNA chain termination following its incorporation into viral DNA. Stavudine triphosphate inhibits cellular DNA polymerases β and γ and markedly reduces the synthesis of mitochondrial DNA. Stavudine is typically administered orally as a capsule and an oral solution. The drug has a very short half-life (1.00 h) thus necessitating frequent administration to maintain constant therapeutic drug levels. However patients receiving stavudine develop neuropathy and lactic acidosis. The side effects of stavudine are dose-dependent and a reduction of the total administered dose reduces the severity of the toxicity.[3]

One of the suitable methods to overcome these problems could be association with biodegradable polymeric carriers such as nanoparticles. The nanometric size of these carrier systems allows efficient crossing of biological barriers, amelioration in tissue tolerance, improved cellular uptake and transport, thus enabling efficient delivery of the therapeutic agents to the target sites like liver, brain and solid tumor.[4–6] Nanoparticles may become one of the successful carriers by overcoming problems caused by infections that are refractory to conventional treatment. Chitosan possesses some ideal properties of a polymeric carrier for nanoparticles such as biocompatibility, biodegradability, non-toxicity, and low cost. It possesses a positive charge and exhibits an absorption enhancing effect. This characteristic can be employed to prepare cross-linked chitosan nanoparticles.[7] Hence, these nanosystems are being used to target drugs to a specific site only in the body, to improve oral bioavailability, to sustain drug effect in the target tissue, to solubilize drugs for intravascular delivery, and to improve the stability of drugs against enzymatic degradation.

The objective of the work was to formulate chitosan nanoparticles containing stavudine by ionic gelation method, evaluate its physicochemical characteristics such as particle size, shape, zeta potential, drug loading capacity and *in vitro* release characteristics.

2. Materials and methods

Stavudine used was a gift sample from Cipla Pvt. Ltd., Mumbai and chitosan from Central Institute of Fisheries Technology, Cochin, India. Glacial acetic acid and sodium tripolyphosphate (TPP) were obtained from Merck Specialties Private Limited, Mumbai, India. All other chemicals used were of analytical grade.

2.1. *Preparation of nanoparticles*[8–15]

Chitosan nanoparticles were prepared by ionic cross linking of chitosan solution with TPP anions. Chitosan was dissolved in aqueous solution of acetic acid (0.25 vv^{-1}) at various concentrations such as 1.0, 2.0, 3.0, 4.0, 5.0 $mgml^{-1}$. Under magnetic stirring at room temperature, 5 ml of 0.84% wv^{-1} TPP aqueous solution was added dropwise using syringe needle into 10 ml chitosan solution containing 10 mg of stavudine. pH was adjusted to 6.0 by adding 0.1 N NaOH. The stirring was continued for about 30 min. The resultant nanoparticles suspensions were centrifuged at 12,000× *g* for 30 min using C24 centrifuge. The formation of the particles was a result of the interaction between the negative groups of the TPP and the positively charged amino groups of chitosan (ionic gelation) (Table 1).

Table 1 – Formulation and physicochemical characterization of stavudine nanoparticles.

S. no	Batch code	Drug: carrier ratio	% Drug entrapment efficiency ± SD	Mean particle size ± SD (nm)
1	FS-1	1:1	62.4 ± 0.63	326 ± 5.04
2	FS-2	1:2	63.38 ± 0.06	342 ± 4.2
3	FS-3	1:3	67.80 ± 0.28	319 ± 8.9
4	FS-4	1:4	72.50 ± 0.82	294 ± 10.5
5	FS-5	1:5	85.8 ± 0.16	212 ± 10.7

FS-1, FS-2, FS-3, FS-4 and FS-5 represent formulations 1–5 respectively, etc.