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Controlled delivery of anti retroviral drug loaded chitosan cross-linked microspheres

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Abstract

The main objective of the study to develop controlled drug delivery system to increase the efficacy of anti retroviral drug, lamivudine against HIV infections. Encapsulation of lamivudine in chitosan microspheres was carried out by cross- linking process with 20% glutaraldehyde. The prepared lamivudine loaded chitosan cross-linked microspheres were evaluated for drug loading, encapsulation efficiency, particle size distribution, surface morphology (SEM), and Fourier Transform Infrared Spectroscopy (FTIR) and in-vitro release studies. The chitosan microspheres shows very smooth surface and its exhibits regular spherical geometry due to higher cross-linking density. FTIR spectrum for cross-linked chitosan microspheres, an additional peak at 1686 cm-1 were observed, which is due to stretching vibrations of C N bond. This peak indicates formation of chain due to reaction between carbonyl group of glutaraldehyde and amine group of chitosan polymer and the percentage of encapsulation efficiency was found to be 54.83-63.70 %w/w. The particle size was ranged from 5-40 µm in size and 68% of the particles lying between 27-37 µm sizes and *in-vitro* release profile showed that cross-linking density of chitosan microspheres were effectively controlled the release of lamivudine. Marked retardation of lamivudine release may provide a useful controlled release of anti retroviral drug therapy.

Key words: Lamivudine, Chitosan microspheres, SEM, FTIR

Introduction

Human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS), commonly referred to as HIV/AIDS, constitute one of the most serious infectious disease challenges to public health globally. AIDS is considered to be an epidemic according to estimates from the UNAIDS/WHO AIDS Epidemic Update, July 2008[1]. Globally, there were an estimated 33 million people living with HIV in 2007. Moreover, in Sub-Saharan Africa

remains most heavily affected by HIV, accounting for 67% of all people living with HIV and for 72% of AIDS deaths in 2007 and globally the percentage of women among people living with HIV has remained stable at 50% for several years. HIV-1 is the globally common infection while HIV-2 is more prevalent in West Africa, and takes a longer time to develop into immunodeficiency from infection than HIV-1[2,3]. HIV infection in the human body results mainly from integration of the viral genome into the host cell for the purpose of cell replication, and AIDS is the advanced stage of the disease caused by HIV infection. The end stage of the disease may be characterised by a spectrum of diseases [4] including opportunistic infections (such as Pnuemocystis carinii and Mycobacteruim tuberculosis), dementia and cancer [3, 4]. Interestingly, HIV has been referred to as a ‘‘master regulator” of cellular gene expression [5] as a means to augment expression of its own genome. An understanding of these processes is critical to developing novel therapeutic strategies for the suppression or elimination of the virus. The development of drugs for HIV infection has undergone substantial progress, currently various drugs are used as anti retroviral therapy and has contributed significantly to improved patient/disease management. But its current use is associated with several disadvantages and inconveniences to the HIV/AIDS patient. Many ARV drugs undergo extensive first pass metabolism and gastrointestinal degradation leading to low and erratic bioavailability. The half-life for several ARV drugs is short, which then requires frequent administration of doses leading to decreased patient compliance [6]. Moreover, a major limitation is that HIV is localised in certain inaccessible compartments of the body such as the CNS, the lymphatic system and within the macrophages. These sites cannot be accessed by the majority of drugs in the therapeutic concentrations required; and the drugs also cannot be maintained for the necessary duration at the site of HIV localization that leads to the development of multidrug-resistance against the ARV drugs [7]. Strategies currently being investigated to overcome these limitations include the identification of new and chemical modification of existing chemical entities, as well as the design and development of novel drug delivery systems that can improve the efficacy of both existing and new ARV drugs. There are growing interests in developing delivery systems for drug targeting to specific sites because of the lack of other effective and practical pharmacological approaches [8]. Chitosan is a cationic biopolymer obtained from N-deacetylation of chitin [9]. Chitin is the principal component of protective cuticles of crustaceans such as crabs, shrimps, prawns, lobsters and cell walls of some fungi such as *aspergillus* and *mucor*. Chitin is a straight homopolymer composed of - (1, 4)-linked *N*-acetyl-glucosamine units while chitosan comprises of copolymers of glucosamine and *N*-acetyl-glucosamine [10-12]. Chitosan is a weak base and is insoluble in water and organic solvents, however, it is soluble in dilute aqueous acidic solution (pH < 6.5), which can convert the glucosamine units into a soluble form R–NH3+ [13]. Lamivudine is a synthetic nucleoside analog that is being increasingly used as the core of an antiretroviral regimen for the treatment of HIV infection [14]. Lamivudine is rapidly absorbed after oral administration with an absolute bioavailability of 86% ± 16%, peak serum concentration of lamivudine (C_{max}) of 1.5 ± 0.5 mcg/ml and mean elimination half-life ($t_{1/2}$) of 5 to 7 hours, thus necessitating frequent administration to maintain constant therapeutic levels [15]. However, patient receiving lamivudine frequently develop various side effects, which are dose-dependent and a reduction of the total administered dose reduces the severity of the toxicity. The development of controlled release of lamivudine would be beneficial in comparison with the current dose regimens. In this study, anti retroviral drug lamivudine loaded microspheres were developed by cross-linking technique with glutaraldehyde by using natural

biocompatible, non-toxic polymer chitosan for controlled release. Microspheres were evaluated for its loading, encapsulation, particle size distribution, surface morphology, FTIR and *in-vitro* release studies.

Materials and methods

Materials

Lamivudine was obtained from Medireich steri lab and Chitosan (Central Institute of Fisheries and technology, Cochin) Sodium Chloride (Labchem)**,** Acetic Acid (Hindustan Laboratory chemical Ltd.,) Liquid Paraffin (S.D Fine Chem Limited)**,** Petroleum ether (Ranbaxy)**,** Sodium metabisulphite (Central Drug House (P) Ltd., Acetone (Rankem)**,** Glutaraldehyde (LOBA Chem Pvt. Ltd)**,** Toluene (Rainbow) (Laboratory reagent)**,** Sodium dihydrogen phosphate (Ranbaxy), Disodium hydrogen phosphate (Indian Research Products). All other chemicals were analytical grade.

Methods

Preparation of chitosan microspheres of Lamivudine

A 4% solution of chitosan in 5% acetic acid containing 2% sodium chloride was prepared and 12 g of prepared viscous solution was weighed, mixed with lamivudine (20% by weight of Chitosan) and dispersed in a mixture of 50 ml of petroleum ether and 70 ml of liquid paraffin containing 2 to 3 drops of sorbitan sesquoleate in a 250 ml beaker at room temperature. The dispersion was stirred using a stainless steel stirrer at 2000 rpm for 5 minutes and glutaraldehyde saturated with toluene was introduced in to the beaker and the stirring was continued for 10 more minutes. The mixture was transferred to a magnetic stirrer. Highly cross-linked microspheres were prepared by adding aqueous (20%) glutaraldehyde at the end of 30 minutes. The cross-linking reaction was allowed to proceed for further 3 hours. The hardened microspheres were then separated by centrifugation, washed 4 times with petroleum ether, once with acetone, once with 5% solution of sodium metabisulphite, three times with water, centrifuged, air dried at room temperature.

Characterization of microspheres

Determination of Lamivudine loading efficiency

The Lamivudine loading in microspheres can be estimated by the equation

$$L = \frac{Q_m}{W_m} X \quad 100$$

Where L = percentage loading of microspheres, W_m = weight of microspheres in grams, Q_M = quantity of Lamivudine present in W_m gram of microspheres.

Determination of encapsulation efficacy of microspheres

The amount of Lamivudine encapsulated in the microspheres was determined by

$$E = \frac{Q_P}{Q_t} X\ 100$$

Where E = the percentage encapsulated microspheres, Q_p = Quantity of drug encapsulated in microspheres (gm), Q_t = Quantity of drug added for encapsulation (gm).

Particle size determination

The size of the microsphere was determined by using calibrated eye piece micrometer. The particle size distribution was plotted and the average size was determined.

Scanning electron microscopy (SEM)

The prepared microspheres were characterized using scanning electron microscopy. The samples for the SEM analysis were prepared by sprinkling the microspheres onto one side of double adhesive stub and the stub were then coated with gold using JEOL JFC 1100E sputter coater. The SEM micrographs of the microspheres were taken using JEOL-JEL -5300 scanning microscope.

Fourier Transform Infrared Spectroscopy (FTIR)

The infrared spectra of lamivudine loaded chitosan microspheres were taken in KBr pellet using AB Bomen MB series (Canada) FTIR instrument.

In- vitro release studies

In vitro release study of drug from microsphere was conducted by using phosphate buffer saline pH 7.4. 250 mg of microspheres were weighed accurately and added to 50 ml of phosphate buffer saline (pH 7.4) in a standard flask. The flask is kept in incubator and maintained at 37^{o}C. The writers are shaken regularly and a sample of 1 ml was withdrawn from the standard flask at regular time intervals and replaced with fresh phosphate buffer saline. Each sample was measured spectrophotometrically at 267 nm to determine the amount of released drug.

Results and Discussion

The chitosan microspheres were prepared by cross-linking technique with 20% glutaraldehyde for controlled delivery of anti retroviral drug lamivudine. The chitosan molecule chains have abundant free amino groups, which can interact with glutaraldehyde to form chitosan microspheres. During the cross-linking and hardening process, water was exuded from the microspheres, and the cross-linking intensity of chitosan microspheres increased with the increase of the concentration of glutaraldehyde.

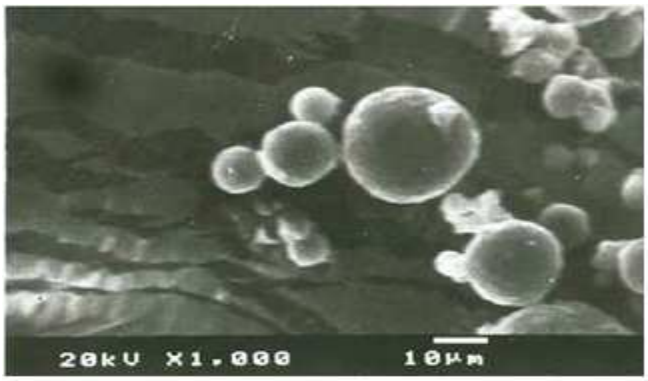


Fig: 1 SEM photograph of Lamivudine loaded chitosan microspheres

The surface morphology of the prepared chitosan microspheres were investigated by Scanning Electron Microscopy **(Fig: 1)**. Lamivudine loaded natural chitosan microspheres prepared by cross-linking with glutaraldehyde appeared to be spherical, smooth, homogeneously distributed without evidence of collapsed particle, suitable for controlled release of anti retroviral drugs. The chitosan microspheres shows very smooth surface and its exhibits regular spherical geometry due to higher cross-linking density. The SEM photograph does not show any aggregation of microspheres and there was no grafting of polymer in chitosan microspheres. In order to precede the in vitro studies of the glutaraldehyde cross-linked chitosan microspheres, drug loading and encapsulation efficiency were determined. The drug loading in chitosan microspheres was found to be 17% w/w and the percentage of encapsulation efficiency was found to be 54.83-63.70 %w/w shown in Table **I.**

Table: I Percentage of loading, encapsulation of chitosan microspheres loaded with Lamivudine

Glutaraldehyde satu-Rated toluene(ml): Aqueous glutaraldehyde Ratio (ml)	Drug : Polymer	Amount of Drug(gm)	Amount of Polymer	Yield in grams	Percentage of loading		Encapsulation %
					Theoretical	Practical	
10:1 5:1	1:5 1:5	0.2 0.2	1.0 1.0	1.05 0.97	16.67 16.67	9.12 10.62	54.83 63.70

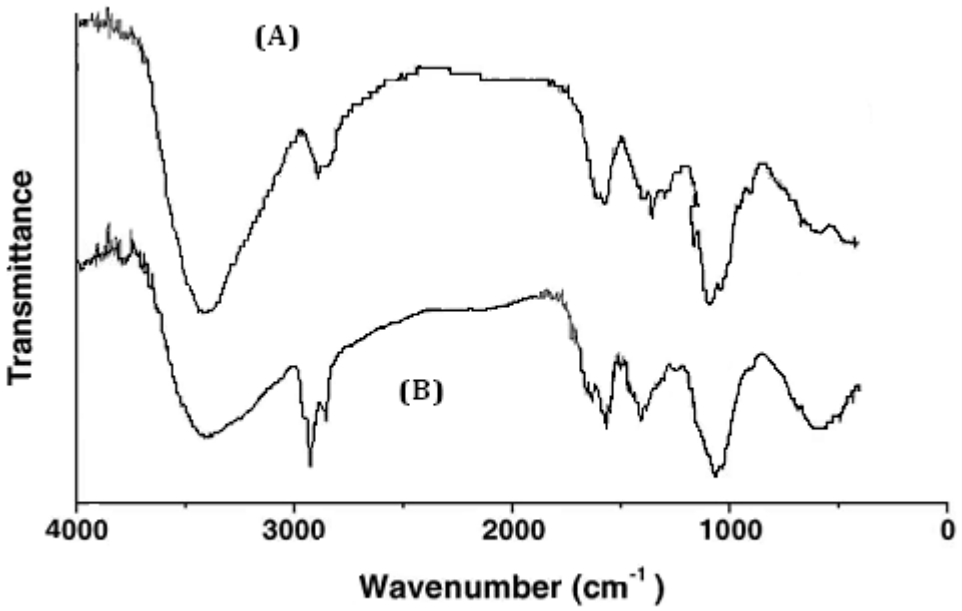


Fig: 2 FTIR Spectra of (A) Chitosan polymer, (B) Lamivudine microspher

Fig. 2 exhibits the FTIR spectrum of chitosan polymer (A), Lamivudine microsphere (B) respectively. As seen in **Fig.2 (A)**, the peak at 3446 cm-1 corresponds to stretching vibrations of hydroxyl group. The backbone of the polymer is manifested through strong peak at 2925cm-1 and 2885 cm-1 due to C-H stretching vibrations. The stretching vibrations of C O are found at 1088 cm-1 and 1022 cm-1 [16, 17].

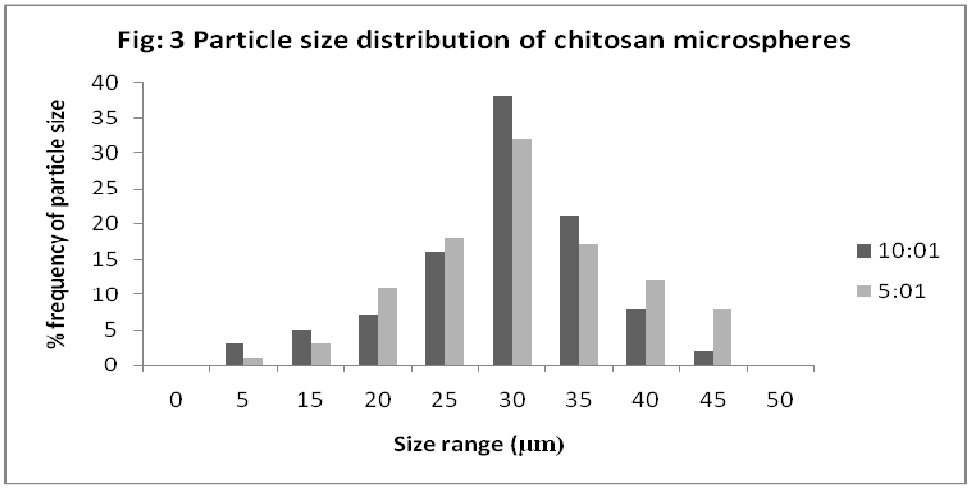
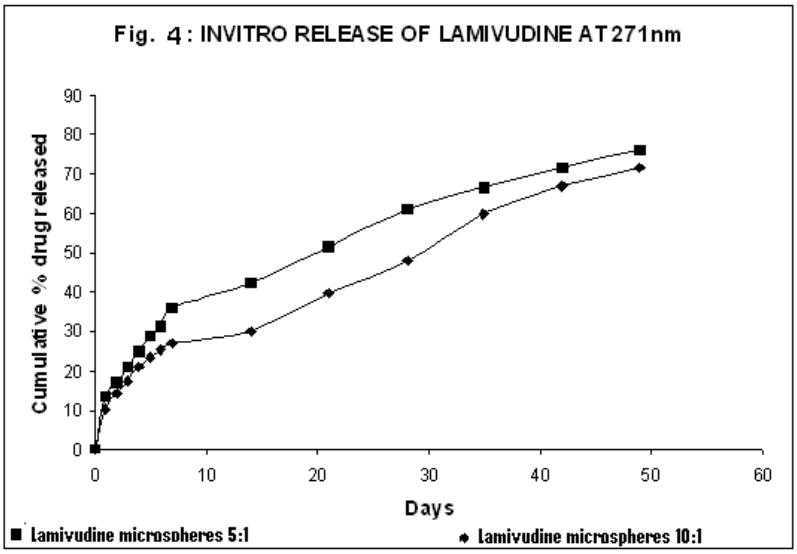


Fig: 4 show the *in-vitro* release profile obtained by representing percentage of Lamivudine release with respect to the concentration of cross-linking density



For cross-linked chitosan microspheres, an additional peak at 1686 cm-1 can be observed in **Fig. 2(B)**, which is due to stretching vibrations of C N bond. This peak indicates formation of chain due to reaction between carbonyl group of glutaraldehyde and amine group of chitosan polymer. The particle size distribution of Lamivudine loaded chitosan microspheres were showed in the Fig: 3. the particle size was ranged from 5-40 µm in size and 68% of the particles lying between 27-37µm. The release from microspheres having high cross-linking density was slower compared to the release from microspheres containing low cross-linking density. Within 14 days 68.72% of the incorporated lamivudine was released from chitosan microspheres of low cross-linking density whereas from microspheres having high cross-linking density, about 22.32% was released. Thus cross-linking effectively controls the drug diffusion from the chitosan microspheres.

An initial burst release was shown in all the batches, which was due to the drug particles adsorbed on the wall of the microspheres. The rest of the drug in the interior of the microspheres was trapped by the chitosan matrix and could not be released in the short term until the microspheres were degraded by enzymes in the biological environment. The chitosan microspheres show marked retardation in the release profiles due to effective cross-linking density. Thus the glutaraldehyde cross-linked chitosan microspheres were potentially useful for controlled release of anti retroviral therapy.

Conclusion

This paper describes the formulation and evaluation of anti retroviral drug lamivudine loaded chitosan microspheres by using cross-linking technique. Our result clearly indicates that prepared chitosan microspheres were controllable and their surface morphology was smooth and spherical. These chitosan microspheres showed marked retardation of the lamivudine release profiles, so they hold promise for applications in anti retroviral drug delivery systems.

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