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# Nanoreticulations of etherified locust bean polysaccharide for controlled oral delivery of lamivudine

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ABSTRACT

Herein, an aqueous solution of etherified locust bean polysaccharide (ELBP) containing lamivudine was reticulated in presence of trivalent aluminium ($Al^{3+}$) ions to nanoscale level (43.82–197.70 nm) by surfactant assisted homogenization-reticulation technique. The variation in aluminium chloride ($AlCl_3$) strength (1.5–3.5% (w/v)) and drug:ELBP weight ratio (0.11–0.43) affected the properties of the nanoreticulations. Regardless of the variables, a maximum of ~44% drug entrapment efficiency was noted. In simulated intestinal fluid (phosphate buffer solution, pH 7.4), the drug release rate was inversely proportional to the strength of $AlCl_3$; but followed a proportional relationship with the drug:ELBP ratio. The mechanism of drug release shifted from Fickian diffusion to anomalous transport as the salt strength was increased above 2.5% (w/v). At intermediate drug:ELBP ratio, the drug release rate was regulated by polymer chain relaxation as opposed to simple diffusion mechanism. Fourier transform infrared spectroscopy did not show any evidence of chemical interaction between the drug and ELBP. Thermal analysis and X-ray diffraction studies suggested amorphous dispersion of drug in the nanoreticulations. Thus, the nanoreticulations were expected to absorb *via* intestine and phagocytosed by the virus-infected hepatic macrophages and hence could be useful for controlled delivery of lamivudine avoiding dose-dependent toxicity of the drug.



## 1. Introduction

In the last few decades, there is an astonishing progress in the field of nanometric delivery of therapeutic agents. Targeting therapeutics to the site of action will have numerous benefits such as reduction of unwanted side effects, reduced toxicity due to low dose requirement, enhanced patient compliance, and greater therapeutic effectiveness [1]. Particles in the nano-size range (~200 nm) are capable of being absorbed intact from the intestine *via* transcellular or paracellular pathways, thereby substantially improving the bioavailability of encapsulated drugs [2] and are more successfully phagocytosed by the HIV-infected macrophages [3]. In addition, oral route is the most popular ones because of its unique advantages [4]. This is the motivation for major research work in recent years focusing on oral nanoparticles [5].

Chemically, lamivudine is 2′,3′-dideoxy-3′-thiacytidine, and is a water soluble potent nucleoside analog reverse transcriptase inhibitor. For the treatment of adults with hepatitis B and co-infected with human immunodeficiency virus (HIV), a dose of 150 mg twice daily has been recommended for conventional tablets. Patients receiving lamivudine frequently develop various side effects like thrombocytopenia, paresthesia, anorexia, abdominal cramps, depressive disorders and skin rashes, which are dose-dependent, and a reduction of total administered dose could reduce the severity of toxicity [6]. It is characterized by its moderate elimination half-life of about 5–7 h [7,8] and thus, controlled release preparations could help to achieve maximum therapeutic benefit of the drug. Nanocarriers have been fabricated by emulsification technique using synthetic polymers such as polyhexylcyanoacrylate [9]; poly (lactic acid) [10]; poly-(lactic-co-glycolic acid) [11]; polybutylcyanoacrylate [12] with the aim to have better cellular targeting, overcoming the pharmacokinetic problems, and enhancing the drug activities for the treatment of viral infections. The particles demonstrated low drug encapsulation efficiency and rapid release of water-soluble drugs [13]. Natural polysaccharides are nontoxic, biodegradable, and can be tailored to obtain better materials for drug delivery applications [14]. The design of nanoparticles using biopolymers is limited in the literature. The nanoparticles based on hydrophilic polymers are negatively charged, show a strong bio-adhesion, and are absorbed by both M-cells and enterocytes [15].

The principle involving $Ca^{2+}$ ion-induced controlled gelation of sodium alginate has been used mostly for the preparation of micro(hydro)gel beads but to a lesser extent for the nanocarriers.

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Other binary nanoparticulate systems have also been investigated by ionic complex formation such as chitosan–alginate [16,17], chitosan–tripolyphosphate (TPP) [18,19], poly-L-lysine–alginate [20,21], chitosan–kappa carrageenan [22], chitosan–dextran sulfate [23]. However, the therapeutic agents are released when the matrix re-dissolves due to the reversible exchange of divalent cations with monovalent ions, especially sodium present in simulated intestinal fluid. Due to their small size and large surface area, the nanoparticles had limited drug loadings and resulted in burst release of the loaded drugs. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available. Thus, it would have been a novel approach if any polysaccharide other than sodium alginate and chitosan is sought.

Locust bean polysaccharide (LBP) was extracted from the seeds of carob tree *Ceratonia siliqua*. *It* consists of α-1,4-linked β-D-mannopyranose backbone with branch points from their 6-positions linked to α-D-galactose (1,6-linked α-D-galactopyranose) [24]. It contains mannose units which makes it a very attractive material to specifically target intestinal M-cells, which over express mannose receptors. Tomizawa demonstrated a significant uptake of liposomal particles *via* rat Peyer's patches, where mannose residues were bonded to liposomal surface [25].

This study was undertaken to design ELBP nanocarriers by a novel surfactant-assisted homogenization-$Al^{3+}$ reticulation technique, and characterize their drug entrapment efficiency and drug release behaviors, preferably. Furthermore, the compatibility of drug in these reticulations was examined by differential scanning calorimetry (DSC), X-ray diffraction (X-RD) and Fourier transform infrared (FT-IR) spectroscopy analyses.

## 2. Materials and methods

### 2.1. Materials

Lamivudine was a gift from Ranbaxy Research Laboratory, Gurgaon, Hariyana, India. Locust bean polysaccharide was purchased from Himedia Pvt. Ltd., Mumbai, India. Monochloroacetic acid was supplied by Loba Chemie Pvt. Ltd., Mumbai, India. Span 80, chloroform, and aluminium chloride were supplied by Merck Ltd., Mumbai, India. All other reagents obtained commercially were of analytical grade and used as received.

### 2.2. Synthesis of ELBP

One gram of dried locust bean polysaccharide (LBP) powder was dispersed in 4 ml water and heated to 90 °C for 10 min for better hydration of the polysaccharide. Then, an ice-cold solution of NaOH was added drop wise to have its strength of $2.5 \times 10^{-4}$ (M). The alkaline polysaccharide mixture was maintained at 15 °C and $1.3 \times 10^{-5}$ (M) aqueous solution of monochloroacetic acid was added over a period of 1 h. Next, the mixture was heated to 65 °C with continuous stirring for an additional hour. The wetted mass was washed with 80% (v/v) methanol:water mixture and adjusted to pH 7.0 with glacial acetic acid. The solvent was decanted and a mass of etherified locust bean polysaccharide (ELBP) obtained was purified by precipitating with methanol and finally dried.

### 2.3. Degree of etherification

ELBP powder was converted into its unionized form by using excess hydrochloric acid (HCl) in 80% (v/v) methanol/water mixture. The mixture was filtered and the residue was successively washed with methanol until washing shows neutrality. The residue was dried to constant weight.

A known amount (200 mg) of unionized sample was placed in 20 ml of water; 5 ml of 0.5 M NaOH were added and magnetically stirred for 3–4 h until the sample dissolved completely. The solution was then back titrated with 0.4 M HCl to a phenolphthalein end point. The degree of etherification (DE) in ELBP was calculated as follows [26]: DE = 0.162A/(1 − 0.058A), where 'A' is miliequivalents of NaOH required per gm of sample.

### 2.4. Nanoreticulations of ELBP

ELBP nanoreticulations (nanoparticles) were prepared by a novel surfactant-facilitated reticulation technique. Firstly, 20 ml 1% (w/v) solution of ELBP was prepared in double distilled water and 10% (w/w) lamivudine was dissolved in this solution. Span 80 was added at a concentration of 2.0% (w/v). Then, 5 ml of 2.5% (w/v) $AlCl_3$ solution was added slowly to this solution and homogenized (Model RQ-127A, Remi Motors Ltd., Mumbai, India) at 5000 rpm for 15 min. ELBP nanoparticles were formed instantaneously and recovered by centrifugation at 8000 rpm for 10 min. The supernatant liquid was decanted from the tube without disturbing the precipitate. The particles, gathered in the form of precipitate on the bottom of the tube were washed with a small volume of chloroform (1 × 10 ml) to remove the residual surfactant and air-dried. Following processing variables were investigated:

(a) Concentration of $AlCl_3$: 1.5, 2.5, 3.5% (w/v)
(b) Drug:ELBP weight ratio: 0.11:1, 0.25:1, and 0.43:1

The percentage product yield was calculated as the weight of the nanoreticulations recovered from each batch divided by total weight of drug and ELBP used to prepare that batch multiplied by 100.

### 2.5. Microscopic examinations

The ELBP solution and Span 80 was homogenized and colored with a water soluble dye, amaranth to identify the micelle or vesicle structure. A drop of dispersion was put on a microscopic slide and observed under an optical microscope (Magnus MLX, Olympus, India) fitted with a digital camera Moticam 1000 (1.3 mega pixel). The image was captured using Motic Images Plus 2.0 software.

The morphology (roundness, formation of aggregates) of nanoreticulations was examined by scanning electron microscope (JEOL-JSM-6360, Japan). A drop of particle suspension in methanol was deposited on a NEM TAPE adhesive paper, dried, and sputtered for 30 s with platinum to obtain a coating thickness of 5 nm. SEM photographs of coated samples were taken at an acceleration voltage of 20 kV with different magnifications.

### 2.6. Size analysis

The size of the ELBP nanoreticulations was measured by Malvern Instrument, Zetasizer DTS Ver.5.03 (Malvern Instruments Ltd, Malvern, UK). The measurements were conducted at a temperature of 25 °C using water as dispersant. Other system details were as follows: cell description: Disposable low volume cuvette; count rate (kcps): 150–300; duration used: 50 s; measurement position (mm): 4.65. Zeta potential of the optimized ELBP nanoreticulations was determined.

### 2.7. Estimation of drug entrapment efficiency

Accurately weighed, 10 mg of drug-loaded nanoreticulations was dispersed in 100 ml of phosphate buffer solution (pH 7.4) and the drug was extracted overnight under mechanical shaking. The solution was warmed at 50–55 °C, filtered, and an