International Journal of Biological Macromolecules xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

A systematic evaluation of hydroxyethyl starch as a potential nanocarrier for parenteral drug delivery

Dhanya Narayanan, Shantikumar Nair, Deepthy Menon*

Amrita Centre for Nanosciences & Molecular Medicine, Amrita Institute of Medical Sciences & Research Centre, Amrita Vishwa Vidyapeetham, Kochi 682 041, Kerala, India

ARTICLE INFO

Article history:
Received 10 October 2014
Received in revised form
29 November 2014
Accepted 4 December 2014
Available online xxx

Keywords:
Hydroxyethyl starch
Nanoparticles
Crosslinking-precipitation
Hemocompatibility
Parenteral drug delivery

ABSTRACT

Development of parenteral nanoformulations is highly challenging due to the stringent demands on stability, reproducibility and high drug loading with minimal excipients. This study focuses on the development of a pharmaceutically acceptable nanomatrix system for parenteral delivery based on Hydroxyethyl Starch (HES), a FDA approved polymer that is relatively unexplored in drug delivery research. HES nanoparticles were prepared through a simple, two-step crosslinking-precipitation route, yielding 160 ± 5 nm, nearly monodispersed spherical particles with high colloidal stability. The utility of this nanocarrier for parenteral delivery was verified by a panel of hemo/cytocompatibility assays at high concentrations (0.05–1 mg/ml) *in vitro* and *in vivo*. HES nanomatrix was found effective in encapsulating two chemically distinct drugs having varying hydrophobicities, with the release behavior being influenced by their chemical nature and drug-matrix interactions. Better *in vitro* efficacy was measured for the nanoencapsulated drug than its bare form, establishing the potential of HES nanocarriers for controlled drug delivery.

1. Introduction

Parenteral administration is a universal therapeutic strategy adopted in clinics for active pharmaceutical ingredients with poor bioavailability and narrow therapeutic index. This route is most preferred in emergency clinical episodes due to the easy access of injected drugs to systemic circulation, thus offering rapid onset of action. However, it is often accompanied by a fast decline in systemic drug levels, necessitating frequent injections to maintain the therapeutic dose, ultimately resulting in patient non-compliance. Further, the ease of systemic access also can potentially increase toxic effects. The use of biodegradable polymeric nanosystems can overcome these limitations by facilitating sustained and slow drug release for long durations, thereby reducing the frequency of injections and improving the toxicity profile and quality of treatment [1]. However, the limited number of acceptable excipients available for injection, the complexity in production, formulational stability, batch-to-batch reproducibility, etc. are issues that need to be addressed while developing parenteral formulations [2,3]. In this regard, the choice of an appropriate biomaterial as well as the route adopted for synthesis becomes extremely vital.

Polysaccharides are natural biopolymers that have gained considerable interest in the field of drug delivery systems [4,5], biomedical imaging [6,7] and tissue engineering [8–10] due to its outstanding merits such as structural diversity, presence of reactive groups, muco-adhesion, etc. The most common polysaccharides studied for drug delivery application include chitin, chitosan, pectin, dextran, chondroitin sulphate, cyclodextrin, cellulose and starch [11–16]. Of the afore-mentioned polysaccharides, starch and its derivatives are relatively less explored for drug delivery applications and is gaining immense attraction due to its versatile properties such as biocompatibility, biodegradability, non-toxicity, ease of availability and low cost, making it amenable for nano-drug delivery. However, a major practical limitation related to starch for drug delivery use is its hydrophilicity. This can be avoided by its modification with hydrophobic units [17,18], which, in turn, would improve stability as well as degradation of the nanocarrier *in vivo*. Nanoformulations based on modified starch such as starch acetate [19], cross-linked starch [20], propyl starch [21], hydrophobically-modified hydroxyethyl starch [22], etc., have been studied for its suitability for controlled drug release. However, hydroxyethyl starch (HES) in its unmodified form, is unexplored for parenteral drug delivery, despite its routine clinical use as a plasma volume expander. Its characteristics such as restoration of

* Corresponding author. Tel.: +91 484 4008750; fax: +91 484 2802020.
E-mail addresses: deepthymenon@aims.amrita.edu, deepsmenon@gmail.com (D. Menon).

http://dx.doi.org/10.1016/j.ijbiomac.2014.12.012
0141-8130/

hemodynamic balance, improvement of hemorheology, sufficiently long intravascular life, absence of hemostasis and anaphylaxis, etc., make HES suitable for such parenteral use [National Research Council, USA, 1963]. Additionally, the rich amylopectin content as well as hydroxyethylation of HES makes it more stable and imparts better half-life *in vivo*, in contrast to native starch which gets cleared from circulation by serum amylase in a short time period [23,24]. All these features render HES an ideal choice for a biomaterial matrix system.

The present study utilizes this polymer, without any further modification, for the first time for developing a nanodrug delivery vehicle. The selected route for the synthesis is a facile crosslinking-precipitation route, which is particularly advantageous for the development of a pharmaceutically translatable drug formulation, owing to the high yield, batch-to-batch reproducibility, easy scalability, etc. The developed HES nano-matrix was well characterized for its size, stability, bio/hemo compatibility and thereafter assessed for its suitability for drug encapsulation, release and efficacy to ensure its utility as a drug carrier for parenteral use.

2. Materials and methods

2.1. Materials

Hydoxyethyl starch [HES] (degree of substitution—0.38–0.45, MW: 110–150 kDa) was purchased from ShanDong Qidu Pharmaceutical, China. Trisodium trimetaphosphate (STMP), Indomethacin and Ibuprofen Sodium were purchased from Sigma Aldrich, USA. Soy Lecithin (MW: 330 Da) was procured from Alfa Aesar, USA. Methanol, Ethanol and Acetone from Merck, India and Liquor ammonia from Qualigens Pvt. Ltd., India. All the chemicals were used without any further purification.

2.2. Preparation of hydroxyethyl starch (HES) nanoparticles

Hydroxyethyl starch (HES) nanoparticles were prepared through a novel, yet simple, two-step crosslinking-precipitation route, with trisodium trimetaphosphate as the crosslinker. Briefly, in the first step, the polymeric solution was prepared by dissolving 200 mg of HES in 5 ml of MilliQ water (aqueous phase) containing 10 μl of liquor ammonia (for alkaline pH to facilitate crosslinking). This solution was then crosslinked by the addition of STMP (0.5 wt% of HES). This HES-STMP mixture was allowed to react for ~3 h at 47 ± 2 °C under magnetic stirring (700 rpm, IKA C-MAG HS 7, Germany). In the second step, crosslinked starch solution was precipitated with methanol (oil phase) under magnetic stirring and sonication (Vibra cell Sonicator, Sonics Inc. USA) for 2 min resulting in the formation of hydroxyethyl starch nanoparticles (HES Nps). These nanoparticles were further stabilized by the addition of lecithin (0.25 wt% of HES) under stirring and sonication. Unreacted excipients were removed from this suspension by centrifugation at 20,000 rpm for 10 min (Hermle Z36 HK, Germany) followed by repeated washing. The pellet was re-dispersed in MilliQ water for further experiments.

2.3. Physical and chemical characterization of hydroxyethyl starch nanoparticles

Gel Permeation Chromotography (GPC, Perkin Elmer, Series 200) analyses of HES and crosslinked HES were performed to evaluate the molecular weight change as a confirmation of crosslinking. Apart from GPC, viscometric analysis (DV-II + Pro, Brookfield, USA) was also done to observe the viscosity changes before and after crosslinking. Raman spectroscopic analysis also was used to confirm crosslinking of HES. A confocal Raman microscope system (WITec alpha 300RA, GmbH, Germany) consisting of a spectrograph [UHTS 300] (600 lines/mm grating, 30 cm focal length) coupled to a Peltier cooled charge coupled device (CCD) detector, with an excitation laser source (wavelength 488 nm) focused onto the sample via an optical fiber (50 μm) was used for the purpose. Scattered light was collected for analysis, wherein the time of each scan was 3 s.

Hydrodynamic particle size and size distribution as well as zeta potential of the prepared nanosuspension were measured by dynamic light scattering (DLS) analysis using NanoZS Zetasizer (Malvern, USA). The samples were evaluated in triplicates after dispersing in MilliQ water at a dilution of 1:10. The morphology of the nanoparticles was examined by Scanning Electron Microscopy [SEM] (JEOL, JSM-6490LA, Japan) for which 1:100 diluted samples were drop casted on an aluminium stub, and sputter coated with gold before imaging. The size and morphology was further confirmed by Atomic Force Microscopy [AFM] (JEOL, SPM 5200, Japan) imaging.

2.4. Lyophilization

HES nanoparticles were lyophilized (Alpha 2-4 LD plus LT, Martin Christ, Germany) without the addition of any cryoprotectants. Prior to lyophilization, the sample was frozen at −80 °C for 4 h. Size measurements were carried out by DLS and SEM before and after lyophilization after redispersing the lyophilized particles in MilliQ water.

2.5. Colloidal stability analysis

Stability of the nanoparticles was studied by dispersing the HES nanoparticles in serum containing media for a time period of 48 h. Stability was also checked by dispersing the same in media with different ionic strengths, viz., MilliQ water, 0.9% NaCl and Phosphate Buffered Saline [PBS]. The tendency of the nanoparticles to aggregate in various media was assessed using DLS.

2.6. Hemocompatibility

To check the feasibility of using HES Nps for intravenous drug delivery, its hemocompatibility assessment becomes very critical. Hemocompatibility studies were performed on bare HES Nps using whole blood from healthy volunteers after obtaining approval from the Institutional Ethical Committee. Blood was collected in tubes containing acid citrate dextrose (ACD) (Blood:ACD—8.5:1.5) from three different donors and pooled before experiment. HES Nps of varying concentrations ranging from 0.05 to 1 mg/ml were used for all the biocompatibility tests. A dilution of 1:9 of nanoparticle to respective biological fluid was maintained for all the experiments.

2.6.1. Hemolysis

The hemolytic property of HES Nps was measured spectrophotometrically by Soret band based absorption of free hemoglobin at 415 nm [25]. 450 μl of blood was treated with 50 μl of sample and incubated at 37 °C for 3 h under mild shaking conditions. PBS and 1% TritonX100 served as negative and positive controls, respectively. The treated sample was then centrifuged at 4000 rpm for 10 min and the plasma obtained was diluted with 0.01% sodium carbonate. The amount of plasma hemoglobin was calculated by measuring the optical density at 380, 415 and 480 nm using a spectrophotometer (Powerwave XS-BioTek, USA).

$$\text{Amount of plasma hemoglobin (mg/dl)} = \frac{2 \times A_{415} - (A_{380} + A_{450}) \times 1000 \times \text{Dilution factor}}{E \times 1.655} \quad (1)$$