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Antihypertensive nano-ceuticales based on chitosan biopolymer: Physico-chemical evaluation and release kinetics

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ABSTRACT

Prime risk factor behind cardiovascular associated mortality and morbidity is hypertension. The main challenge with antihypertensive (AHT) drug therapy is their extreme hydrophobic nature and very low oral bio-availability; which result into higher dosage/frequency and associated side effects of drugs. The main objective of this study was to fabricate AHT nano-ceuticals in hydrophilic carriers of natural origin to improve drugs' solubility, protection and sustained release. AHT nano-carrier systems (NCS) encapsulating captopril, amlodipine and valsartan were fabricated using chitosan (CS) polymer by ionic gelation assisted ultra-sonication method. Drug encapsulation efficiencies of 92 ± 1.6%, 91 ± 0.9% and 87 ± 0.5% were observed for captopril, valsartan and amlodipine respectively. Scanning electron microscopy (SEM) based analysis had revealed that captopril loaded polymeric NCS were regular, smooth and without any agglomeration. FTIR analyses of drug loaded and empty NCS demonstrated that drugs were molecularly dispersed inside the nanoparticles via week hydrogen bonding. Captopril and valsartan have demonstrated grafting reaction with N—H group of chitosan. Zeta sizer results had confirmed that average size of chitosan nanoparticles was below 100 nm. Encapsulation of captopril had reduced the surface charge value from +52.6 ± 4.8 to +46.5 ± 5.2 mV. Controlled release evaluation of highly encapsulated drug captopril had revealed a slow release *in vitro* from NCS in physiological buffer. Thus, here reported innovative AHT nano-ceuticals of polymeric origin can improve the oral administration of currently available hydrophobic drugs while providing the extended-release function.

1. Introduction

According to World Health Organization (WHO), cardiovascular diseases (CVDs) are the foremost contributor to morbidity and mortality worldwide. WHO data had revealed that CVDs are responsible for 17.3 million deaths annually; which are approximately 31% of the total global death-count (Mendis & Chestnov, 2014). Among those, only heart attacks and stroke were responsible for 7.3 million and 6.2 million deaths respectively (Mendis & Chestnov, 2014). Among all other predisposing factors behind CVDs (obesity, physical inactivity, smoking), hypertension is considered as the major risk factor which causes 7.5 million deaths worldwide (Alwan, 2011; WHO, 2014).

Normal systolic and diastolic blood pressure (BP) in adults is recognized as 120/80 mmHg. But if the ratio for systolic and diastolic BP is raised to 140/90 mmHg, then it is termed as hypertension. If hypertension is left untreated then it can lead to enlargement of the heart and eventually to heart attack, heart failure and stroke (Thomas & Duffin-Jones, 2015). Drugs prescribed for cardiovascular treatments are considered as one of the largest category of therapeutic drugs. Major therapeutic features of the antihypertensive drugs are covered by three main classes which includes; Angiotensin Converting Enzyme Inhibitors (ACEI), β-blockers (BBs) and Calcium Channel Blockers (CCBs). ACEI and BBs block the cascade of hormonal system called renin angiotensin system while CCBs reduce the influx of calcium ions into the cells which helps to reduce the blood pressure (Coates, 2003; Elliott & Ram, 2011). Renin

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angiotensin system (RAS) is the most powerful hormonal system of the body with endocrinal properties. RAS is a peptidergic system and plays a central role in arterial BP control. Known components of local or tissue specific RAS system are renin, angiotensinogen, ACE, angiotensin peptides (ANG I and II), and angiotensin receptors AT1 and AT2 (Bonnet, Cooper, Carey, Casley, & Cao, 2001).

All the major classes of AHT drugs used for the treatment of CVDs have many drawbacks. Antihypertensive drugs produce extremely hypotensive effect after oral administration (Gupta, Prajapati, Balamurugan, Singh, & Bhatia, 2007) and inhibit vascular cell growth by increasing apoptosis which leads to cancer. Due to short half-life of these drugs, reflex tachycardia can occur which can be controlled by sustained release. Other side effects of antihypertensive drugs included cough, skin rashes, and loss of hair (Kumbhare et al., 2014) headache and accumulation of bradykinin, swelling in ankles, flushing, palpitation and rarely gingival hyperplasia due to high dosage (Aldemir, Begenik, Emre, Erdur, & Soyoral, 2013). Almost all of these antihypertensive drugs are available in oral tablet forms. Nevertheless, 80% of the available AHT drugs have low oral bioavailability. The oral bioavailability of drugs like valsartan, amlodipine, and captopril are very low and sometimes irregular. Therefore, more advanced delivery systems are needed to reduce the dosing frequency, increase the bioavailability and deliver the drug as extended release with minimal side effects (Gupta et al., 2007; Puchalska, Marina Alegre, & García López, 2015).

Nano-encapsulation provides the solution for all these problems by increasing the oral bioavailability of hydrophobic agents, augmenting plasma half-life and minimizes the side effects associated with AHT drugs by reducing their dosage requirements and intake-frequency (Mulla, Hiremath, & Sharma, 2012; Vikas, Arvind, Ashish, Gourav, & Vipasha, 2011). Biopolymeric nanoparticles (NPs) can increase the stability of many drugs by controlling their pharmacokinetic behaviour (Rawat, Singh, Saraf, & Saraf, 2006).

Chitosan is a linear polysaccharide which has high biodegradability, biocompatibility and non-toxicity (Rishabha et al., 2010). Due to the presence of amino groups, chitosan acts as a basic polymer and makes electrolytic complexes with the oppositely charged molecules. Chitosan has natural mucoadhesive properties which is useful for the prepartion of bioadhesive nanoparticles. Hydrophobic and ionic interations between the cationic group of chitosan and anionic structure of the mucus are responsible for its mucuadhesive nature. Similarly the presence of thiol groups in chitosan can assist to form disulphide bond with mucus layer (Bansal, Sharma, Sharma, Pal, & Malviya, 2011). Interactions between the poly-anionic drugs and chitosan are more distinct due to ionic cross linkings, which form better complexes for sustained release over long period of time.

Due to colon specific degradation by microbes in last part of gastrointestinal tract, chitosan based nano-carrier systems (NCS) are extensively used in the encapsulation of many drugs for site secific delivery (Kean & Thanou, 2010; Sonia & Sharma, 2011). All these properties of chitosan make this polymer a perfact drug delivery system for AHT drugs as well to enhace their oral bioavailabity by increasing the first pass metabolism of the drug, reduce dosage, reduce dosage-associated side effects and to control the release of AHT drug *in vitro* and *in vivo* over longer period of time.

Carbohydrate based nano-carrier systems emerge as a solution for major drawbacks in the current antihypertensive treatments. Purpose of the current study was to design, formulate and characterize not yet reported nano-ceuticals; which may increase the oral bioavailability by over-coming hydrophobicity, reduce the drug dosage as well as increase the sustained release of drug by encapsulating antihypertensive drugs in chitosan based NCS. This polymer is natural and biocompatible to human body (Fernández-Quiroz et al., 2015).

2. Materials and methods

2.1. Materials

Chitosan with medium molecular waight having 85% of degree of deacetylation was purchased from Sigma-Aldrich (USA). Tripolyphosphate (TPP-molecular formula: $Na_5P_3O_{10}$) was purchased from Sigma. Acetic acid (molecular waight: 60.05 g/mol) was purchased from Riedel-de Haen chemicals. Antihypertensive drugs captopril, valsarten and amlodopine was kindly provided by CCL Pharmaceutical (Pvt), Pakistan.

2.2. Quantification of drugs by Nano-photometer

Stock solution (1 mg/mL) of valsartan, amlodipine and captopril was prepared by dissolving 0.01 g of drug in 10 mL of deionized water. Working solutions were made by various serial dilutions separately for each drug. Wavelength selected after wave scan for amlodipine, valsartan and captopril were $\lambda = 363$ nm, $\lambda = 292$ nm and $\lambda = 272$ nm, respectively by Nano-spectrophotometer (Implen). Optical density (OD) values were recorded for each sample. This practise was repeated with each drug dilution (triplicate) and then respective standard curves were prepared.

2.3. Fabrication of chitosan nanoparticles

Chitosan nanoparticles were prepared according to the protocols reported previously (Elwerfalli, Al-Kinani, Alany, & ElShaer, 2015; Vaezifar et al., 2013) with slight modifications. Chitosan solution was prepared by mixing 0.3% (w/v) chitosan in 1% (v/v) acetic acid solution. One percent (w/v) TPP solution was formulated in deionized water. For drug loaded chitosan nanoparticles, 50 mg of the drug was dissolved in 2 mL of one percent w/v TPP solution (TPP to drug ratio was 1:2.5). Afterwards 1 mL of the TPP solution was added drop wise to 25 mL of chitosan solution. This mixture was kept on magnetic stirring for 30 min. Further, it was sonicated with ultra-sonicator of 25 kHz for 30 min (Sononzap). Drug loaded NPs were separated by centrifugation at 12,000 rpm for 12 min. Supernatant was discarded and the nanoparticles were stored at refrigeration temperature (4 °C) for further characterization.

2.4. Verification of nanoparticles fabrication

AFM studies were performed to confirm the nano-scale size of prepared chitosan particles. The AFM images were acquired using AFM (Agilent Pico Plus). A drop of prepared nano particles was air dried on glass slides ($1 \times 1\ cm^2$). Imaging was carried out in tap mode in air with standard Si cantilever having a spring constant of 0.6 N/m and an estimated tip radius of 10 nm.

2.5. Encapsulation efficiency of antihypertensive drugs

After centrifugation of sonicated samples, free or non-encapsulated drug in the supernatent was measured by checking its optical density (OD) value with Nanophotometer (Implen). Asorbance was taken at $\lambda = 363$ nm, $\lambda = 292$ nm and $\lambda = 272$ nm for amlodopine, valsarten and captopril respectively. By comparing OD value of each drug with the standard curve, specific concentration of the encapsulated drug was calculated. Encapsulation efficiency was calculated according to the formula given below.

$$EE\% = \frac{\text{Encapsulated drug(Total drug} - \text{unencapsulated drug)}}{\text{Total drug}} \times 100$$