Research Article

Formulation, Optimization and Characterization of Chitosan Monodisperse Microparticles for Sustained Delivery of Hydrochlorothiazide HCl

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Abstract

Background: Hydrochlorothiazide HCl (HCTZ) is first line medication in the management of hypertension, however its poor solubility and GI irritation warrants long term application. The aim of present study was to prepare hydrochlorothiazide HCl (HCTZ) loaded monodisperse microparticles of chitosan (CS) for sustained delivery.

Methods: In the present study chitosan microparticle were prepared using ionic gelation method. The effect of input parameters such as concentration of chitosan and pH of CS solution on the mean particle size (PS), zeta potential (ZP), polydispersity index (PDI) and in-vitro drug release were investigated. In-vitro drug release from the microparticles was studied using Franz diffusion cell. Structural formation and surface morphology was investigated using FTIR and SEM, respectively.

Results: PS of CS microparticles ranges from 0.87±0.066 to 1.96±0.011 µm, ZP between 33.43±0.80 mV to 53.6±2.06 mV, PDI values from 0.271±0.063 to 0.842±0.073. The optimized CSMP- 3 (1.5% chitosan, pH 3) showed PS 1.67±0.073 µm, ZP 53.6±2.06 mV, PDI 0.481±0.053, %EE 80.8±2.27% and HCTZ release of around 80% in 8 hours. FTIR reveals successful formation of crosslinked structure. SEM shows irregular surface owing preparation procedure. Drug kinetics modeled with Korsemeyer–Peppas equation revealed non-Fickian diffusion mechanism.

Conclusion: Drug permeation study showed more than 70% of drug permeated through membrane. In conclusion, the CS microparticles can be used for the sustained delivery of HCTZ which would reduce the dosing frequency and improved patient compliance.

Introduction

Drug delivery systems are developed to ensure maximum concentration of drug at the target site by overcoming physiological barriers.1 Fabrication of a biocompatible carrier for a candidate drugs is the need of the time. The carrier molecules are expected to have high loading efficiency and should provide an optimal drug release at the site of action. Chitosan (CS), a natural cationic amino polysaccharide, obtained from natural sources, is a biodegradable and biocompatible polymer that can be considered as suitable carrier for both small and large molecules.2 In order to discover the effectiveness of CS in preparing microceuticals and nanoceuticals, the entrapment of CS has been extensively studied.3,4 Hydrochlorothiazide (HCTZ) is an efficacious diuretic agent used in the management of congestive heart failure and hypertension. It described with poor oral bioavailability.5,6 Studies performed in healthy fasting volunteers represents 70% gastrointestinal uptake, while it was linear over the dose range of 5-75 mg.7,8 The drug belongs to BCS class II. The solubility of HCTZ in water is 0.7 mg/mL and its membrane permeability is also rated poor (i.e., logP= 0.15).9,11 Furthermore, free HCTZ released from immediate release conventional dosage forms results in an injury to the intestinal epithelium which warrants its long term use in the chronic diseases.12 This observation has driven in an increased interest of formulation scientists to develop alternate formulations which satisfactorily address the aforementioned challenges. A variety of techniques are reported in literature to obtain a sustained release of HCTZ. These include controlling membrane-based drug delivery systems, matrix tablets and microcapsules based on natural and synthetic polymers. Among these, microparticles are more popular as the multiparticulate based design is more reliable and offers a broader safety margin in terms of drug release as compared to the monolithic reservoir based system.
In the present study, CS was selected as natural polymer for the development of monodisperse microparticles for oral delivery of hydrochlorothiazide HCl. Optimization was performed by preparing nine formulations of CS microparticles (CSMP-1 to CSMP-9) using a formulation design. The three levels of two variables (i.e. concentration of chitosan and its pH) were investigated. The effects of these factors were evaluated by particle size (PS), polydispersity index (PDI), zeta potential (ZP), entrapment efficiency (%EE), in-vitro drug release and permeation studies. Furthermore, the morphology of prepared microparticles was studied using SEM and compatibility of formulation components was evaluated using FTIR.

Materials and Methods

Materials
Chitosan was purchased from Fisher Scientific Bioblock, Illkirch, France. Acetic acid was purchased from Fisher Scientific Loughborough, UK. Sodium tripolyphosphate (TPP) was from Sigma-Aldrich (Buchs, Switzerland). Methanol was obtained from Riedel-de Haën (Seelze, Germany). Hydrochloric acid, sodium hydroxide from Merck (Darmstadt, Germany) and potassium dihydrogen phosphate (Geldenaaksehaan, Belgium) were of analytical grade. The Hydrochlorothiazide HCl (HCTZ) was gift from China.

Preparation of CS microparticles
In the present study CS microparticles were fabricated using TPP as crosslinker. In essence, the present method is a minor modification of previously reported method.\(^{13-15}\) Briefly, CS was dissolved in 1% (w/v) aqueous solution of acetic acid to prepare different concentrations of CS (0.5, 1 and 1.5%) at 400 rpm for 12 hours by using magnetic stirrer (Stuart, UK). These solutions were sonicated and degassed for 10 minutes. HCTZ solution (10% w/v) was prepared separately and mixed with polymeric solution under continuous stirring for 15 minutes. The pH of these solutions was adjusted to the specific values (3, 4 and 5) (Table 1) by adding 0.1 M NaOH solution or 0.1 M HCl solution.

Aqueous solution of TPP (0.1% w/v) as a crosslinker was added drop wise using 26-gauge needle to chitosan/drug solutions of varying composition under continuous stirring at 1000 rpm for 30 minutes. Chitosan colloidal microparticles formed as a result of crosslinking of TPP with CS were centrifuged at a speed of 10,000 rpm/min for 15 minutes. The separated microparticles deposited at bottom of Eppendorf tubes were collected and stored at room temperature for further use. An illustration presenting the preparation of CS microparticles is shown in Figure 1. The detailed compositions of nine formulations (CSMP-1 to CSMP-9) prepared during optimization are described in Table 1.

Physicochemical characterization
Determination of particle size (PS) and polydispersity index (PDI)
The mean particle size, polydispersity index (PDI) of CS/TPP/HCTZ microparticles was examined by
using Zetasizer (Malvern instruments, UK Ver.7.11). A suspension of microparticles was prepared in distilled water at pH 7. All the aggregated particles were separated by using sonicator for 5 minutes. The suspension was poured in disposable cuvettes having 10mm diameter and the particles were counted at a rate of 150 to 250.2 kcps.

Table 1. Various formulations during optimization of CSMPs at different pH and chitosan concentrations.

| Formula Code | pH | Concentration of Cs (%) |
|--------------|----|-------------------------|
| CSMP-1       | 3  | 0.5                     |
| CSMP-2       | 3  | 1.0                     |
| CSMP-3       | 4  | 0.5                     |
| CSMP-4       | 4  | 1.0                     |
| CSMP-5       | 5  | 0.5                     |
| CSMP-6       | 5  | 1.0                     |
| CSMP-7       | 5  | 1.5                     |

**Determination of zeta potential (ZP)**
The charges on HCTZ-loaded chitosan (CS/TPP/HCTZ) microparticles was determined by zetasizer 3000 HAS (Malvern Instrument, UK). Samples were dispersed in water, sonicated and zeta potential was determined.

**Estimation of entrapment efficiency**
Entrapment efficiency (%EE) of HCTZ was determined by indirect method. The supernatant solution obtained after centrifugation of drug-loaded microparticles was assayed by UV spectrophotometer. Absorbance (A) of each sample was recorded at 272nm. The standard curve was used to estimate the concentration of free HCTZ in the supernatant. Entrapment efficiency was calculated by using the following equation.

\[
EE\% = \frac{\text{total amount of HCTZ} - \text{free HCTZ}}{\text{total amount of HCTZ}} \times 100
\]

**Scanning electron microscopy**
Surface morphology of the prepared microparticle was investigated using a Hitachi scanning electron microscope S3400-N (Japan). Samples were scanned at appropriate magnifications.

**Fourier transformed infrared spectroscopy (FTIR)**
Drug polymer interaction was determined by using Fourier Transform Infrared Spectrophotometer (Bruker Germany). The ATR-FTIR spectra of chitosan, TPP, HCTZ and drug loaded microparticles (CSMP-9) were recorded. The FTIR spectrum was recorded over the wavelength range 4000-400 cm⁻¹.

**In-vitro drug release kinetics of HCTZ**
The percentage drug release from HCTZ-loaded CS (CS/TPP/HCTZ) microparticles was determined using USP dissolution apparatus II. Microparticles weighing equivalent to drug concentration of 30 mg were introduced in 900 ml of phosphate buffer pH 6.8 at 100 rpm and 37°C. At discrete intervals (0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 4, 5, 6, 7 and 8 hours), aliquots of drug solution (5 ml) was taken and replaced by fresh phosphate buffer solution. HCTZ absorption was recorded by UV-visible spectrophotometer at 272 nm and percentage release of drug was determined.

**Pharmacokinetic analysis of release data**
Model dependent and model independent methods were used to delineate best fit release kinetics. These were zero-order, first order, Higuchi, and Korsmeyer-Peppas release kinetic model.16-20

**Permeation from chitosan microparticles**
To study permeation of HCTZ across the dialyzing membrane, the modified Franz Diffusion cell was used. Freshly prepared phosphate buffer solution of pH 7.4 was transferred to the receptor chamber. A pre-hydrated dialyzing membrane was employed to partition donor and receptor chamber. The jacketed receptor compartment was water circulated to achieve a temperature of 37±1 °C. Microparticles of selected formulation were poured on the dialyzing membrane and the contents of the receptor chamber were stirred at speed of 250rpm. Aliquot of 0.5 ml was taken from receptor chamber by using micropipette at discrete time intervals (5, 15, 30, 45, 60, 90, 120, 180, 300 and 480 minutes). After each withdrawal, the receptor chamber was replaced by 0.5ml freshly prepared phosphate buffer solution in order to ensure the sink conditions. These samples were further diluted and analyzed by UV spectrophotometer at 272 nm in order to determine the concentration of HCTZ.21,22

**Statistical analysis**
The data was analyzed by using Microsoft Excel 2010. Significance of data was evaluated using one-way ANOVA. Differences was considered significant at p ≤ 0.05.

**Result and Discussion**
Ion gelation method was used to prepare CS-TPP microparticles.14 This technique encompassed ionic interaction, in which positive amino groups on CS molecules were cross linked with negative phosphate ions of sodium TPP. The proposed structural formation of CS-TPP ionic crosslinking has been presented in Figure 2. The HCTZ drug molecules could be physically entrapped into the particles.

**Physicochemical characterization**

**Effect of chitosan concentration on PS, PDI and ZP**
The effect of different concentration of chitosan (CSMP-1 to CSMP-9) on PS PdI and ZP was studied and results are presented in Table 2. The PS ranges from 0.878±0.066 (CSMP-1) to 1.963±0.011 µm (CSMP-9). The results indicate that increasing chitosan concentration generally leads to increase in mean particle size. The particle size distribution curve for CSMP-3 is shown in Figure 3A. These findings are in agreement with the previously

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In the study, the reported results suggest that the formation of intermolecular hydrogen bonding and inter-molecular electrostatic repulsion due to NH$_3$ groups along the CS chains is responsible for the increase in particle size. Increasing the CS concentration enhances the interaction and aggregation of polymeric molecules, forming counter TPP ions. This leads to the formation of a single large particle.

The ZP of CS microparticles was recorded as 43.6±1.02 mV for CSMP-1, 42.5±1.12 mV for CSMP-2, and 53.6±2.06 mV for CSMP-3 at pH 3, increasing with the CS concentration. The ZP values at pH 4 were 38.1±0.26 mV for CSMP-4, 41.9±1.70 mV for CSMP-5, and 41.9±1.70 mV for CSMP-6. At pH 5, the ZP was 33.4±0.80 mV for CSMP-7, 41.9±1.70 mV for CSMP-8, and 37.5±0.63 mV for CSMP-9. The ZP curve of CSMP-3 is presented in Figure 3B. The increase in ZP could be attributed to a positive surface charge on CS-TPP arising from residual amine groups of the CS. ZP of microparticles increases linearly with increasing the CS concentration, indicating potential stability and resistance to aggregation.

**Effect of pH of CS solution on PS and ZP**

Increasing the pH of the CS solution affects the physicochemical parameters of prepared CS-TPP microparticles. The solubility of CS is pH-dependent, with increasing solubility at higher pH values due to the protonation of NH$_3$ groups. The pH of the CS solution can influence the microparticle's size and ZP, affecting the stability and aggregation potential. The results showed that at 0.5% CS concentration, the stability and aggregation potential are influenced by the pH of the CS solution.

**Table 2. Optimization of particle size (PS), zeta potential (ZP), polydispersity index (PDI) and percentage entrapment efficiency (%EE).**

| Formula Code | pH  | CS concentration (%) | PS (µm)   | ZP (mV)   | PDI        | %EE         |
|--------------|-----|-----------------------|-----------|-----------|------------|-------------|
| CSMP-1       | 3   | 0.5                   | 0.878±0.066 | 43.6±1.02 | 0.271±0.063 | 74.7±1.16   |
| CSMP-2       | 3   | 1.0                   | 1.565±0.081 | 42.5±1.12 | 0.538±0.029 | 78.1±2.40   |
| CSMP-3       | 3   | 1.5                   | 1.672±0.073 | 53.6±2.06 | 0.481±0.053 | 80.8±2.27   |
| CSMP-4       | 3   | 0.5                   | 1.411±0.403 | 38.1±0.26 | 0.354±0.018 | 66.3±2.55   |
| CSMP-5       | 4   | 1.0                   | 1.688±0.090 | 39.1±0.62 | 0.559±0.038 | 70.1±2.85   |
| CSMP-6       | 4   | 1.5                   | 1.689±0.091 | 41.9±1.70 | 0.531±0.038 | 74.2±2.30   |
| CSMP-7       | 5   | 0.5                   | 1.475±0.112 | 33.4±0.80 | 0.811±0.058 | 66.2±2.13   |
| CSMP-8       | 5   | 1.0                   | 1.699±0.082 | 35.2±0.72 | 0.799±0.056 | 65.6±3.18   |
| CSMP-9       | 5   | 1.5                   | 1.936±0.011 | 37.5±0.63 | 0.826±0.073 | 67.1±2.20   |
concentration, the PS recorded from 0.878±0.066 (CSMP-1), 1.41±0.403 (CSMP-4), 1.475±0.112 µm (CSMP-7) as the pH of CS solution was adjusted to 3.0, 4.0 and 5.0 respectively. This effect is explained by protonation behavior of CS which give higher surface charge to microparticles and thus resulted in higher extent of electrostatic repulsion. Similar trends were observed for higher concentrations of chitosan (Table 2).

The results of ZP from formulations prepared using CS at pH value 3, 4 and 5 shows a reduction from 43.65±1.02 mV (CSMP-1), 38.1±0.26 mV (CSMP-4), to 33.43±0.80 mV (CSMP-7). Similar trends were observed in solution of chitosan at higher concentrations (1 & 1.5% each prepared at 3 different pH values). As the pH of CS solution was increased, the protonation of NH$_2$ groups was discouraged, resulting in a reduced surface charges and therefore less ZP and vice versa at lower pH values. These findings are in line with previously reported data. Ko and co-workers observed that deviation of protonation on the CS molecules was the result of increase in the pH of CS solution. At basic pH, the majorities of the amino groups on the CS molecules were in NH$_2$ form and did not contribute to the surface charge of CS microparticles. In contrast, at acidic pH, the majorities of NH$_2$ groups on CS molecules were protonated (NH$_3^+$) and imparted net positive charge on surface of particles.

**Effect of chitosan concentration and pH on entrapment efficiency**

The results of the entrapment efficiency of HCTZ in chitosan-TPP microparticles (CSMP-1 to 9) are presented in Table 2. Entrapment efficiency was performed at three pH (3, 4 and 5) levels. The three pH were investigated at three concentrations of chitosan (i.e. 0.5, 1.0 and 1.5 %). The decrease in pH has shown pronounced effect on protonization of chitosan molecules which results in increased entrapment of HCTZ upto 80% was observed. The investigation on effect of chitosan concentration on entrapment efficiency has shown the positive effect. The results revealed that EE increases with the concentration of CS. The possible reason for this effect may be the protonation of amino group (at pH 3 to 5) of chitosan solution.

**Scanning electron microscopy**

The SEM image of selected formulation (CSMP-3) is presented in Figure 4. The microparticles presents an irregular shape and showed uniform particle size distribution. The microparticle appears to be aggregated in cluster. A possible explanation to this observation is improved polymeric interaction due to H-bonding of the functional groups.
Fourier transform infrared radiations analysis
FTIR analysis of chitosan, TPP, HCTZ and drug loaded microparticles (CSMP-3) has been presented in Figure 5. CSMP-3 showed a peak at 3311.37 cm\(^{-1}\) for N-H stretching and another peak at 1646.69 and 1636.12 cm\(^{-1}\) for C=C stretching which might indicate the presence of HCTZ. The FTIR spectrum of TPP showed a peak at 2361.67 cm\(^{-1}\) which is associated with stretching of O-H group. There were no major shifts of characteristic peaks in the spectrum of drug loaded microparticles.

In-vitro release and kinetic study of HCTZ microparticles
The results presented in Figure 6 shows release behavior of HCTZ from Chitosan-TPP microparticles (CSMP-1 to 9) at various pH levels. As pH of reaction mixture decreased (from 5 to 3), the release profile of model drug was also decreased. This effect might be due to the increased polymer density of the crosslinked polymer at lower pH. Additionally, ionic reaction between chitosan and TPP is pH dependent which ultimately results in improved ionic crosslinking density at acidic pH. At this pH, the extent of ionization is high and results in complete ionic crosslinking. The release of drug from microparticles prepared with the 1.5 % chitosan solution was lower than...
those produced with the 0.5% chitosan solution. The drug release from CSMP-3 was recorded at consistent rate hence, it was considered optimal in this study. These results are attributed to the viscosity of chitosan solution which, in turn, depends upon its concentration in the reaction medium. The increased viscosity of the solution resulted in the formation of stronger walls of crosslinked polymer matrix thus showed lower swelling and drug release (Table 3). The $R^2$ value revealed that all formulation followed the Korsmeyer–Peppas model with release exponent value less than 0.5 (non-Fickian diffusion).

**In-vitro permeation across membrane using Franz diffusion cell**

In the present study, three formulations (CSMP-3, CSMP-6 and CSMP-9) were investigated for permeation studies through model dialyzing membrane using Franz diffusion cell. The permeation profiles are presented in Figure 7. The amount of drug observed in receptor chamber during 24 hours was approximately 51, 59 and 73 % from formulations CSMP-3, CSMP-6 and CSMP-9, respectively. The permeation profile can be divided into two zones; initial burst release region followed by second sustained release. The initial burst release of the drug provided higher drug concentration over the membrane which continued to permeate in sustained manner up to the investigated study period.

**Conclusion**

Chitosan-TPP microparticles were prepared by ionic crosslinking method at different pH and polymeric concentrations to study the release behavior HCTZ. The drug release was significantly reduced when microparticles were fabricated using higher concentration of chitosan and crosslinked under acidic environment. The optimized formulation showed no interaction between formulation components using FTIR and revealed acceptable permeability through model membrane.

**Conflict of Interests**

The authors claim that there is no conflict of interest.

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