

PHYSICOCHEMICAL CHARACTERIZATION OF PROPRANOLOL-LOADED CHITOSAN NANOPARTICLES FOR A BUCCAL DRUG DELIVERY SYSTEM

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Received: 13 Jan 2020, Revised and Accepted: 23 May 2020

ABSTRACT

Objective: This study aimed to characterize the physicochemical properties, including pH, zeta potential, and particle size of propranolol-loaded nanoparticles that were incorporated into a buccal transmucosal drug-delivery system.

Methods: An ionotropic gelation technique was used to formulate propranolol-loaded chitosan nanoparticles. Chitosan used as the nanoparticle base, using tripolyphosphate (TPP) as a cross-linking agent. The effects on nanoparticle physical properties, including pH, zeta potential, and particle size were examined when various chitosan [0.150-0.300 % (w/v)] and propranolol contents (0-40 mg) were used during the preparation. The effects of using chitosan solutions with different pH values on nanoparticle properties were also determined.

Results: The pH values of all nanoparticles ranged between 4.14–4.55. The zeta potentials of the prepared nanoparticles ranged between 22.6–52.6 mV, with positive charges. The nanoparticle sizes ranged from 107–140 nm, which are within the range of suitable particle sizes for transmucosal preparations.

Conclusion: The pH values, zeta potentials, and particle sizes of the nanoparticle formulations were influenced by the concentrations of chitosan and propranolol and by the pH of the initial chitosan solution. The relationships between nanoparticle properties and all factors primarily depended on the ionic charges of the components, especially chitosan. Our study provides beneficial physicochemical knowledge for the further development of chitosan-based nanoparticles containing propranolol for buccal drug delivery systems.

Keywords: Nanoparticles, Chitosan, Propranolol, Buccal, Drug delivery system, Ionic gelation

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DOI: <http://dx.doi.org/10.22159/ijap.2020v12i4.38009>. Journal homepage: <https://innovareacademics.in/journals/index.php/ijap>

INTRODUCTION

Nanoparticles have been researched considerably in pharmaceutical fields for their potential to carry drugs or active substances directly to target sites, due to their intrinsic properties to control drug release, to protect drugs from hazardous environments, and to increase drug absorption and permeation through mucosal membranes [1, 2]. The buccal mucosa is an attractive target site for the systemic delivery of drug-loaded nanoparticles to avoid first-pass metabolism and drug degradation in the gastrointestinal tract [3-6]. Various natural polymers have been used to improve the cell permeation of buccal drug delivery, including chitosan, alginate, agarose, and gums [7]. Chitosan is a popular polymer due to intrinsic properties, including low toxicity, biocompatibility, biodegradability, mucoadhesion, and membrane permeability [3, 8]. Chitosan facilitates the penetration of drugs, using both transcellular and paracellular transportation through the mucus membrane [4, 9, 10]. Transcellular transport occurs through epithelial cell transcytosis. For the paracellular route, the positive charges on chitosan interact with negatively charged mucus components, resulting in the reorganization of the tight junction, which opens the epithelial junction [10, 11]. However, chitosan not only interacts with negatively charged mucus but hydrophobic and hydrogen bonding are also involved [3]. In addition to enhancing drug penetration, chitosan has also been examined as a potential carrier for targeted drug therapies. The activity of an anticancer preparation (a mixture of fluorouracil and quercetin) against pancreatic cancer cells increased when delivered directly to targeted cells using chitosan nanoparticles. The surface amine groups of chitosan nanoparticles facilitated the quick uptake of both drugs into the cancer cell [12]. Additionally, the cationic chitosan molecules that are used to prepare the nanoparticles can interact with anionically charged small molecules. Several methods have been described to prepare nanoparticles using chitosan, such as ionotropic gelation, coprecipitation, microemulsion, emulsification solvent diffusion, solvent evaporation, and reverse micellar methods [4,13]. Ionotropic gelation is one of the most common techniques for the generation of nanoparticles used for drug incorporation, which

involves an ionic interaction between the positive charges of chitosan and the negative charges of polyanion molecules, such as tripolyphosphate (TPP) [14, 15]. Ionotropic gelation is a simple technique and has many advantages, such as mild conditions, preparation in aqueous environments, and low toxicity [4].

Propranolol, a non-selective- β -adrenergic antagonist, has been used for the treatment of cardiovascular disorders, including hypertension, cardiac arrhythmia, and angina pectoris [16]. The primary problem associated with the oral administration of conventional propranolol tablets is the first-pass metabolism. The absorption of propranolol via the gastrointestinal tract generally occurs rapidly and nearly completely soon after ingesting the drug. However, low bioavailability (25%) has been reported due to high first-pass metabolism [1, 17]. To solve this problem, the nanoparticle-based formulations of propranolol have been developed [1, 18, 19]. Only a few reports have characterized propranolol-loaded nanoparticles. Duangjit et al. reported the effects of different chitosan molecular weights and propranolol concentrations on the physical properties of nanoparticles [19]. However, no comprehensive information is currently available regarding the effects of propranolol concentrations on nanoparticle properties, covering the range of doses that are currently available in commercial tablet forms, and the effects of the initial chitosan solution pH have not been previously reported.

To optimize the nanoparticle formulations, the characteristic properties of nanoparticles should be determined. In this study, we characterized propranolol-loaded chitosan nanoparticles in terms of pH, zeta potential, and nanoparticle size. Additionally, the effects of altering the chitosan solution pH on the nanoparticle properties were also evaluated.

MATERIALS AND METHODS

Materials

Chitosan (MW 20 kDa, 85% degree of deacetylation) was obtained from Seafresh Chitosan Lab. Co (Bangkok, Thailand). Propranolol HCl was purchased from PC drug Co. Ltd. (Bangkok, Thailand). All other chemicals used were of analytical grade and used as received.

Preparation of nanoparticles

Propranolol-loaded chitosan nanoparticles were prepared using the ionotropic gelation technique [19]. Seven concentrations of chitosan solutions [0.150, 0.175, 0.200, 0.225, 0.250, 0.275, and 0.300% (w/v)] were dissolved in a 1% (v/v) acetic acid solution by adding 10 ml of the chitosan solution and continuously stirring, using a magnetic stirrer at room temperature. Different concentrations of propranolol (10, 20, 30, and 40 mg in 1 ml of water) were individually mixed into the chitosan solution. After the mixture was stirred for 5 min, 5 ml 0.1% TPP aqueous solution was added, and the mixture was stirred an additional 5 min to form the nanoparticles. To examine the effects of the chitosan solution pH on the formation of nanoparticles, the pH values of the chitosan solutions (pH 3, 3.5, 4, 4.5, 5, 5.5, and 6) was adjusted using 2.0 N HCl or 2.0 N NaOH, to reach the desired pH, before the addition of propranolol and TPP. Nanoparticles were then investigated with regard to pH, zeta potential, and particle size.

Physicochemical properties the nanoparticles

The pH values of the nanoparticle were measured using a pH meter (Mettler Toledo seven easy, Switzerland). The zeta potentials of the nanoparticles were evaluated using a zeta potential analyzer, Zeta Plus (Brookhaven Instruments Co., New York, NY, USA). The samples were dispersed in distilled water, with gentle stirring, at a volume ratio of approximately 1:50 before the experiment [19]. The particle sizes of the nanoparticles were determined using the dynamic light scattering technique (Horiba, LA-950, Kyoto, Japan), and nanoparticles were dispersed in distilled water, with gentle stirring

before measurement [19]. Each experiment was performed in triplicate.

Statistical analysis

The experiments were performed in triplicate. The data were compared by one-way analysis of variance (ANOVA), using IBM SPSS statistics V26 software. Data were determined to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

Effects of chitosan and propranolol concentrations on the physicochemical properties of nanoparticles

Propranolol-loaded nanoparticles were prepared using the ionotropic gelation technique. The chitosan, containing positively charged amino groups, was cross-linked to the negatively charged TPP. To develop suitable nanoparticles for a buccal propranolol delivery system, the physicochemical properties of various nanoparticle formulas were evaluated. The characterization of the nanoparticles, including pH values, zeta potential, and particle sizes, were performed. The nanoparticle formulations were composed of chitosan at seven concentrations [0.150–0.300 % (w/v)] and five levels of propranolol (0, 10, 20, 30, and 40 mg). The observed pH values of the nanoparticles ranged between 4.14–4.55, as shown in fig. 1. The pH values of the nanoparticles increased in a chitosan concentration-dependent manner. At the same concentrations of chitosan, the pH values of the nanoparticles increased slightly with higher propranolol concentration. Therefore, the pH values were determined to primarily be influenced by the basicity amino moiety of the chitosan.

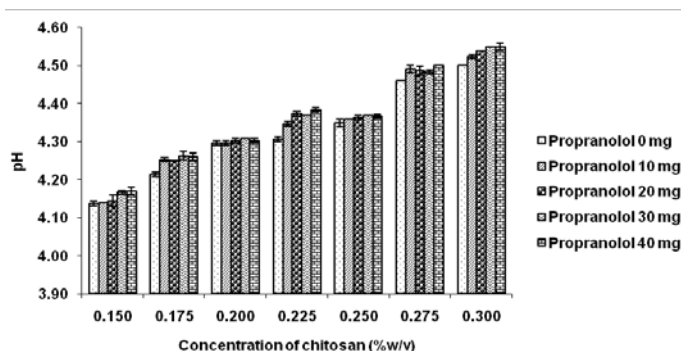


Fig. 1: Effect of chitosan and propranolol concentrations on the pH values of the nanoparticles (mean±SD, n=3)

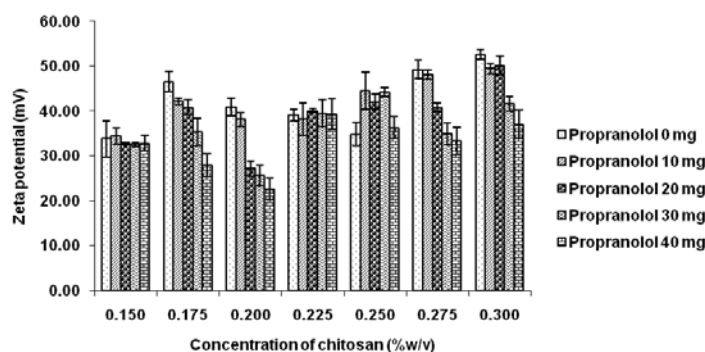


Fig. 2: Effect of chitosan and propranolol concentrations on the zeta potential of the nanoparticles (mean±SD, n=3)

The zeta potential of the nanoparticle reveals the surface potential difference between the dispersion medium and the dispersed particle. Zeta potential is used to determine whether the nanoparticle is stable within the dispersed system, as the surface charge prevents the aggregation of nanoparticles [20]. The zeta potentials of the prepared nanoparticles ranged between 22.6–52.6 mV, with positive charges (fig. 2). Due to the pH effects shown in fig. 1, all nanoparticles were acidic, with the pH values below 4.6. In this condition, the free amine groups of the chitosan, with pKa values of

6.5, were protonated; therefore, they exhibited overall positive charges [21]. The pKa values of chitosan were demonstrated to affect the zeta potential of the nanoparticles [22].

At the same concentration of chitosan, the zeta potential tended to decrease with increasing amounts of propranolol. This result was in accordance with a previous study that reported that decreasing zeta potentials when propranolol was included at concentrations greater than 2-fold the concentration of chitosan [1]. Reduced zeta potential with

additional drug quantities was also observed in anion drug-loaded nanoparticles [10, 23], due to the potential interactions between the cations in the chitosan amino groups and the anion in propranolol. Therefore, the nanoparticle formation with TPP may be disrupted, leading to reduced positive charges on the nanoparticle surface. At fixed propranolol concentrations, the zeta potential fluctuated with changing chitosan concentrations. However, the zeta potentials of nanoparticles containing greater than 0.2% chitosan tended to increase. This result agreed with those reported by Al-Kassas *et al.*, who reported that the zeta potential increased with increased chitosan concentrations, due to the cationic nature of chitosan [1].

The effects of different chitosan and propranolol amounts on the nanoparticle sizes were also observed using the dynamic light scattering

technique. Because smaller drugs are taken into the cells in larger quantities than larger drugs [24], the goal is to develop the smallest particles that can be achieved with good stability. All nanoparticles provided similar particle sizes, which ranged from 105–140 nm (fig. 3). The smallest nanoparticles were established with the preparation containing 0.250% chitosan. Our results were all within the range of optimal nanoparticle size of buccal administration, which has been reported as approximately 100–300 nm [5], and the particle sizes in our study were also smaller than those in previous reports [1, 25]. Although the smallest particles were found in the 0.250% chitosan preparation, the formulas containing 0.200% and 0.225% chitosan were viewed as the optimal concentrations because they produced nanoparticles with consistent sizes, which showed no significant differences regardless of the propranolol concentration.

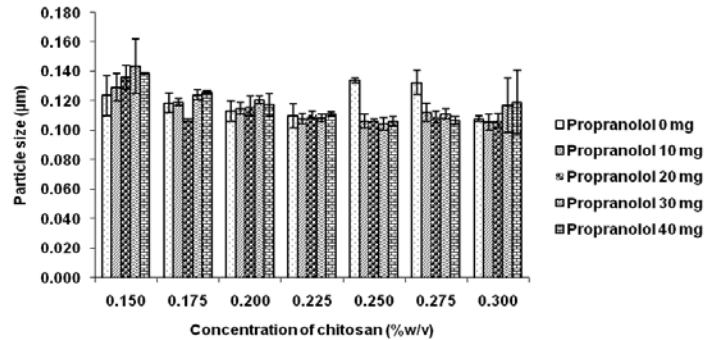


Fig. 3: Effect of chitosan and propranolol concentrations on the particle size of the nanoparticles (mean \pm SD, n=3)

Effect of chitosan solution pH on the physicochemical properties of nanoparticles

Due to the presence of free amino groups throughout the chitosan structure, the pH environment can affect the ionization of these groups, leading to alterations in the ionic crosslinking properties associated with nanoparticle formation. Therefore, the effects of chitosan solution pH prior to nanoparticle preparation were also investigated. The 0.200% (w/v) chitosan solution was chosen, due to particle sizes and zeta potential determinations in a varied pH environment. The chitosan solution pH value was varied, to optimize the nanoparticle stability. The pH values for all nanoparticles ranged from 3.53–6.95, with no differences between formulations with and without propranolol. The pH values of the nanoparticles changed depending on the initial pH value of the chitosan solution, as shown in fig. 4. The zeta potentials of nanoparticles ranged between 7.50–46.90 mV, with the propranolol-loaded samples showing reduced zeta potentials compared with nanoparticles containing no drug (fig. 5). The zeta potential values of the nanoparticles fluctuated with the increased pH of the chitosan solution. However, the addition of the high-pH

chitosan solution tended to decrease the zeta potential. The decline in the zeta potential that is observed with increasing chitosan pH values was in agreement with previous results [26, 27], resulting from the decreased protonation degree on the chitosan structure with increased pH.

The pH value of the initial chitosan solution also affected the nanoparticle size. Altering the pH changes the ionizable groups on the chitosan, which changes the electrical network and the swelling behavior. As shown in fig. 6, the particle sizes of all nanoparticles ranged between 113–681 nm, with the propranolol-loaded nanoparticles being smaller than empty nanoparticles. The particle sizes reduced when the chitosan pH increased from 3.0 to 4.5. At pH values above 4.5, the sizes of the nanoparticles were remarkably larger. These results agree with previously described data [26, 28]. At low chitosan solution pH values, high degrees of protonated amino groups repulse the chitosan molecule, thus causing larger particles. The smallest particle sizes were generated using the pH 4.5 chitosan solutions. Once the pH of chitosan exceeded 4.5, the protonation of the amino group decreased, leading to agglomeration and the generation of larger particles [26, 28].

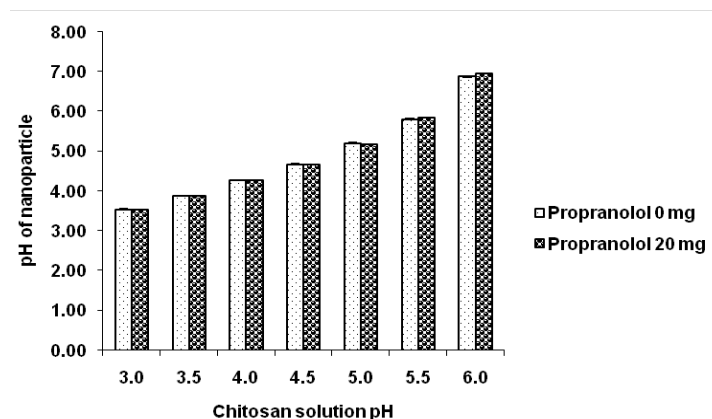


Fig. 4: Effect of chitosan solution pH on pH of the nanoparticles (mean \pm SD, n=3)

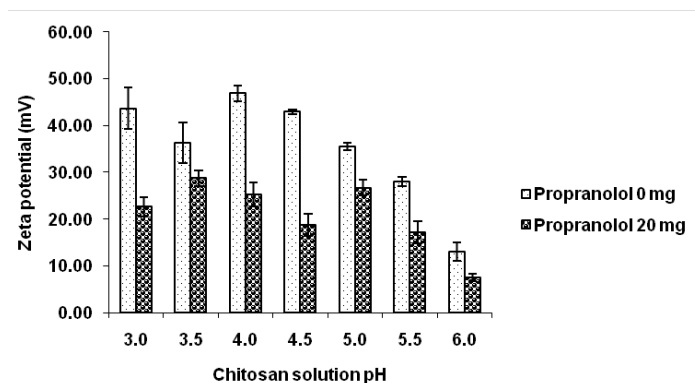


Fig. 5: Effect of chitosan solution pH on the zeta potential of the nanoparticles (mean \pm SD, n=3)

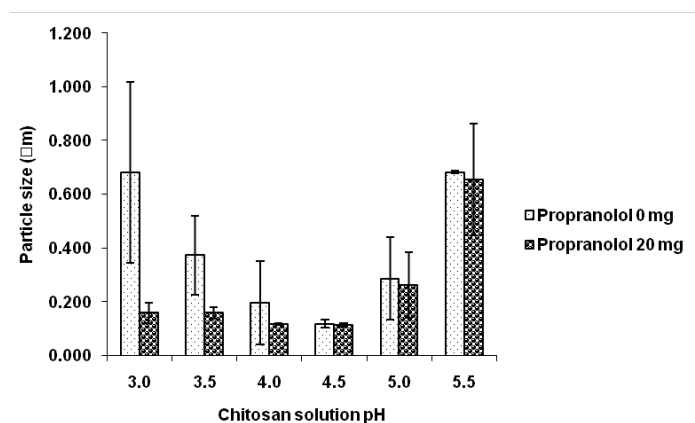


Fig. 6: Effect of chitosan solution pH on the particle size of the nanoparticles (mean \pm SD, n=3)

CONCLUSION

Propranolol-loaded nanoparticles were prepared using the ionic gelation technique, with various concentrations of chitosan and propranolol. The pH, zeta potential, and particle size of the formulations were investigated. The nanoparticle pH varied depending on both the concentration and the pH value of the chitosan solution, whereas the zeta potential depended only on the amount of propranolol. Additionally, the pH of the chitosan solution exhibited effects on nanoparticle size. Therefore, we obtained additional useful information to support the development of a propranolol-HCl transmucosal delivery system. However, drug penetration studies must still be performed to confirm the developed formulations.

ACKNOWLEDGMENT

The author gratefully acknowledges the Thammasat University under the TU Research Scholar, Contract No. TP 2/68/2556 for the financial support. In addition, the author would like to thank Assoc. Prof. Manee Luangtana-Anan, Ph. D. for kindly giving chitosan samples. Grateful thanks also go to Faculty of Pharmacy, Silpakorn University for supporting facilities and equipment.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The author declares no conflict of interest

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