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Original Article

OPTIMIZATION OF DILTIAZEM HYDROCHLORIDE NANOPARTICLES FORMULA AND ITS RELEASE KINETICS EVALUATION

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ABSTRACT

Objective: The purpose of this study was to determine the optimum formula of diltiazem HCl-loaded chitosan nanoparticles due to variations in the speed and duration of stirring and evaluating the release kinetics *in vitro* using DDSolver.

Methods: The method used to prepare nanoparticles is ionic gelation. The ionic gelation method involves an ionic cross-linking between cations on the backbone of chitosan and anion, such as sodium tripolyphosphate (Na TPP).

Results: Stirring speed of 1200 rpm and stirring time of 2 h produce an optimum response. The optimum formula has an entrapment efficiency of 71.10%, a particle size of 110.2 nm, and a polydispersity index of 0.268. The dry powder of diltiazem HCl nanoparticles produced a drug loading of 66.14±1.71% and a yield of 34.07±0.73%. The FT-IR showed ionic interaction (cross-linking) between ammonium ions from chitosan and phosphate ions from Na TPP. Scanning electron microscopy (SEM) analysis showed a particle size of 150 µm, a spherical shape, and rough surface morphology. *In vitro* release profiles indicated prolonged release, which follows the Korsmeyer Peppas model.

Conclusion: It can be concluded that increasing the speed and duration of stirring will improve drug entrapment and reduce the particles size variation. The dry nanoparticles release mechanism is by diffusion and matrix erosion.

Keywords: Diltiazem hydrochloride, Chitosan, Nanoparticles, *In vitro* release kinetics

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INTRODUCTION

Diltiazem HCl has a short half-life (3-4 h) due to rapid elimination from the blood circulation and is available for about 40% [1]. The approach to overcoming the problem of diltiazem HCl is by using a chitosan nanoparticles delivery system. Chitosan is the most applied natural polymer for nanoparticles. Chitosan has an excellent mucoadhesive characteristic and low immunogenicity is non-toxic, biocompatible, and biodegradable [2]. Formulation of Diltiazem HCl loaded into chitosan nanoparticles will increase the residence time of short half-life drugs at the absorption site, control the drug release rate to maximize the therapeutic effect [3], increase intracellular penetration, and enhancing drug absorption [4].

A polymeric nanoparticle delivery system is a colloidal particle with about 10-1000 nm formulated using a polymer matrix used as drug carriers by trapping, dissolving, encapsulating, adsorption, or adhering to the matrix [5]. The ionic gelation technique is a method used to prepare diltiazem-loaded chitosan nanoparticles. Among others methods such as emulsification, coacervation/precipitation, spray-drying, emulsion droplet coalescence method, reverse micellar method, sieving, and ionic gelation methods) the ionic gelation method is simple and could encapsulate a wide range of molecules [6], which occurs through electrostatic interactions between positively charged and negatively charged to form coacervates in nanometer-sized [7]. In this study, we use chitosan as the polymer that reacts with Na TPP to form an electrolyte complex.

The previous study has shown that formulation of diltiazem HCl nanoparticles based on a polyelectrolyte complex between chitosan and natural gum prolonged the release of diltiazem HCl [8]. Controlled release diltiazem HCl was also produced in microparticle preparations using bovine serum albumin [9]. Enhanced release of diltiazem HCl is produced through the formulation of chitosan and Eudragit®RSPM microparticles [10].

The effect of TPP as the crosslinkers on the size, polydispersity, and charge of chitosan nanoparticles have been studied [11]. Besides the crosslinker, the speed and duration of stirring in the manufacture of nanoparticles report affecting the size of particles [12]. Particle properties such as size are essential in the cellular uptake, protein adsorption, and accumulation of the nanoparticles and their distribution throughout the body [13]. Optimum stirring speed and time can reduce particle size and increase entrapment efficiency drug. The speed and duration of stirring that are too low or high can also cause particle size instability and form aggregates. Hence further research is needed to optimize the speed and duration of stirring to produce nanoparticles with good characteristics [14].

The results of the literature study show that no researchers have conducted the same research like this study. Therefore, this study aimed to determine the optimum formula due to variations in the speed and duration of stirring in the preparation of diltiazem HCl-loaded chitosan nanoparticles. Besides that, this study's objective is to evaluate the release kinetics *in vitro* using DDSolver, an Add-In program in excel, to facilitate the modeling of dissolution data in a convenient way to quickly and easily report dissolution data.

MATERIALS AND METHODS

Materials

Materials used in this study were Diltiazem HCl (99,9%) from Supriya Lifescience, Ltd, India. Chitosan (96.24% deacetylation degree, BM 102 KDa) was purchased from CV. Chimultiguna, Cirebon. Na TPP (food grade) from Graha Jaya Chemical, Indonesia. Tween 80 from PT. Brataco Chemika, Indonesia. Glacial acetic acid from Merck, Germany, and all other chemicals was a pharmaceutical grade.

Instruments

The instruments used in this study were PSA (Particle Size Analyzer), Horiba Scientific SZ 100, SEM (TM3000 Hitachi), Spectrophotometer FT-IR (Alpha Bruker), centrifuge (Hermle Labortechnik GmbH, Germany), spectrophotometer UV-Vis (Genesys 10S, Thermo Scientific, USA), oven (Memmert, Germany), mixer four blades propeller (IKA RW 20 digital), pH meter (Elmetron CP-502), hotplate (IKA C-MAG HS4, Germany), vortex mixture (Thermo Scientific), analytical balance (Adventurer TM Ohaus, USA), and Design Expert software version 11.00.

Optimization of the formula

Optimization in this study uses a factorial design with two-factor levels. Four different formulations of diltiazem HCl nanoparticles with different speeds and stirring duration seen in table 1. The formulas are according to Almalik *et al.* (2017) with modification. The speed and duration time was obtained from the preliminary study.

Table 1: Formula components and optimized variables

Formulas code	Diltiazem HCl (0.037%)	Chitosan (0.07%)	Na TPP (0.1%)	Tween 80 (1%)	Stirring speed and duration
(1)	40 mg	90 ml	10 ml	8 ml	700 rpm for 1 h
A	40 mg	90 ml	10 ml	8 ml	1200 rpm for 1 h
B	40 mg	90 ml	10 ml	8 ml	700 rpm for 2 h
AB	40 mg	90 ml	10 ml	8 ml	1200 rpm for 2 h

Preparation diltiazem HCl nanoparticles

Nanoparticles preparation according to [15] with modification. The diltiazem HCl solution in tween 80 was added to 90 ml of chitosan (0.07% w/v) solution during stirring using a magnetic stirrer. The Na TPP solution (0.1%, 10 ml) was added dropwise to the chitosan-diltiazem HCl solution [15].

Entrapment efficiency

Entrapment efficiency was determined by determining levels of free diltiazem HCl in the supernatant using a UV-Vis spectrophotometer at the maximum wavelength (236 nm) [16]. The calculation of entrapment efficiency using the following equation 1.

$$\%EE=\frac{\text{Initial drug added}-\text{free drug content}}{\text{Initial drug added}}\text{x}100\% \ldots\ldots (1)$$

Particle size and polydispersity index

Nanoparticles size and their distribution were analyzed using a Particle Size Analyzer [17].

Determination of the optimum formula

The selection of the optimum formula is determined based on the desirability value that is close to one and meets the response criteria.

Preparation of nanoparticles powder

The optimum formula is centrifugation at a speed of 13000 rpm for 30 min to separate the precipitate. The precipitate was dried using an oven at 60 °C for 24 h to obtain nanoparticles powder.

Drug loading

Nanoparticles powder equivalent to 10 mg of diltiazem HCl dissolves in 50 ml of HCl solution of pH 1.2. The absorbance was then measured using a UV-Vis spectrophotometer at the maximum wavelength (236 nm) [17]. The calculation of drug loading using equation 2.

$$\%\text{Drug Loading} = \frac{\text{Weight of drug adsorbed in nanoparticles}}{\text{Weight of nanoparticles}} \text{ x } 100\% \ldots\ldots\ldots\ldots (2)$$

Yield

The yield was calculated by comparing the amount of nanoparticles powder produced from the preparation with the total ingredients used in the formulation [18]. The yield calculation using equation 3.

$$\%\text{Yield} = \frac{\text{Weight of obtained nanoparticles}}{\text{Total weight of initial drug and polymer}} \text{ x } 100\% \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Fourier-transform infrared spectroscopy (FT-IR)

FT-IR analysis was performed on pure diltiazem HCl, chitosan, Na TPP, and diltiazem HCl nanoparticles powder. The sample scanning at a wavenumber of 4000-400 cm^{-1}. The IR spectra of pure diltiazem HCl were compared with the diltiazem HCl nanoparticles powder [19].

Scanning electron microscope (SEM) analysis

The nanoparticles powder samples were mounted directly on the SEM to obtain digital images of the nanoparticles [14].

***In vitro* dissolution**

A dissolution study of diltiazem HCl nanoparticles carries out using the USP Apparatus 1 (Basket). Diltiazem HCl nanoparticles 25 mg were put into a basket and immersed in a chamber containing 900 ml of HCl buffer solution pH 1.2. The apparatus runs at 100 rpm, and the temperature maintains at 37±0.5 °C. Five milliliters of samples were taken at time intervals of 0.5, 1, 2, 3, 4, 5, 6, 8 h and replaced with the same volume of blank dissolution medium. Drug concentrations were measured using a UV-Vis spectrophotometer at 236 nm [17].

Drug release kinetics

The release model for diltiazem HCl nanoparticles was determined to fit the dissolution data into zero-order, first-order, Higuchi, Hixson Crowell, and Korsmeyer Peppas kinetic equations. The appropriate release kinetics model was determined with the DDSolver program using the non-linear regression approach [20].

RESULTS

Entrapment efficiency is a parameter that indicates the ability of the polymer to trap the drug. The effect of speed, duration of stirring and their interaction on the entrapment efficiency, particles size, and polydispersity was seen in table 2. The software design expert analyzes the entrapment efficiency, particles size, and polydispersity index data to produces the equation 4, 5, and 6.

Entrapment efficiency =+63.41+4.57*A+2.76*B+0.3650*AB ..... (4)

Particles Size =+102, 08+4.08*A+2.98*B+1.07*AB .... (5)

Polydispersity index =+0.3053–0.0327*A–0.0067*B+0.0022*AB . (6)

A is stirring speed

B is stirring time.

Table 2: Physicochemical characteristic of diltiazem HCl nanoparticles

Formulas code	Speed and stirring duration	EE±SD (%)	Particle size (nm)	The polydispersity index (PI)
1	700 rpm 1 h	56.44±0, 51	96.1±31.0	0.347
A	1200 rpm 1 h	64.85±1.06	102.1±72.0	0.277
B	700 rpm 2 h	61.23±0.36	99.9±34.3	0.329
AB	1200 rpm 2 h	71.10±1.03	110.2±30.3	0.268

The FTIR wavenumber of some functional groups of diltiazem HCl ingredients seen in table 3.

Table 3: Functional groups of diltiazem HCl nanoparticles ingredient

Code	Functional groups	Wavenumber (cm^{-1})				
		Theoretical wave number	Diltiazem HCl	Chitosan	Na TPP	Diltiazem HCl nanoparticles
A	O-H stretching	3424		3370.99		
B	-CONH2 (Amida)	1655		1647.56		
C	C=O	1742.1680	1745.09 1680.12			1744.67 1683.48
D	N-H stretching (primer amine)	3415	3312.52	3370.99		3300
E	C-N stretching	1220	1237.90 1217.95			1218.03
F	C-N bending	1610 1532		1607.57		1540.46

The spectra of diltiazem HCl nanoparticles and its components seen in fig. 1.

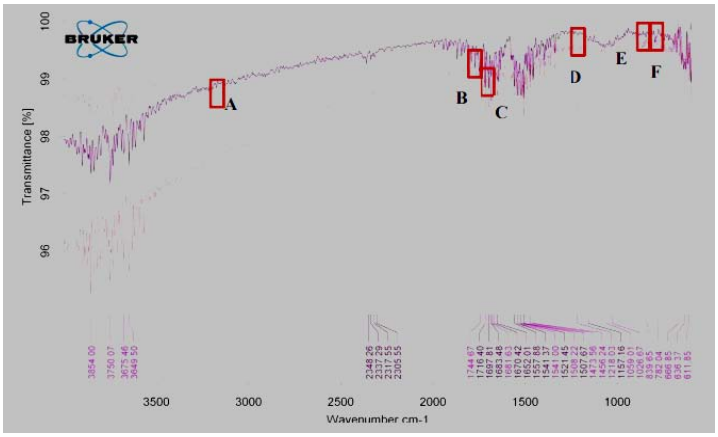

Fig. 1: FTIR spectra of diltiazem HCl (pink), chitosan (green), Na TPP (brown), diltiazem HCl nanoparticles (purple)

The diltiazem HCl nanoparticles have a spherical shape with rough surface morphology (fig. 2).

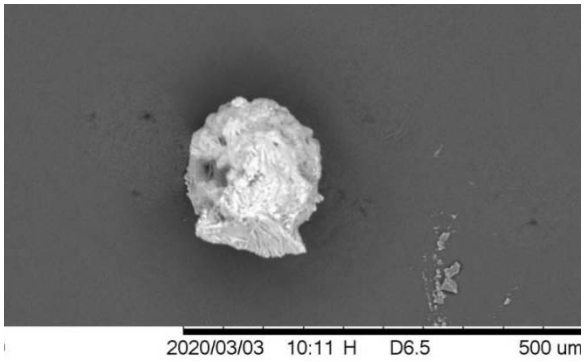

Fig. 2: SEM image of diltiazem HCl nanoparticles

The amount of diltiazem HCl release from nanoparticles after eight h was 73.94% (fig. 3).

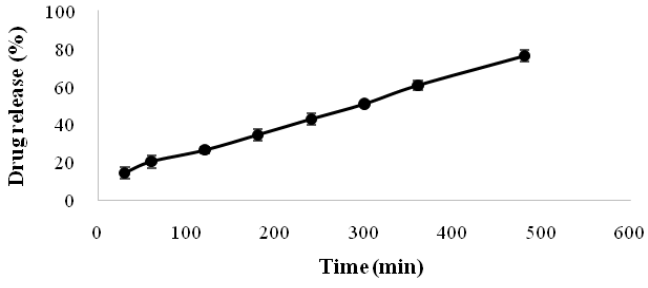

Fig. 3: The release profile of diltiazem HCl nanoparticles drug release kinetics

The most appropriate model selection for release kinetics is base on the highest value of adjusted R2 and MSC and the smallest AIC, MSE, WSS values seen in table 4 [21].

Fig. 4 shows the distribution between the observation results and the prediction. The best model shows the distribution of observation data close to the prediction data.

Table 4: Release statistical kinetics parameters of diltiazem HCl nanoparticles

Parameters	Zero-order	First-order	Higuchi	Hixson crowell	Korsmeyer peppas
R^2adjusted	0.9215	0.9647	0.9526	0.9627	**0.9706**
AIC	54.1315	46.3157	49.7054	47.0442	**45.4396**
MSC	2.3405	3.2089	2.8323	3.1280	**3.3063**
MSE	42.3738	19.3729	25.1264	20.3665	**16.0383**
WSS	338.9903	154.9828	201.0116	162.9323	**112.2680**

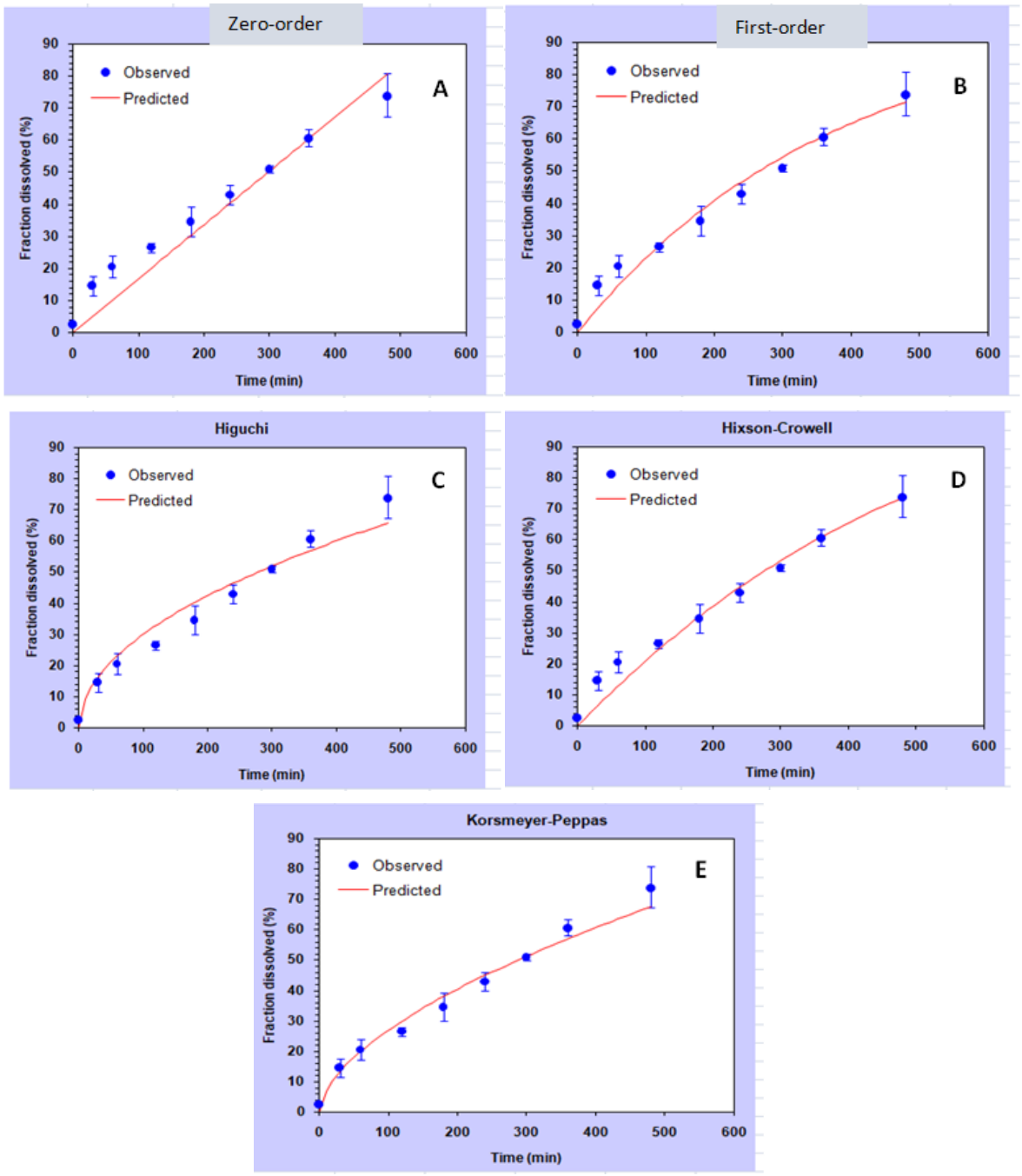

Fig. 4: Release profile of diltiazem HCl nanoparticles against time in the release kinetics model of (A) Zero-order, (B) First-order, (C) Higuchi, (D) Hixson-Crowell, (E) Korsmeyer Peppas

DISCUSSION

The equation 4 shows that the speed, duration of stirring, and interaction increase entrapment efficiency. However, the interaction effect is smaller than the single factor effect on entrapment efficiency. The increasing stirring speed and duration causes high shear forces that accelerate the dispersion process. Therefore, the amount of drug absorbed in the nanoparticles will increase. Nanoparticles with a particle size of 100-200 nm are ideal sizes because they can reduce the elimination of nanoparticles and have a prolonged circulation time, thus prolonging the duration of drug action and increasing targeting efficiency to specific sites [22]. The equation 5 shows that the speed, duration of stirring, and interaction increase the particle size. The interaction effect is smaller than the speed and duration of stirring. Based on [14], increasing stirring speed and duration will reduce particle size. Maximum stirring speed and time can increase particle size because small particle sizes were unstable, hence could form larger particles. On the other hand, the speed and duration of stirring reduce the polydispersity index, while the interaction between the two factors increases the polydispersity index. The interaction effect is smaller than the single effect of the speed and duration of stirring. High stirring speed can form more stable droplets, while slow stirring speed is not enough to form stable droplets so that aggregates will form. The high speed and duration of stirring cause the energy to be distributed well. Hence the molecules were getting the same energy to produce homogenous particle size distribution [23].

The optimum formula produces at a stirring speed of 1200 rpm and two h with the entrapment efficiency of the optimum formula is 71.10 %, a particle size of 110.2 nm, and a polydispersity index of 0.268. The desirability value is 0.843. Drug loading determines how efficiently the formula used in preparation reduces the amount of material/matrix administration [24]. The optimum formula of diltiazem HCl nanoparticles produced a good drug loading value of 66.14% and a yield value of 34.07±0.73 %.

The FT-IR spectra of diltiazem HCl nanoparticles showed a peak shift from wave number 1607.57 cm-1 to 1540.46 cm-1, indicating an amine group (C-NH2) bending of chitosan. A phosphate group (P=O) of Na-TPP is found at a wavenumber of 1157.25 cm-1. The amine group causes the shift in peak in chitosan binding to a phosphate group. This shifting indicates that there has been an ionic interaction (cross-linking) between ammonium ions from chitosan and phosphate ions from Na TPP. The results also show no sharp peak shift in the wavenumber produced in the spectra of diltiazem HCl nanoparticles. Hence, there was no interaction between diltiazem HCl with chitosan and Na-TPP during preparation that could affect the therapeutic effect of diltiazem HCl (fig. 1).

The particles size, shape, and surface characteristics affect the biodistribution, drug release properties [25], penetration to the cell membrane [26], and contact between the particles, which causes aggregation. The results of the SEM analysis showed the particle size on a micrometer scale (150 µm) due to the occurrence of agglomeration after drying. The rough surface is caused by the low viscosity of the polymer, which causes the cross-linking strength to be less intense so that it is easy to shrink.

The diltiazem HCl release from nanoparticles follow the Korsmesyer Peppas (table 4) model with an n (release exponent) value of 0.582. Rosuvastatin calcium nanoparticles are also released from chitosan nanoparticles following Korsmesyer Peppas and could effectively sustain drug release for a prolonged period [27].

The value of n of<0.45 shows the drug release mechanism from spheres follows a Fickian diffusion (Case I transport). If 0.45<n<0.89, then the release mechanism is non-Fickian (anomaly), and if the value of n>0.89 hence drug release is dominated by matrix erosion mechanism (Case II transport) [28]. Hence, the diltiazem HCl release mechanism was non-Fickian diffusion or anomaly (combination of diffusion and matrix erosion). Anomalous drug release mechanisms are characterized by polymer relaxation when enzyme degradation occurs so that the drug trapped in the matrix will come out [29].

CONCLUSION

The present study verified a significant influence of the stirring speed and duration on the mean particle size, drug entrapment efficiency, and size distribution index of diltiazem HCl nanoparticles. Further, the parameters of speed and duration of stirring need to be considered in diltiazem HCl nanoparticles production to optimize diltiazem HCl for the intended pharmaceutical applications. DDSolver fitting releases data of diltiazem HCl loaded chitosan nanoparticles into Korsmeyer-Peppas dissolution models. It can be explained that the release of diltiazem HCl from chitosan nanoparticles is by a combination of diffusion and matrix erosion.

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AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICT OF INTERESTS

Declared none

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