Evaluation of in vitro release kinetics of Capsaicin-loaded chitosan nanoparticles using DDSolver

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**ABSTRACT**
The present aim is to evaluate the release profile and its release kinetics of encapsulated capsaicin from chitosan nanoparticles using the software DDSolver. The release study was performed by using a dialysis technique in PBS solutions with different pHs (1.2, 6.8 and 7.4) to mimic the different gastrointestinal tract and circulatory system pH ranges as a releasing medium. The nanoparticles were prepared using o/w emulsification and ionotropic gelation technique under optimal condition obtained from response surface methodology (RSM) design as described in our previous study. These nanoparticles were around 180 nm in average hydrodynamic size and encapsulation efficiency percentage around 70%, respectively. In vitro drug release study suggested that the chitosan nanoparticles can potentially use to controlled and sustained release of capsaicin over at least 96. The kinetic release analysis results by DDSolver software indicated that Weibull model was suggested to be the best dynamic models with highest $R^2_{adjusted}$ and model selection criteria (MSC) and lowest Akaike information criterion (AIC), respectively, for capsaicin loaded chitosan nanoparticles. The release mechanism of capsaicin from nanoparticles was found to be Fickian diffusion. The results suggest that the chitosan nanoparticles can be applied for the controlled and sustained release of capsaicin in the gastrointestinal tract and circulatory system.

**INTRODUCTION**
Capsaicin (CAP, trans-8-methyl-N-vanillyl-6-nonenamide) is a natural bioactive found in hot peppers. Works of the literature suggested that CAP show a broad spectrum of biological and pharmacological activities such as anticancer, antioxidant, anti-inflammatory, antimutagenic (Porfido et al., 2016) and so on. Among the various advantageous of CAP, poor water solubility, low stability and bioavailability have limited its clinical application (Rollyson et al., 2014). Therefore, the formulation of polymeric nanoparticulated drug delivery system is designed to enhance the bioavailability of CAP.

Polymeric nanoparticles are matrix drug delivery system that can be prepared with natural (i.e., protein, polysaccharides, chitosan, alginate, cellulose and cellulose) or synthetic polymers (i.e., PLA, PLGA, Eudragit, carbopol, poly (vinyl alcohol) with a par-
article in the nano-sized range of 10-1,000 nm (Chan et al., 2010).

The polymeric nanoparticles made by both natural or synthetic polymers are preferred to obtain a controlled and sustained release of drug from improving the life quality of patents (Bolhassani et al., 2014). However, to avoid the toxicity caused by the synthetic polymer, the naturally occurring polymer, especially chitosan (CS), is suited for medical and pharmaceutical applications because of its promising properties including biodegradable, biocompatible, non-toxic, mucoadhesive and so on (Mohammed et al., 2017). Chitosan is a positively charged naturally occurring polysaccharide that is mainly obtained from crustaceous shells; it is considered as non-toxic polymer.

The US FDA has approved it as safe for use in human. CS can quickly occur polyelectrolyte complexation with negatively charged cross-linking agents such as sodium tripolyphosphate (TPP) to form CSNPs which have demonstrated an active drug carrier for delivery and controlled release of several drugs and bioactive compounds (Mohammed et al., 2017). Although, the CSNPs has been extensively used as the nanocarrier for delivery of drugs and bioactives (Garg et al., 2019), the using of CSNPs for controlled release of CAP has not been reported.

CAP has poor aqueous solubility; thus, dissolution is one of the rates limiting step in their absorption and bioavailability (Fattori et al., 2016; Rollyson et al., 2014). Information on the dissolution mechanisms and kinetics is significant to approximate the absorption of CAP-loaded polymeric nanoparticles.

Various mathematical models were established to analyzed releasing data such as the zero-order, first-order, Higuchi, Korsmeyer-Peppas, Weibull and so on. However, these mathematical models need the initial value for each parameter in the mathematic equation and require the user analyzed the equations manually (Zhang et al., 2010).

DD solver is a menu-driven Microsoft Excel add-in program coded in Visual Basic for Applications (VBA), which can effectively be used to analyze data from in vitro drug release/dissolution experiment (Murtaza et al., 2012).

Calculation using Excel provides several advantages over another statistical program such as simplicity of use, extensive availability, and high flexibility (Zhang et al., 2010). Therefore, this study was aimed to investigate the releasing data of CAP-CSNPs using DDSolver. The CAP-CSNPs were prepared under the optimal condition as described in our previous work.

MATERIALS AND METHODS

Materials

Capsaicin (CAP, 8-Methyl-N-vanillyl-trans-6-nonenamide) from Capsicum sp. and sodium tripolyphosphate (TPP) were purchase from Sigma Chemicals Company (St. Louis, MO, USA). Chitosan (CS) with MW 75 kDa with %DD ~ 84% was kindly gifted from Marine Bio Resources company (Samut Sakorn, Thailand). Polysorbate 80 (Tween 80®) was purchased from Thermo Fisher ACROS Organics™ company (Geel, Belgium). Ultrapure water (Type I) was obtained from the Milli-Q® water purifier system (Millipore, France). Dialysis bag with MW-cutoff 35 kDa, Cellu-Sep® T1 was purchased from Membrane Filtration Products company (Texas, USA). All other chemicals and reagents used in this study were purchased from Carlo-Erba reagents company (Val de Reuil, France) in analytical grade and used directly without purification.

Preparation of capsaicin loaded-chitosan nanoparticles

CAP-CSNPs were prepared by o/w emulsification and ionotropic gelation techniques as described in our previous report. (Kulpreechanan and Sarasittiyanarkarn, 2020). First, 1 mL of ethanolic CAP solution (1 mg/mL) was added dropwise to 18 mL chitosan solution (0.11% w/v) containing non-ionic surfactant polysorbate 80 (1.55% w/v) using an automatic syringe pump (NE 100, New Era, Pump System company, USA) with 1,000 rpm stirring using a magnetic stirrer (IKA® C-Mag HS7, Staufen, Germany) at ambient temperature for 30 min. 9 mL of aqueous TPP solution (0.25 mg/mL) was then added to this suspension, and the stirring was maintained for 90 min under ambient temperature. After completion of the reaction, the nanosuspension was
Table 1: Release kinetic modelling of CAP from capsaicin loaded chitosan nanoparticles in PBS (pH 1.2, 6.8 and 7.4)

<table>
<thead>
<tr>
<th>Kinetic models</th>
<th>pH</th>
<th>Parameter</th>
<th>$R^2_{\text{adjusted}}$</th>
<th>AIC</th>
<th>MSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero order (F=kt)</td>
<td>1.2</td>
<td>$k_0 = 1.127$</td>
<td>0.7404</td>
<td>89.8341</td>
<td>0.9152</td>
</tr>
<tr>
<td></td>
<td>6.8</td>
<td>$k_0 = 0.563$</td>
<td>0.6312</td>
<td>78.3464</td>
<td>0.5350</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>$k_0 = 0.351$</td>
<td>0.6481</td>
<td>67.9434</td>
<td>0.4481</td>
</tr>
<tr>
<td>First order F = 100/[1-Exp(-k_t)]</td>
<td>1.2</td>
<td>$k_1 = 0.032$</td>
<td>0.9734</td>
<td>64.7669</td>
<td>3.1941</td>
</tr>
<tr>
<td></td>
<td>6.8</td>
<td>$k_1 = 0.008$</td>
<td>0.7457</td>
<td>74.2573</td>
<td>0.9067</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>$k_1 = 0.004$</td>
<td>0.7118</td>
<td>65.7459</td>
<td>0.6479</td>
</tr>
<tr>
<td>Higuchi F = kH.t^{0.5}</td>
<td>1.2</td>
<td>$k_H = 9.662$</td>
<td>0.9671</td>
<td>67.1240</td>
<td>2.9798</td>
</tr>
<tr>
<td></td>
<td>6.8</td>
<td>$k_H = 4.906$</td>
<td>0.9343</td>
<td>59.3684</td>
<td>2.2603</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>$k_H = 3.055$</td>
<td>0.9286</td>
<td>50.3974</td>
<td>2.0432</td>
</tr>
<tr>
<td>Korsmeyer-Peppas F = k_{KP}.t^n</td>
<td>1.2</td>
<td>$k_{KP} = 10.413$, n = 0.481</td>
<td>0.9642</td>
<td>68.8960</td>
<td>2.8187</td>
</tr>
<tr>
<td></td>
<td>6.8</td>
<td>$k_{KP} = 6.349$, n = 0.435</td>
<td>0.9374</td>
<td>59.6739</td>
<td>2.2325</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>$k_{KP} = 3.744$, n = 0.449</td>
<td>0.9268</td>
<td>51.5058</td>
<td>1.9424</td>
</tr>
<tr>
<td>Hixon-Crowell F = 100/[1-(1-k_{HC}.t)^3]</td>
<td>1.2</td>
<td>$k_{HC} = 0.009$</td>
<td>0.9412</td>
<td>73.4987</td>
<td>2.4003</td>
</tr>
<tr>
<td></td>
<td>6.8</td>
<td>$k_{HC} = 0.002$</td>
<td>0.7096</td>
<td>75.7201</td>
<td>0.7737</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>$k_{HC} = 0.001$</td>
<td>0.6911</td>
<td>66.5080</td>
<td>0.5786</td>
</tr>
<tr>
<td>Weibull F=100.[1-Exp{[(t-T_i)^{3/\alpha}]})</td>
<td>1.2</td>
<td>$\alpha = 11.657$, $\beta = 0.706$, $T_i = 0.703$</td>
<td>0.9966</td>
<td>43.5537</td>
<td>5.1225</td>
</tr>
<tr>
<td></td>
<td>6.8</td>
<td>$\alpha = 10.614$, $\beta = 0.402$, $T_i = 1.715$</td>
<td>0.9741</td>
<td>50.6595</td>
<td>3.0520</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>$\alpha = 16.635$, $\beta = 0.372$, $T_i = 1.902$</td>
<td>0.9720</td>
<td>41.6337</td>
<td>2.8399</td>
</tr>
</tbody>
</table>

$F$ is the fraction percentage of drug release at the time $t$; $k_n$ is the release constant of kinetic model, $n$ is the release exponent use for indicating the drug release mechanism; $\alpha$ is scale parameter, $\beta$ is shape parameter, $T_i$ is location parameter (Papadopoulou et al., 2006). Data shown in table was expressed as the mean ± standard deviation, obtained from three independent samples.

Characterization of nanoparticles

Particle size and polydispersity index (PDI)

The average hydrodynamic size and polydispersity index (PDI) of nanoparticle suspension dispersed in ultrapure water were measured using dynamic light scattering technique (DLS) on the Zetasizer Nano (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK). All measurements were performed in triplicate. Transmission electron microscope (TEM, H-9500, Hitachi High Technology America company, CA, USA) was used to examine the morphology of prepared nanoparticles.

Encapsulation efficiency

The encapsulation efficiency of CAP in CSNPs was then kept in a light-proof cabinet at ambient temperature for 12 h to assure accomplished formation and uniform particles prior analysis.

quantified using an indirect method as described in our previous report (Kulpreechanan and Sorasitthiyanukarn, 2020). After ultracentrifugation of nanosuspension at 45,000 rpm, 4°C for 30 min, the amount of CAP remaining in the supernatant (unloaded of free CAP) was measured using UV-Vis spectrophotometer (Agilent Technologies, CA, USA) at 280 nm as described in our previous report (Kulpreechanan and Sorasitthiyanukarn, 2020). The % EE was then calculated through Equation (1).

\[
\%EE = \left( \frac{\text{Total CAP} - \text{Free CAP}}{\text{Total CAP}} \right) \times 100
\]  

(1)

In vitro drug release study

The CAP release from CSNPs was examined for 96 h using dialysis technique as the protocol described by Cheredy et al. (2013) with minor modifications. The dialysis bag was hydrated and removed, the preservative and other contaminants by 12 h incubation in ultrapure water before proceeding to release experiment in the next step. The nanosuspension (20 mL) were separately transferred into dialysis bag and tied at both ends with sealing clip and then immersed in 150 mL releasing medium at 37°C with at 150 rpm stirring using temperature-controlled, shaking water bath. The releasing medium used in this experiment was PBS solution with three different pHs (1.2, 6.8 and 7.4) containing ethanol (50% v/v) with maintaining sink condition of CAP (Cheredy et al., 2013). Aliquots (2 mL) of the sample was withdrawn at 0, 30, 1, 2, 4, 6, 12, 24, 48, 72 and 96 h, respectively. The fresh releasing medium with an equivalent volume was quickly added after each sampled to maintain a constant total volume of the system. Finally, the cumulative release percentage of CAP from CSNPs was measured using UV-Vis spectrophotometer as described above.

Drug release kinetics

The release kinetics of encapsulated CAP from nanoparticles was determined by fitting the releasing data obtained from in vitro release experiment into different kinetic models available in DDSolver software including zero order, first order, Higuchi model, Korsmeyer-Peppas model, Hixson-Crowell models, and Weibull model, respectively. The best-fitted kinetic model between the releasing data with a kinetic model was selected based on three statistical parameters are R^2 adjusted, MSC and AIC, respectively. Nearest to 1 of R^2 adjusted with maximum MSC and minimum AIC indicates the best-fitted kinetic model (Zhang et al., 2010). Finally, the analysis of release kinetic was performed by DDSolver software.

RESULTS AND DISCUSSION

Characterization of nanoparticles

The average particle size and PDI value of the nanoparticle were 183 ± 23 nm and 0.38 respectively, indicating a narrow size distribution of nanoparticles. TEM image, as shown in Figure 1, indicating that the obtained nanoparticles were spherical with well dispersed and narrow size distribution. The encapsulation efficiency was 75 ± 3 %. All of these results were almost similar with those reported previously (Kulpreechanan and Sorasitthiyanukarn, 2020), which indicated the repeatability and reproducibility of Box-Behnken design (BBD) and response surface methodology (RSM) in design and optimized the preparation method for CAP-CSNPs formulation.

In vitro release study

The in vitro cumulative release percentage of CAP from CAP-CSNPs shown in three different pHs of PBS solution, mimicking the gastrointestinal tract and physiological environment, respectively were shown in Figure 2. In this study, an initial burst drug release was observed for all pH environments which may be due to drug adsorption on the nanoparticle surface, and the sustained release pattern can be attributed to slow drug diffusion and subsequent diffusion in chitosan polymeric matrix. Besides, the pH of the PBS solutions was mainly influenced by the release rate of CAP from CSNP. The cumulative release of CAP from CSNPs over 24 h at pH 1.2, 6.8 and, 7.4 was 93%, 70% and 50%, respectively, which may suggest that the release of CAP from CSNPs at lower pH was faster than neutral or higher pH. This may be due to the well swollen and then dissolve in lower pH of chitosan content int the nanoparticles, resulting in an erosion of nanoparticles matrix and the fast release of CAP.

Evaluation of in vitro kinetics with DDSolver program

As a result of applying in vitro cumulative release study obtained to various kinetic models using DDSolver program, adjusted determination coefficient R^2 adjusted, MSC and AIC found were shown in Table 1. Therefore, the Weibull model was found to be the best fitted kinetic model for CAP release from nanoparticle from all pH. The exponent β value is used as an indicator to investigate the transport mechanism of a drug through the nanoparticle matrix. The value of β ≤ 0.75 indicate Fickian diffusion, 0.75 < β < 1 indicates the combined mechanism of Fickian diffusion and Case II transport and β > 1 indicates the drug transport follows a complex release mechanism (Papadopoulou et al.,
The β in this study was found to be 0.706, 0.402 and 0.372 for pH 1.2, 6.8 and 7.4, respectively, indicates that the CAP release from CSNPs was mainly controlled by Fickian diffusion in all releasing medium.

CONCLUSION

Based on the results, it can be concluded that encapsulation of CAP by CSNPs enhanced after the sustained release of CAP. The in vitro release data evaluated with DDSolver showed that the kinetics release of CAP-CSNPs followed the Weibull model through the Fickian diffusion mechanism.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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REFERENCES


