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Formulation and Characterization of Carbamazepine Chitosan Nanoparticle

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Abstract

Background*: Carbamazepine is an antiepileptic drug used to treat trigeminal neuralgia and pain associated with neurological disorders. The drug belongs to class II of the Biopharmaceutical Classification System (BCS), which has low solubility. Hence, dissolution is a rate-limiting step.* ***Objective****: This study aimed to determine the best formula for carbamazepine nanoparticles based on physical characteristics and determine the effect of chitosan and Na-TPP concentration variation on nanoparticle characterization.* ***Methods****: The carbamazepine chitosan nanoparticles were prepared using ionic gelation method with a concentration of 0.1% w/v carbamazepine and the ratio of chitosan and Na-TPP concentrations of 0.2%:0.1% (F1), 0.2%:0.2% (F2), and 0.3%:0.1% w/v (F3). The parameters evaluated included particle size, polydispersity index, zeta potential, particle morphology, and entrapment efficiency. Statistical analysis was conducted on the evaluation data using One Way ANOVA.* ***Results****: The results showed that the effect of increasing the concentration of chitosan reduced particle size ($p<0.05$), increased zeta potential ($p<0.05$), and had no effect on the value of entrapment efficiency ($p>0.05$). Furthermore, F3 had a particle size of 169.8±13.71 nm with a polydispersity index of 0.378±0.02, the zeta potential of +28.80±2.44 mV, entrapment efficiency of 84.3±7.50%, and spheric particle morphology which was measured using Transmission Electron Microscope (TEM).* ***Conclusion****: Therefore, F3 with the ratio of chitosan and Na-TPP concentrations of 0.3%:0.1% was the formula that provided the best characteristics of chitosan carbamazepine nanoparticles.*

Keywords*: carbamazepine, chitosan, epilepsy, nanoparticle*

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INTRODUCTION

Carbamazepine is an effective anticonvulsant drug that controls the grand mal and psychomotor convulsions (Zafrul & Halim, 2014). However, the drug's weakness is that it is practically insoluble in water (113 µg/mL at 25^{o}C) (Sethia & Squillante, 2002). Therefore, carbamazepine is categorized in the Biopharmaceutical Classification System (BCS) class II, which indicates a slow dissolution rate and high membrane permeability (Nair et al., 2012). The factor that causes its practically non-dissolved nature in water is due to the existence of an aromatic group, double bond, and hydrophobic long hydrocarbon chain (Wardiyah, 2016). Meanwhile, a drug that is difficult to dissolve in water, especially an oral drug, has its absorption controlled by the dissolution rate in the gastrointestinal tract (Nair et al., 2012).

The use of nanoparticle technology is a promising approach to increasing carbamazepine solubility. Moreover, reducing the particle size into the nanometer range increases the contact width of the compound surface with the medium, increasing solubility, dissolution rate, and drug permeability due to the increasing penetration capacity into the cell (Martien et al., 2012).

In this study, chitosan, a chitin derivative polysaccharide was used as the polymer. It is often used as a polymer in the formation of nanoparticles due to its nontoxic, mucoadhesive, biodegradable, biocompatible, and hydrophilic nature. The amino group in chitosan creates uniqueness in its structural aspect compared to other polysaccharides, which gives it a cationic character. This character incurs a strong electrostatic interaction between chitosan and anionic drug. Therefore, its usage is very good within the drug transport system (Harahap, 2012).

One method to synthesize chitosan nanoparticles is through ionic gelation, which reduces the particle to nanometer size. The principle is based on the electrostatic interaction between the positive amino group (-NH_2) in chitosan and the negative charge group of the polyanion, namely natrium tripolyphosphate (Na-TPP) (Harahap, 2012). Moreover, Na-TPP has superiorities as the cross-linking material is not poisonous, affordable in price, stable, and has more negative charges than other polyanions, which causes stronger interaction (Marrisa, 2017). The prevalence of this ionic gelation method, among other methods, includes its simplicity, ease to obtain, and no heating involved in the process (Irianto & Muljanah, 2011). Meanwhile, azithromycin with a carbonyl group similar to carbamazepine is successfully bound with an amino group in chitosan to form azithromycin chitosan nanoparticles. These nanoparticles have a size of 172.9 nm (PDI 0.265) and a zeta potential of +41.3 mV, which is obtained by mixing 0.1% of chitosan and 0.1% of Na-TPP (Mannuela, 2016). Therefore, carbamazepine has the potential to be formulated into nanoparticles. A previous study by Mardliyati et al., (2012) formulated nanoparticles without active substance and the particle size obtained was less than 100 nm with chitosan concentrations Na-TPP of 0.2%:0.1%. Hence, the ratio of concentrations of chitosan and Na-TPP will determine the success of nanoparticles formed by the ionic gelation method.

In this study, the formulation of carbamazepine nanoparticles was carried out using the ionic gelation method by varying the concentrations of chitosan and Na-TPP. The best formula was obtained by examining the characteristic results, which include the smallest particle size below 1000 nm, polydispersity index less than 0.5, biggest zeta potential of approximately ±30 mV, and highest adsorption efficiency evaluation above 60%.

MATERIALS AND METHODS

Materials

The materials used include carbamazepine pharmaceutical grade (MCF-Zhejiang Jiu, China), pro-analyzed (p.a) grade low molecular weight chitosan with a deacetylation degree of 83% and viscosity of 108 Cps (Sigma-Aldrich-Elo Karsa Utama), Na-TPP p.a (Sigma-Aldrich-Elo Karsa Utama), aquadest, acetate acid glacial (Merck), aqua demineralization, and methanol p.a (Merck).

Tools

The tools used include Particle Size Analyzer (PSA) (Malvern Instrument) and UV spectrophotometer (Shimadzu UV-1900).

Methods

Preparation of chitosan and Na-TPP solutions

The chitosan solutions were prepared by dissolving 50 mg or 75 mg of chitosan in 25 mL of 0.5% v/v acid acetate solution using the magnetic stirrer (LabTech) for 10 minutes to obtain two different concentrations of chitosan, which is 0.2% b/v and 0.3% b/v. Acetic acid 0,5% solution was prepared by diluting 0.5 mL acetic acid glacial in approximately 100.0 mL of aquadest (Iswandana et al., 2013). Furthermore, the Na-TPP solution concentrations of 0.1% b/v and 0.2% b/v were

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prepared by dissolving each of the 10 mg and 20 mg of Na-TPP in a 10mL measuring flask of aquadest (Iswandana et al., 2013).

Preparation of carbamazepine chitosan nanoparticles

Carbamazepine of 12.5 mg was dissolved with 12.5 mL methanol to obtain a 1 mg/mL concentration. It was added to 25 mL chitosan solution and stirred at 350 rpm using a magnetic stirrer (LabTech). A total of 5 mL Na-TPP was dripped into the mixture of chitosan-carbamazepine at room temperature of (±25°C) and stirred for 10 minutes at 350 rpm using the magnetic stirrer (LabTech) (Mannuela, 2016), to obtain a total dispersion volume of 42.5 mL nanoparticles. Meanwhile, the formula of carbamazepine chitosan nanoparticles is shown in Table 1.

The carbamazepine nanoparticle suspensions were then subjected to evaporation using a water bath at a temperature of 40ºC for 24 hours to eliminate the methanol. Furthermore, freeze-drying (VirTis benchtop K 2KBTXL-75) was conducted to obtain the powder of carbamazepine chitosan nanoparticles (Mannuela, 2016). Before the freeze-drying, the transmittance percentage was measured using a UV spectrophotometer (Shimadzu UV-1900) at a wavelength of 650 nm and a blank containing distilled water to ensure the clear solution had a particle of nanometer size. When the transmittance was above 90%, a particle size within the nanometer range was achieved (Huda & Wahyuningsih, 2016).

Characterization of carbamazepine chitosan nanoparticles

Particle size and polydispersity index

The particle size was analyzed with Particle Size Analyzer (PSA) (Malvern Instrument) using the technique of Dynamic Light Scattering (DLS) (Rasmussen et al., 2020). The parameters analyzed were average particle diameter and polydispersity index. Subsequently, the sample of nanoparticle powder was diluted with deionized aqua (Jazayeri et al., 2016) using the ratio of 4:5 b/v before being analyzed.

Zeta potential

Zeta potential was measured based on Laser Doppler Electrophoresis (LDE) method using the Zeta Sizer tool similar to Particle Size Analyzer (Malvern Instrument) (Rasmussen et al., 2020). Also, the potential can be measured by determining the particle speed and charge in the electricity field. The analysis of Zeta potential was carried out by dissolving the powders of carbamazepine chitosan with deionized aqua (Jazayeri et al., 2016) under the ratio of 4:5.

Morphology

The particle morphology was determined using Transmission Electron Microscopy (TEM) (JEM-1400) by dripping the sample of 10 µL into the grid and holding it for 1 minute. The residue volume within the grid was absorbed with filter paper and dried for 30 minutes before it was finally observed (Wulandari & Nugroho, 2020). This assay was done on the best formulation of carbamazepine nanoparticles (F3).

Entrapment efficiency

The entrapment efficiency was determined by measuring the concentration of free carbamazepine in the dispersion medium. Subsequently, 10 mg of the carbamazepine nanoparticle powder was dispersed into the 10 mL methanol and kept overnight before being centrifuged at 5000 rpm for 10 minutes. The supernatant was further filtered with a 0.2 µm membrane filter and analyzed with a UV spectrophotometer (Shimadzu UV-1900) at 280.4 nm, and the result obtained was considered a free drug (Arya et al., 2015). Whilst the total drug was calculated as the initial amount of carbamazepine added in the formula (12.5 mg). The percentage (%) of entrapment efficiency is calculated using Equation 1 below.

$$\text{Entrament Efficiency} = \frac{\text{total drug} - \text{Free drug}}{\text{Total drug}} \text{ x } 100\% \quad \ldots\ldots \quad (1)$$

Table 1. Formula of carbamazepine chitosan nanoparticles

Formula (F)	Carbamazepine (% b/v)	Chitosan (% b/v)	Na-TPP (% b/v)	The actual amount in formulation (mg)		Weight ratio Chitosan: Na-TPP (w/w)
				Chitosan	Na-TPP	
1	0.1	0.2	0.1	5	0.5	10:1
2	0.1	0.2	0.2	5	1	5:1
3	0.1	0.3	0.1	7.5	0.5	15:1

Volume ratio of Chitosan : Na-TPP is 5:1

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Data analysis

The data analysis was based on the characterization results of carbamazepine chitosan nanoparticles of F1, F2, and F3. The formula which fitting the ideal characteristic of nanoparticles, such as having the smallest particle size (<1000 nm), polydispersity index of less than 0.5, the zeta potential of approximately ±30 mV, the highest entrapment efficiency value above 60% and a spherical morphology was considered as best formula in this study. (Mannuela, 2016). Furthermore, the characterization results in particle size, zeta potential, and entrapment efficiency of all formulas were analyzed statistically using SPSS 25.0 under normality and homogeneity tests to evaluate the effect of concentration variation between chitosan and Na-TPP in the nanoparticle formation. When the data were homogenously ($p \geqslant 0.05$) and normally distributed ($p \geqslant 0.05$), the statistics test was continued with One Way ANOVA and Post Hoc, and when the data were not, Kruskal-Wallis and Mann Whitney non-parametric tests are to be conducted. Meanwhile, the data have significantly different meaning in statistics when parametric or non-parametric tests gives 0.05.

RESULTS AND DISCUSSION

The first observation was carried out against the maximum concentration of carbamazepine which can be dissolved in methanol. Moreover, all materials, such as chitosan, Na-TPP, and carbamazepine, must dissolve in their respective solvents (Wu et al., 2005). The observation of the solubility of carbamazepine in methanol was carried out by visual selection to produce a clear solution without any hovering particle. Based on the observation, the maximum concentration that sufficiently dissolved carbamazepine within methanol was the carbamazepine concentration of 0.1% b/v. Also, low viscosity chitosan is a weak base (pKa of 6.5) therefore, it was well-dissolved in an acid solution having a pH value of less than 6 (Mannuela, 2016). In the acidic pH, the amino group of chitosan ($-NH_2$) is protonated, causing chitosan to be a polycationic ($-NH_3^+$) molecule that can dissolve in water and interact through ionic bonding with the anionic drug. However, when the pH was more than 6, the amino group in chitosan was deprotonated and unable to form a crosslinking with other counter ions (Harahap, 2012). In addition, Na-TPP was well-dissolved in the aquadest because, in neutral pH (pH above the value of pKa), it is ionized into polyphosphoric ion ($P_3O_{10}^{5-}$) and natrium ion (Na+). Subsequently, the polyphosphoric ions ($P_3O_{10}^{5-}$) of TPP interact with the cationic group of chitosan (NH_3^+) (Lam et al., 2006).

Carbamazepine chitosan nanoparticle formation via the ionic gelation method involved a cross-link reaction between Na-TPP (polyanionic), chitosan (polycationic), and drug (Iswandana et al., 2013). The interaction between carbamazepine (a drug used in this study), chitosan, and Na-TPP forming a nanoparticle, is illustrated in Figure 6. The carbamazepine entrapment between chitosan molecules was due to the interaction between the carbonyl group of carbamazepine and the NH_3^+ group of chitosan. Higher electronegativity of atom O than C in the carbonyl group induce the resonance within the C-O covalent bond, which displaces the one pair electron onto atom O to form an intermediate with a negative charge on oxygen atom (C□□-O□□□□□ (Dwiyanti, 2014). Subsequently, the cation amino in chitosan bind with O- atom in the carbamazepine carbonyl group via ionic interaction. The formation of nanoparticles is then completed by inter- and intra-molecular cross-linkage between the chitosan and Na-TPP molecule, which further entrapped carbamazepine inside the particle (Figure 1).

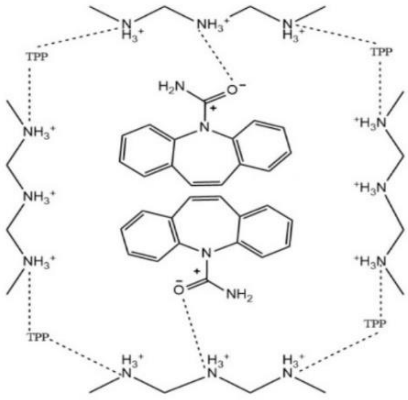

Figure 1. Illustration of chemical interactions formed between carbamazepine, chitosan, and TPP

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The preliminary study was conducted to determine the stirring speed and time in preparing carbamazepine chitosan nanoparticles. Based on the results (not published), the best stirring condition was at 350 rpm for 10 minutes, based on the formation of the clear suspension without any hovering particle. According to Apriliyati (Apriliyati et al., 2020), the stirring speed and time affect the particle size and distribution of the nanoparticles. A longer stirring period leads to smaller particle size. However, too long and quick stirring increases the particle size and distribution. A previous study stated that at a higher stirring speed and time, a turbid unclean solution is formed. Therefore, the transmittance percentage value becomes smaller, and the particle size becomes bigger (Abdassah, 2017). According to Fan et al., (2012), the increasing stirring speed accelerates the dispersion of Na-TPP, which affects the shifting power of chitosan and causes the broken interparticle bond-forming aggregation. Therefore, stirring with optimum speed and time makes the formed particle more uniform and equal (Syaputra et al., 2020). Meanwhile, the stirring needs to be maintained to avoid air bubbles within the solution which can inhibit the interaction of the chitosan amino group and polyanion of Na-TPP during the formation process of nanoparticles (Kurniawan, 2012).

Before drying the dispersed particle, a preliminary test based on the measurement of the transmittance percentage was conducted using a UV spectrophotometer to view the solution clarity. A target for good nanoparticle dispersion clarity was the transmittance percentage of over 90% (Huda & Wahyuningsih, 2016), and all formulae met the target. Results of transmittance percentage values of the three formulae were not significantly different ($p>0.05$). These results failed to provide any information because it was on the preliminary test to estimate the size within the nanometer range and could not precisely identify the particle size. Therefore, the particle size was further analyzed using the Particle Size Analyzer (PSA).

The transmittance percentage was similar to the particle size since it was affected by the Brownian Motion, which causes the particles to collide in an irregular direction. Similarly, it is defined as the repulsion motion of inter-particles with the same charge. This Brownian Motion can also maintain the colloid stability against nanoparticles to avoid easy precipitation. Moreover, lesser Brownian Motion occurs due to larger particle sizes that combine to become aggregate and precipitate easily (Abdassah, 2017; Martien et al., 2012). This process causes the transmittance percentage value to become smaller. Visually, the dispersion of nanoparticles appeared transparent and clear (Figure 2), and none of F1, F2, and F3 hovered. The dry powder from freeze-drying also became white, light like cotton (Figure 2), and without odor.

Figure 2. Carbamazepine chitosan nanoparticle in the form of dispersion (a), and powder (b)

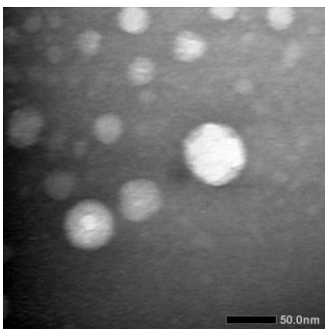

Figure 3. TEM image of carbamazepine chitosan nanoparticles at 80000x magnifications

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Table 2. Characterization result of carbamazepine chitosan nanoparticles

Formula (Chitosan: Na-TPP)	Weight ratio Chitosan: Na-TPP (w/w)	Particle size (nm)	Polydispersity index	Zeta potential (mV)	Entrapment efficiency (%)
F1 (0.2%:0.1%)	10:1	422.4±39.22	0.512±0.07	+7.76±2.03	88.00±1.73
F2 (0.2%:0.2%)	5:1	233.5±36.43	0.353±0.03	+17.37±2.67	89.00±2.64
F3 (0.3%:0.1%)	15:1	169.8±13.71	0.378±0.02	+28.82±2.44	84.30±7.50

Particle size and polydispersity index

From Table 2, the particle size results of all formulas were categorized as nanoparticles because they fall within the range of 10-1000 nm (Laili et al., 2014). The increased concentration of chitosan often produced the bigger the particle size (Mannuela 2016). However, based on Table 2, the higher concentrations of chitosan and NA-TPP produced a smaller particle size ($p<0.05$). This may be due to when the chitosan concentration was increased, not all chitosan molecules are dissolved to form a linear long chain. Some were still in a coiled state (a smaller and compact form). Therefore, a smaller particle was formed when TPP interacted with the polymer still in the coiled state. In low concentrations of chitosan, such as in F1, the polymer was still in the position of a dilute regime (the linear long-chain state), which causes it to move freely. Therefore, it is difficult for the TPP molecule to interact with chitosan, which leads to higher size (Sreekumar et al., 2018). This finding was supported by the study of Cai (2017), which stated that the size of chitosan nanoparticles not only depends on chitosan concentration but also on the concentration of counterions. If the concentration or weight ratio between the counterions to chitosan was low, crosslinking between polymers is not optimal, which results in a more loose density between two polymers, increasing the size of the particles.

Meanwhile, the higher the counterions to chitosan ratio tends to generate smaller particles due to a higher crosslink density. However, if the concentration of TPP is excessive, this condition can cause aggregation between two chitosan particles resulting in a much bigger size. Therefore, an optimization in the ratio of counterions to chitosan is needed.

The polydispersity index results in Table 2 showed that only F2 and F3 have a value less than 0.5. Therefore, it can be concluded that F2 and F3 formulations resulted in a homogenous particle size because the lower value of PDI signifies a narrower size distribution of the particles. A homogenous size of particles in nanoparticle formation is needed to maintain physical stability by preventing the particle from aggregating (Mardliyati et al., 2012). F3 was the best formula from these two parameters because it has the smallest yet homogenous size of the particles. Thus, it can be easily absorbed in the administration site due to the increase in contact surface area. Smaller particles tend to have a higher solubility and a faster dissolution rate. Oxcarbazepine, an anti-epileptic drug encapsulated into nanoparticles (with the size of 170 nm) was proven to successfully penetrate the Blood-Brain Barrier (BBB) (Lopalco et al., 2015) in the same manner as carbamazepine.

Similarly, the nanoparticle of zaleplon also had a better anti-epileptic effect than free drugs with nanoparticle sizes closer to 200 nm (Haggag et al., 2021). In this study, F3 nanoparticles having a size of 169.8±13.71 nm is the potential to be used as a vehicle to transport carbamazepine and other epileptic drugs to the target site via oral administration. However, the carbamazepine nanoparticle should be further tested in vitro and in vivo to determine its performance.

Zeta potential

Zeta potential is a parameter that can be used to predict the nanoparticle stability-based resultant in inter-particle charge, which affects the interparticle repulsion (Mannuela, 2016). The zeta potential is an electrostatic potential that exists on the nanoparticle surface (Mannuela, 2016). Based on the result shown in Table 2, a higher concentration of chitosan concentration increased the zeta potential value to approximately +30 mV ($p<0.05$. A previous study stated that nanoparticle which has zeta potential value closer to or higher than ±30 mV is stable, thus preventing further aggregation and flocculation (Mohanraj & Chen, 2006). This is because the repulsion force is bigger than the attraction force, which leads to an increase in its physical stability (Fitri et al., 2019). Since F3 has the highest value of zeta potential, therefore, it was assumed that F3 has the best physical stability.

Moreover, the value of zeta potential was affected by the chitosan concentration. Zeta potential could have a positive or negative value based on the net charge of the polymer used in the formation of the nanoparticles.

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In this study, all the formulas resulting a positive zeta potential due to the positive charge of chitosan (Arya et al., 2015). A positive charge of zeta potential is beneficial in the drug's transport through the membrane because it facilitates the interaction with a negative charge of the cell membrane and increases its contact length (Bernkop-Schnürch, 2005). The average size and PDI of the resulting nanoparticles also influenced the value of zeta potential. As seen in Table 2, formulas with lower nanoparticles have higher zeta potential. This was due to the distance between two particles being far enough to prevent aggregation, as smaller particles dispersed better in the medium. On the contrary, the bigger particle will have a narrow inter-particle distance, which induces attraction and low zeta potential value (Bernkop-Schnürch, 2005).

Entrapment efficiency

The entrapment efficiency describes the percentage of carbamazepine entrapped into a nanoparticle. According to Mannuela (2016), an ideal nanoparticle should have an entrapment efficiency value ≥60%. From the result shown in Table 2, all formulas have an entrapment efficiency of ≥60%. All three formulas have entrapment efficiency values between 84-89%, but it is not significantly different in statistics ($p>0.05$).

Selection of best formula

Table 2 showed that among the three formulas, F3 produced the smallest particle size of 169.8±13.71 nm, with a good polydispersity index of 0.378±0.02, the highest value of zeta potential (28.80±2.44 mV), and good entrapment efficiency (84.3±7.50%). Therefore, this showed the weight ratio between chitosan and NA-TPP of 15:1 was the optimal condition to produce nanoparticles of carbamazepine in this study. F3 was further subjected to morphology analysis using TEM.

Morphology of carbamazepine chitosan nanoparticles

The analysis result of the TEM morphology of carbamazepine chitosan (F3) is shown in Figure 3. Based on the result, the particle resulting from the F3 formula have spherical-shaped with varied sizes. Most of the particles have sizes lower than 50 nm. A spherical-shaped particle could easily penetrate the cellular membrane, t. Therefore, its penetration was better than that of non-spherical (Martien et al., 2012; Wissing et al., 2004).

Contrary to this result, analysis of the particle using another instrument (Malvern zeta sizer) indicate a formation of the bigger particle but the monodisperse distribution of the particle (PDI <0.5). This was due to a difference in the size determination method in the two instruments. The particle size from a zetasizer was calculated from the hydrodynamic radius of the particle and cumulant average size of all of the particle population, which was also affected by the shape of the particle. However, TEM measurement only shows an image from a tiny portion of the sample which cannot reflect all the particles in the sample. Another reason is that PSA measured the distribution of the dispersion and its reduction. Therefore, its hydrodynamic coverage became broader and produced a bigger particle size, while TEM showed the actual size of the nanoparticle without weighing on the influence of the medium. Therefore a much smaller size is produced (Sulistiawaty et al., 2015).

CONCLUSION

Based on this study, the increase in chitosan concentration affected the decrease in the particle size ($p<0.05$) and the increase in the value of zeta potential (<0.05), but did not influence the entrapment efficiency value ($p>0.05$). Therefore, the best formula is from the ratio of chitosan and NA-TPP concentrations of 0.3%:0.1% b/v (F3), with the particle size of 169.8±13.71 nm, polydispersity index of 0.353±0.03, a zeta potential of +28.82±2.44 mV, entrapment efficiency of 89±2.64%, and spherical particle morphology.

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AUTHOR CONTRIBUTIONS

Conceptualization, C.A.E.; Software, A.N.Z.; Methodology, C.A.E.; Validation, C.A.E., A.N.Z.; Formal Analysis, C.A.E., A.N.Z.; Investigation, C.A.E., A.N.Z.; Resources, C.A.E., A.N.Z.; Data Curation, C.A.E., A.N.Z.; Writing - Original Draft, C.A.E., A.N.Z.; Writing - Review & Editing, C.A.E., A.N.Z., W.P., D.A.A.; Visualization, C.A.E., A.N.Z.; Supervision, C.A.E., A.N.Z.; Project Administration, C.A.E., A.N.Z.; Funding Acquisition, C.A.E.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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