

Sodium Triphosphate Effect on Encapsulation of Vitamin B₆ into Chitosan-Alginate Nanoparticles and Its *In Vitro* Drug Release Study

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Abstract: The in-vitro drug release study of vitamin B₆ encapsulated into sodium tripolyphosphate crosslinked chitosan-alginate (B₆-TCA) nanoparticles aims to determine the effect of sodium tripolyphosphate on the encapsulation efficiency of vitamin B₆ and effectiveness of the nanoparticles to release vitamin B₆. The focus of this research is synthesizing and characterizing TCA nanoparticles to encapsulate vitamin B₆ as an effective delivery system by studying the kinetics release of vitamin B₆. The research resulted in the formation of coarse solid powder nanoparticles in yellowish-white color with a nanoparticle size of 22.55 nm. Sodium tripolyphosphate decreased the percentage of encapsulation efficiency in the B₆-TCA nanoparticles as its concentration increased. However, the increasing sodium tripolyphosphate causes a slower release of vitamin B₆ from nanoparticles. The encapsulation efficiency of vitamin B₆ is 82.04%. The optimum composition of B₆-TCA nanoparticles ratio is 2:1:1.5:2, where Korsmeyer-Peppas kinetics model suited its better with the Fickian diffusion mechanism of 0.989 and has the smallest reaction rate constant of 0.039 occurred within 6 h.

Keywords: alginate; chitosan; nanoparticles; vitamin B₆; sodium tripolyphosphate

■ INTRODUCTION

Vitamins are needed by the human body, and play a significant role in the growth and maintenance of the body. Vitamins are defined as organic compounds needed by the body in tiny amounts for growth and maintaining the body's metabolism [1]. There are "fat-soluble" and "water-soluble" vitamins based on how they are absorbed, stored, and removed from the body [2]. Fat-soluble vitamins have a broader range of physiological functions than water-soluble vitamins [3]. They play a crucial role in the muscles and heart function, immune system, easy flow and clotting of blood, and eye health [2-3]. The human body cannot produce vitamins except vitamins D and B₃. This means that most vitamins must be obtained from outside the human body [1].

Vitamin B₆ is a water-soluble vitamin. In the body, this vitamin plays a role in the amino acids, carbohydrates and fats metabolism. Apart from that, vitamin B₆ is also responsible for synthesizing neurotransmitters in brain cells and helps the production of serotonin and

norepinephrine hormones, which influence mood. Vitamin B₆, along with vitamin B₁₂ and folic acid, help to control homocysteine levels in the blood [4]. Vitamin B₆ takes a part as a coenzyme in over 150 biochemical reactions and makes it a vital molecule in most changes in the human body. It is active in the metabolic processes of carbohydrates, lipids, amino acids, and nucleic acids, and participates in cellular signaling. Vitamin B₆ is an antioxidant with the ability to reduce the advanced glycation end product (AGE) levels which is associated with diabetes, heart disease, cancer, or the prognosis of COVID-19 [5], anemia, skin rashes, depression, and weak immune system. Vitamin B₆ is often combined with vitamins B₁ and B₁₂ as neurotrophic vitamins. The combination of vitamins B₁ and B₁₂ will improve the nervous system. This illustrates the importance of obtaining enough vitamin B₆ in the human body.

Vitamins have important physiological functions, including being anti-inflammatory, immunoregulatory, antioxidative, etc. Unfortunately, the chemical

structures of vitamins are extremely sensitive to light exposure, high temperatures, oxygen, and extreme pH levels [6]. The crucial role of vitamin B₆ in maintaining body health based on scientific literature led to the conclusion that vitamin B₆ is a vital molecule, and its role cannot be overestimated. Therefore, it is necessary to find a way to load vitamin B₆ and make it more stable and controlled during its delivery in the human body. One of the ways is to load vitamin B₆ into nanoparticles.

The choice of material as a polymer matrix must be considered because the material must have biodegradable, edible, and non-toxic properties. Alginates consist of (1,4) connected β -d-mannuronic and α -l-guluronic acids, each in pyranose conformation, organized in homogeneous (MM or GG) and heterogeneous (MG or GM) blocks [7-8]. The COO⁻ groups of guluronic acids from different chains strongly bind with divalent cations such as calcium. This interaction forms a water-insoluble and thermally reversible three-dimensional lattice called "egg box" [9]. Alginate has the disadvantage that it easily releases active compounds which makes it unsuitable for active compound release purposes [10].

One of the biopolymers suitable for alginate coatings is chitosan. Chitosan consists of D-glucosamine and N-acetyl-D-glucosamine copolymer [11] and includes a positive ionic charge. These properties allow chitosan to interact with negatively charged substances such as lipids, cholesterol, fat, proteins, ions, etc. Moreover, chitosan stands out for its biodegradability, bioavailability, adsorption properties, and non-toxicity. These properties make it valuable in various package applications in multiple fields, including pharmaceutical and biomaterial purposes, drug carriers, wound treatment, chelating agents, meal packaging, nutritional supplements, etc. [10,12]. When the amino group of chitosan interacts with the carboxylate group of alginates, a complex chitosan-alginate will be formed with strong electrostatic interaction. This interaction enhances the ability to maintain microcapsules during encapsulation and control the compound release. This positive charge of chitosan is important in drug delivery systems because it plays a role in the interaction with the drug it delivers [13].

The use of chitosan as an oral drug delivery material needs to be modified due to its unstable nature in acidic media. Chitosan can be modified by combining it with certain polymers or cross-linking agents to make it more inert and resistant in acidic media [14]. Sodium tripolyphosphate is a cross-linking agent that forms electrostatic bonds with polycationic polymers, such as chitosan, by a technique called ionic gelation. Sodium tripolyphosphate is physiologically nontoxic when it is compared with glyoxal, glutaraldehyde, and other chemical cross-linkers. Additionally, it offers the advantage of being water-soluble and requires only a single-step reaction for ionic gelation. Sodium tripolyphosphate with its high charge density (six ionic groups), guarantees high cross-linking density with chitosan amine groups through ionic gelation. The use of chitosan-sodium tripolyphosphate as a nanocarrier enabled the encapsulation of drugs, which included risperidone [15], influenza vaccines [16-17] and gene delivery [18].

Considering the importance of vitamin B₆ in human life, it is necessary to study the process of vitamin B₆ encapsulation in TCA nanoparticles. The effectiveness of B₆-TCA nanoparticles when applied to the delivery system will be conducted by studying the kinetics release of vitamin B₆. The stability of vitamin B₆ and a higher percentage of encapsulation efficiency on B₆-TCA nanoparticles are expected to be achieved. Furthermore, B₆-TCA nanoparticles are expected to control the release of vitamin B₆ under certain conditions, allowing side effects on the body to be minimized.

■ EXPERIMENTAL SECTION

Materials

Sodium alginate, chitosan, sodium tripolyphosphate, pyridoxine (vitamin B₆), CaCl₂, sodium hydroxide (NaOH), hydrochloric acid (HCl), 1% (v/v) acetic acid solution, aquabidest, and phosphate buffered saline (PBS) with a pH of 7.2 were obtained from Merck and Sigma Aldrich with pro analytical quality.

Instrumentation

The instruments used include laboratory glassware, pH meter, analytical balance, centrifugation (Sorvall Biofuge Primo), magnetic stirrer, UV-vis spectrophotometer (GENESYS 50 Thermo Scientific), scanning electron microscope (SEM-EDX, JSM-6510 JEOL/EO), transmission electron microscopy (TEM, JEM-1400 JEOL/EO), and Fourier transform infrared spectrophotometer (FTIR, Shimadzu Prestige-21).

Procedure

Preparation of B₆-TCA nanoparticles

Preparation of chitosan-alginate nanoparticles is primarily based on ionic interactions between the negative charge of the alginate solution and the positive charge of the chitosan solution. Chitosan was dissolved in 1% acetic acid solution with numerous concentrations (0.5, 1.0, 1.5 and 2.0%), alginate was dissolved in aquabidest with numerous concentrations (1.0, 1.5, 2.0 and 2.5%) while vitamin B₆ is dissolved in aquabidest. Rajaonarivony's method is used to synthesize sodium tripolyphosphate crosslinked chitosan-alginate (TCA) nanoparticles with modifications.

An amount of 5 mL of alginate solution is mixed with 10 mL of vitamin B₆ solution. The mixed solution was added to 10 mL of chitosan solution drop by drop while stirring with a magnetic stirrer (1,000 rpm). Then sodium tripolyphosphate was added with various concentrations (0.5, 1.0, 1.5 and 2.0%) while stirring at constant speed at room temperature. B₆-TCA nanoparticles are formed instantly. The suspension of B₆-TCA nanoparticles was maintained with stirring for 240 min for further crosslinking of the nanoparticles. B₆-TCA nanoparticles were collected by centrifugation (5,000 rpm) for 30 min, followed by further filtration using filter papers and then dried.

Characterization of B₆-TCA nanoparticles

B₆-TCA nanoparticles were characterized using a FTIR. The powder state of the sample was prepared and formed with KBr, pressed to form pellets. FTIR spectra are decided in the wavenumber region between 400–4000 cm⁻¹. The size of B₆-TCA nanoparticles was characterized using TEM with a scale of 20–500 nm. B₆-

TCA nanoparticle morphology was characterized by SEM.

Encapsulation efficiency (EE) of B₆-TCA nanoparticles

An amount of vitamin B₆ (5 mg) encapsulated nanoparticles in dry conditions were put into 100 mL of aquabidest, then shaken until the vitamin B₆ was perfectly extracted from the nanoparticles. The UV-vis spectrophotometer was used to analyze the extracted vitamin B₆ at its maximum wavelength (λ_{max}), with aquabidest as a blank solution. Encapsulation efficiency was calculated in Eq. (1):

$$\%EE = \frac{\text{Vitamin B}_6 \text{ concentration in nanoparticle}}{\text{Vitamin B}_6 \text{ concentration}} \times 100\% \quad (1)$$

Evaluation of in vitro release of B₆-TCA nanoparticles

The release of vitamin B₆ in B₆-TCA nanoparticles was determined *in vitro*, where 20 mg of nanoparticles were put into a vial containing 20 mL of PBS solution (pH 7.2 and 25 °C) and incubated in an incubator shaker at 80 rpm at 25 °C. The release study was conducted by taking 5 mL of sample to analyze the concentration of vitamin B₆. The release study was evaluated at 30 min time intervals for 6 h. The amount of vitamin B₆ released was calculated using UV-vis spectrophotometer at λ_{max} of vitamin B₆. The concentration of vitamin B₆ released from the nanoparticles was calculated using the linear regression equation ($y = a + bx$) of the standard curve of vitamin B₆. The concentrations of vitamin B₆ data at each observed time were converted into a C_t vs t for the zero-order, ln C_t vs t for the first order, %C_t vs t^{1/2} for Higuchi model kinetics and log %C_t vs log t for the kinetics of the Korsmeyer-Peppas model. The release constant (k) of vitamin B₆ was determined from the data obtained.

RESULTS AND DISCUSSION

The research resulted in coarse solid powder nanoparticles with yellowish-white color. One of the factors that influence the efficiency of encapsulation and efficiency of drug release from the matrix is the addition of crosslinker agents in the matrix. Therefore, the effect of adding sodium tripolyphosphate was studied.

FTIR Characterization

The FTIR spectra of chitosan, alginate, sodium tripolyphosphate, vitamin B₆ and B₆-TCA nanoparticle

are represented in Fig. 1(a–e). The characteristic peak of chitosan (Fig. 1(a)) showed the amine group and the hydroxyl group at 3449 cm^{-1} . The carbonyl stretching ($\text{C}=\text{O}$) of the secondary amide (amide I band) is found at 1651 cm^{-1} . The peaks in wavenumbers 1419 and 1381 cm^{-1} stood for the $\text{N}-\text{H}$ stretching of the amide and ether bonds (amide III band) and the $\text{N}-\text{H}$ stretching (amide III band). The wavenumbers of 1080 and 1026 cm^{-1} are secondary hydroxyl groups and primary hydroxyl groups [19–21].

Chitosan's FTIR spectrum is different from alginate's (Fig. 1(b)) because it has NH_2 groups while alginate has carboxylic groups. The NH_2 group in chitosan was shown by wavenumbers of 3449 and 1651 cm^{-1} , while the carboxylic group in alginate was shown by wavenumbers of 1620 and 1420 cm^{-1} [1,22–24]. Sodium tripolyphosphate (Fig. 1(c)) has a peak of 800 (antisymmetric stretching of the $\text{P}-\text{O}-\text{P}$ bridge), 1093 (symmetric and antisymmetric stretching vibrations in PO_3 group), 1150 (symmetric and antisymmetric stretching vibrations in PO_2 group), and 1211 cm^{-1} ($\text{P}=\text{O}$ stretching) [25]. Vitamin B_6 (Fig. 1(d)) also showed a characteristic peak at 1281 , 1543 and 1620 cm^{-1} indicating the presence of $\text{C}-\text{O}$, aromatic $\text{C}=\text{C}$ and $\text{C}=\text{O}$ stretching vibration.

From Fig. 1, there has been a shift in the characteristic spectra of chitosan from wavenumber 1597 to 1528 cm^{-1} . This happens due to the interaction $-\text{NH}_3^+$ from chitosan with $-\text{COO}^-$ from alginate. Interaction occurs in an electrical interaction, which causes the $-\text{NH}$ group bending frequency on the amino to decrease. The frequency drop can be related to the amount of energy dropped during the interaction process, and this bond can strengthen the matrix of the nanoparticles. In addition, the wavenumbers 1026 cm^{-1} in chitosan and 1033 cm^{-1} in alginate had a change in wavenumber to 1103 cm^{-1} , which indicates the stretching vibrations of $\text{C}-\text{O}-\text{C}$. Sodium tripolyphosphate's wavenumber at 1150 cm^{-1} shifted to 1172 cm^{-1} , indicating the presence of $\text{P}=\text{O}$ group in sodium tripolyphosphate [26]. The interaction between tripolyphosphate ions in sodium tripolyphosphate and ammonium ions in chitosan with the shifted wave number indicates that chitosan and sodium tripolyphosphate were successfully crosslinked during the synthesis of nanoparticles.

SEM-EDX Characterization

B_6 -TCA nanoparticle's surface morphology and elemental composition before and after the release of

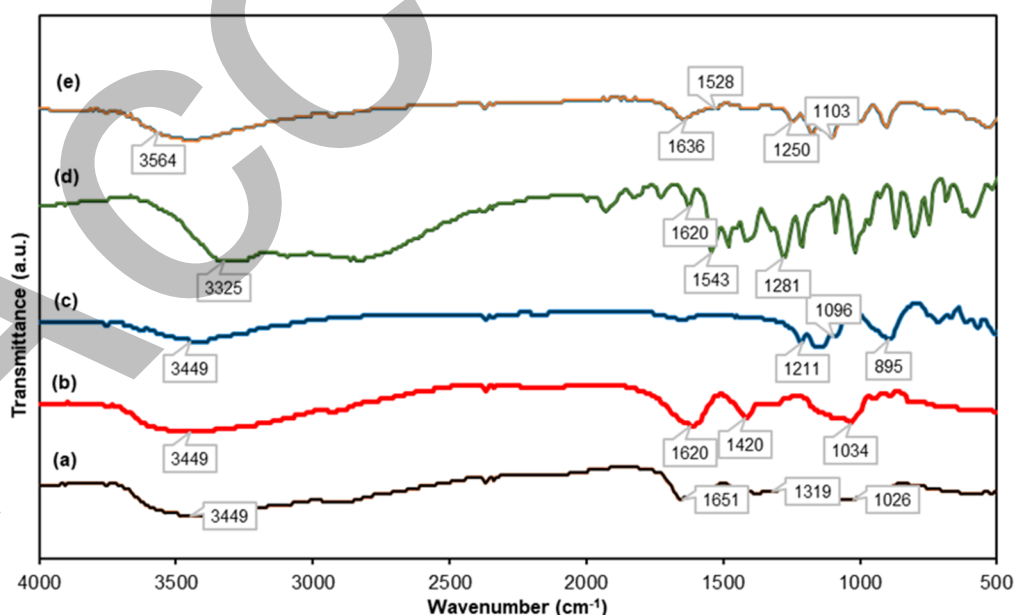


Fig 1. FTIR spectra of (a) chitosan, (b) alginate, (c) sodium tripolyphosphate, (d) vitamin B_6 and (e) B_6 -TCA nanoparticles

vitamin B₆ was figured out with SEM-EDX. SEM images taken with a magnification of 200 times on nanoparticles before and after the release of vitamin B₆ are represented in Fig. 2. Before vitamin B₆ is released, nanoparticles exhibit a rough, irregular, and slightly porous surface that can form aggregates, as shown in Fig. 2(a). There is a distinction between the nanoparticles before and after release in Fig. 2(b). In the SEM image of B₆-TCA nanoparticle, before vitamin B₆ is released, the loss of the porous structure in the nanoparticles is feasible because the surface of the nanoparticle has been covered by vitamin B₆, which may have filled the pores. Table 1 provides information on the elemental composition of B₆-TCA nanoparticles before and after release.

The release process in B₆-TCA nanoparticles can be understood by analyzing the composition changes of the nanoparticle constituent elements before and after the release process. In Table 1, there was a composition decrease in C and N elements of the nanoparticles. Before the release process, the C element had a weight percentage of 38.23% and decreased to 35.46% after the release process. As for N element, the weight and atomic percentages were 19.86%, then decreased to 15.69%. There was an increase in the composition of O element from 27.05 to 46.95%. The percentage of elements in the nanoparticle product decreased after the release of vitamin B₆, except O element. This is possible due to the oxidation process of certain groups, such as hydroxyl, aldehyde and other groups, which causes the increase in elemental. The decrease in these elements, which was not too significant, indicated that there was no damage (erosion) to the polymer carriers during the release process.

TEM Characterization

The inner morphology and average size of the B₆-TCA nanoparticles were studied using TEM and represented in Fig. 3(a and b) with a magnification scale of 100 and 50 nm. The TEM images of the B₆-TCA nanoparticles have a spherical but relatively irregular shape with some darker parts than the others. This characteristic indicates the tendency of B₆-TCA nanoparticles to form aggregates. B₆-TCA nanoparticles have an average particle size of 22.55 nm based on the size distribution graph in Fig. 4. The result suggests that the ionic gelation method yields smaller particle sizes.

Encapsulation Efficiency (EE) of Vitamin B₆

Drug encapsulations enhance the therapeutic effect of compounds that are difficult to dissolve, fragile or aggressive along with minimising the side effects. On the other hand, formulating bioactive compounds with high encapsulation efficiencies remains a big challenge during pharmaceutical development [27]. Encapsulation enhances the delivery of drugs to targeted

Table 1. Elemental composition of B₆-TCA nanoparticles before and after vitamin B₆ released

Element	Weight (%)	
	Before release	After release
C	38.23	35.46
N	19.86	15.69
O	27.05	46.95
Na	0.25	1.48
P	7.27	-
Cl	0.30	0.14
Ca	7.04	0.27

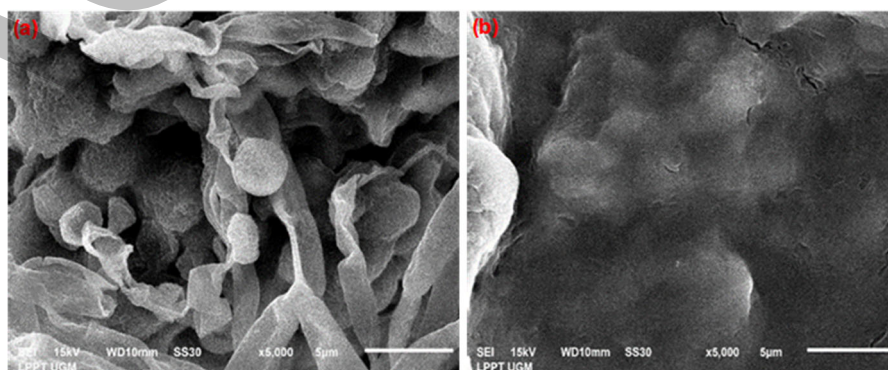


Fig 2. SEM image of B₆-TCA nanoparticles (a) before and (b) after vitamin B₆ release

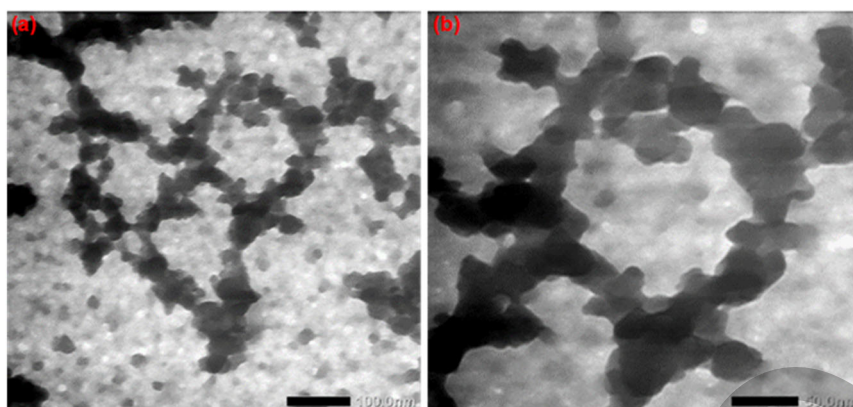


Fig 3. TEM image of B₆-TCA nanoparticles: (a) 100 nm and (b) 50 nm scale

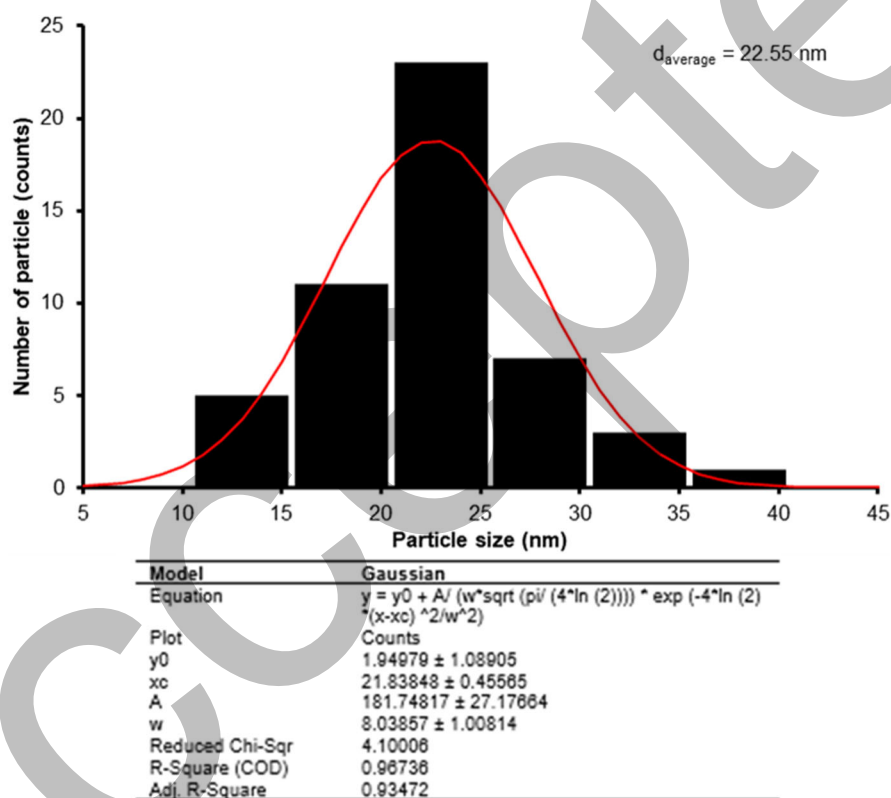


Fig 4. Particle size distribution of B₆-TCA nanoparticles

sites, increases drug stability, prolongs pharmacological activity by continuously releasing the active molecules locally and reduces side effects, thereby enhancing the efficacy and safety of therapies [28].

The technique of encapsulation involves packaging, adsorption, or coating active substances in solid, liquid, or gas forms in a matrix. Encapsulation techniques have the advantage of protecting and controlling the release of active ingredients under specific conditions to minimize

the potential side effects [29]. The preservation of bioactive properties can be effectively improved by encapsulation, which enables the administration of sensitive vitamins and their metabolization [27]. The encapsulation efficiency parameter is used to determine how much vitamin B₆ is packed into the matrix. The alginate-chitosan polymer was evaluated for its ability to bind vitamin B₆ and form nanoparticle complexes by conducting encapsulation efficiency testing. The test was

conducted by measuring the amount of vitamin B₆ in the matrix and compared with the total vitamin B₆ used in the preparation.

The results of calculations for the encapsulation efficiency of vitamin B₆ with variations in vitamin B₆, chitosan, alginate, and sodium tripolyphosphate concentration are displayed in Tables 2 and 3. In the various concentrations of sodium tripolyphosphate, the optimum encapsulation efficiency value in the ratio of vitamin B₆:chitosan:alginate:sodium tripolyphosphate is 2.0:1.0:1.5:0.5 of 95.91%. As the concentration of sodium tripolyphosphate increased, the percentage of encapsulation efficiency decreased. This is because chitosan-tripolyphosphate crosslinks increase with sodium tripolyphosphate concentration, making the bond network tighter and the free space in the nanoparticle system reduced so that the vitamin B₆ encapsulated by the matrix also decreases.

Evaluation of In Vitro Release of B₆-TCA Nanoparticles

Phosphate buffer saline (PBS) solution with a pH value of 7.2 was used to release vitamin B₆ from nanoparticles using aquabidest at 25 °C. Samples were taken and analyzed using UV-vis spectrophotometer to study the kinetics release. The effect of sodium tripolyphosphate concentrations on nanoparticle preparations was studied by varying the concentrations of vitamin-chitosan-alginate-sodium tripolyphosphate, namely with a composition ratio of 2.0:1.0:1.5:0.5; 2.0:1.0:1.5:1.0; 2.0:1.0:1.5:1.5 and 2.0:1.0:1.5:2.0 (Tables 2 and 3). The effect of varying sodium tripolyphosphate concentrations on the synthesis of B₆-TCA nanoparticles is depicted in Fig. 5.

From Fig. 5, it is known the addition of sodium tripolyphosphate causes a slower release of vitamin B₆ from nanoparticles. An increase in the concentration of

Table 2. Influence of vitamin B₆, chitosan, and alginate concentrations on encapsulation efficiency (%EE) of vitamin B₆ in B₆-TCA nanoparticles

Influence of composition	Nanoparticle products vitamin B ₆ -chitosan-alginate-sodium tripolyphosphate	%EE
Variation concentrations of vitamin B ₆	0.5:1.0:1.0:2.0	77.37
	1.0:1.0:1.0:2.0	90.49
	1.5:1.0:1.0:2.0	96.35
	2.0:1.0:1.0:2.0	97.65
Variation concentrations of chitosan	2.0:0.5:1.0:2.0	83.69
	2.0:1.0:1.0:2.0	89.84
	2.0:1.5:1.0:2.0	88.08
	2.0:2.0:1.0:2.0	82.16
Variation concentrations of alginate	2.0:1.0:1.0:2.0	94.34
	2.0:1.0:1.5:2.0	96.42
	2.0:1.0:2.0:2.0	95.95
	2.0:1.0:2.5:2.0	89.41

Table 3. Encapsulation efficiency of vitamin B₆ in various concentrations of sodium tripolyphosphate

Nanoparticle products vitamin B ₆ -chitosan-alginate-sodium tripolyphosphate	%EE
2:1:1.5:0.5	95.91
2:1:1.5:1.0	92.57
2:1:1.5:1.5	85.01
2:1:1.5:2.0	82.04

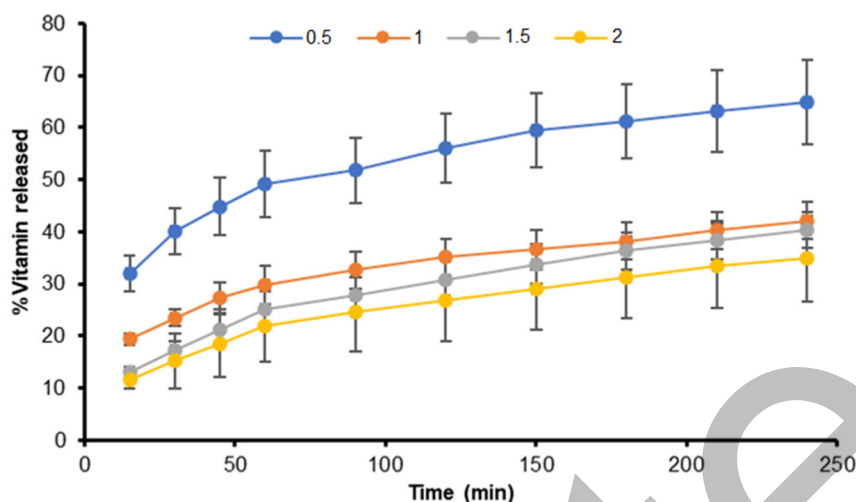


Fig 5. Effect of variations of sodium tripolyphosphate concentrations on the release of vitamin B₆

sodium tripolyphosphate will result in higher cross-links with chitosan so that the path of diffusion becomes obstructed [30]. Sodium tripolyphosphate can be used as a medium to control the slow release of vitamin B₆, because the slower the release of vitamin B₆ from the nanoparticles, the lower the dose of the drug required. Using zero order, first order, Higuchi and Korsmeyer-Peppas models, the kinetics of vitamin B₆ release from B₆-TCA nanoparticles was investigated. Table 4 summarizes the results of calculating the *in vitro* release parameters of vitamin B₆ from B₆-TCA nanoparticles.

According to the data in Table 4, the ratio of 2.0:1.0:1.5:2.0 in the composition has the highest linearity value (R^2), which is the closest to 1 in the Korsmeyer-Peppas kinetics model, amounting to 0.989 and has the lowest reaction rate constant, which is equal to 0.039. The small reaction rate indicates that the process of releasing vitamin B₆ is slow. In addition, the n value for the product composition ratio of 2.0:1.0:1.5:2.0 is less than 0.45. This indicates that the release of vitamin B₆ from the TCA nanoparticles occurred through a Fickian diffusion process without being followed by erosion of the carrier polymer. The composition ratio of 2.0:1.0:1.5:2.0 resulted in a vitamin B₆ %EE of 82.04%. The %EE obtained in this study is above the average of other vitamins encapsulated in other polymers (nano)microparticles, where the previous research resulted in an average of %EE in the range of 27–45% [31].

Table 4. Parameter calculation results for *in vitro* release of vitamin B₆ on B₆-TCA nanoparticles

Model and Parameter	Score
Zero-order	
R^2	0.926
K_0 (min^{-1})	9.660×10^{-4}
First-order	
R^2	0.946
K_1 (min^{-1})	2.605×10^{-4}
Higuchi	
R^2	0.984
K_H ($\text{min}^{-1/2}$)	1.970×10^{-2}
Korsmeyer-Peppas	
R^2	0.989
K_{KP} (min^{-n})	0.039
N	0.400

Possible interactions between vitamin B₆ and TCA nanoparticles could involve the interaction between alginate and chitosan which is shown in Fig. 6. This interaction entails the negatively charged carboxyl group in alginate and the positively charged amino group in chitosan. The interaction between chitosan and sodium tripolyphosphate occurred between the negative group of polyanions in sodium tripolyphosphate and amine groups in chitosan. Fig. 7 shows the possible interaction between vitamin B₆ with TCA nanoparticles due to the interaction between hydrogen bonds between hydroxyl groups from vitamin B₆ and chitosan.

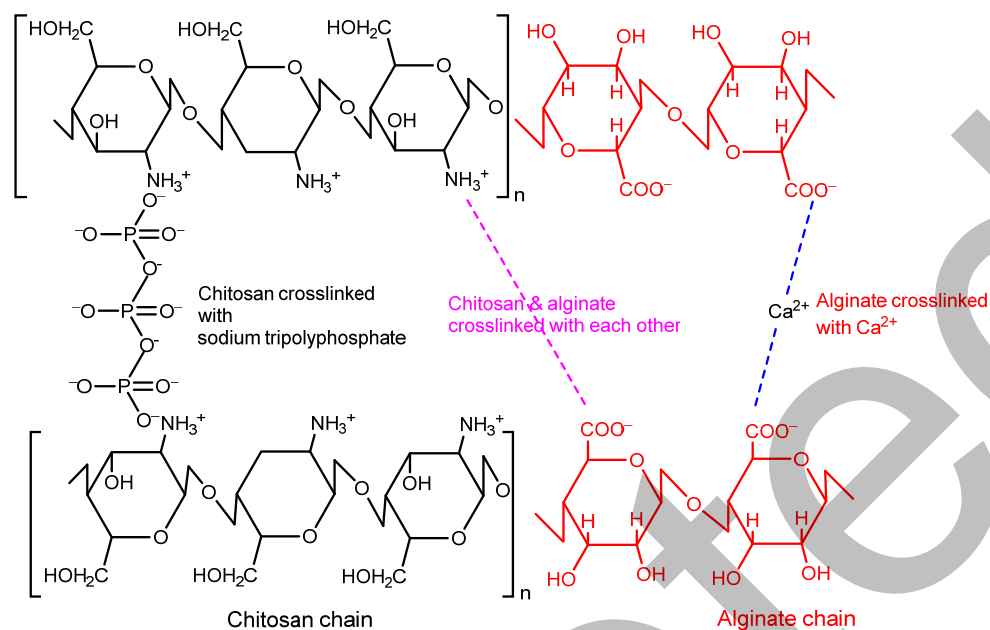


Fig 6. Structure of TCA nanoparticles

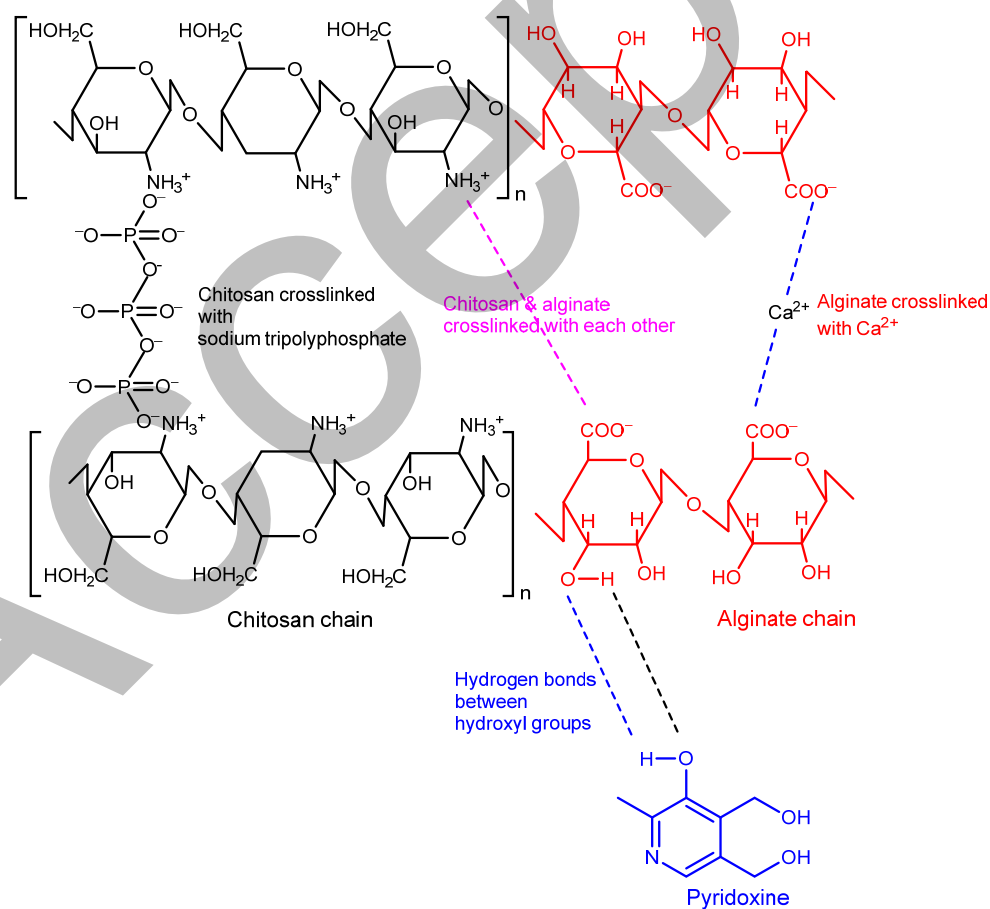


Fig 7. Possible interactions between vitamin B₆ and TCA nanoparticles

■ CONCLUSION

B₆-TCA nanoparticles were successfully synthesized, and the resulting nanoparticles had an average particle size of 22.55 nm. The optimum composition of B₆-TCA nanoparticles ratio is 2.0:1.0:1.5:2.0 followed by Korsmeyer-Peppas kinetics as its best-fitted model with the Fickian diffusion mechanism, amounting to 0.989 and has the smallest reaction rate constant, which is equal to 0.039 occurred within 6 h. The results show that the encapsulation efficiency of vitamin B₆ in this study reaches 82.04% and is above the average encapsulation efficiency of other vitamins in polymers.

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■ CONFLICT OF INTEREST

The authors declare no conflict of interest.

■ AUTHOR CONTRIBUTIONS

Aulia Rahman: investigation, formal analysis, writing – review & editing. Suherman: validation and review. Adhitasari Suratman: supervision, conceptualization, review.

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