# Chapter 10
# Synthesis of Chitosan-Folic Acid Nanoparticles as a Drug Delivery System for Propolis Compounds


**Marselina Irasonia Tan and Adelina Khristiani Rahayu**

**Abstract** This chapter discusses the method to synthesize chitosan-folic acid nanoparticles. Developments in nanotechnology provide an alternative drug delivery system. Chitosan is a polymer that can be utilized to make nanoparticles because it is biodegradable, non-toxic and inexpensive. These characteristics are important for the drug delivery system. To optimize this system, a specific ligand conjugated to the nanoparticle can aid in directing the nanoparticle to the cell target. Folic acid can be used as a ligand to direct nanoparticles to cell targets with high folic acid receptors such as tumor cells. In this research, a chitosan nanoparticle was synthesized to deliver propolis to the cell target. Since propolis bioavailability in the body is relatively low, its bioavailability needs to be improved by encapsulating it in nanoparticles. The purpose of this study is to synthesize folic acid conjugated chitosan nanoparticles that encapsulate propolis compounds. The effect of the molar ratio between chitosan:folic acid:sodium tripolyphosphate (TPP), chitosan molecular weight, encapsulated propolis concentration, sonication in the synthesis of chitosan-folic acid-containing propolis nanoparticles (NP-KF-P), and chitosan-folic acid conjugate nanoparticles (NP-KF-blanks) were also studied. The encapsulation efficiency of propolis in NP-KF and Fourier Transform Infrared Spectrophotometer (FTIR) of the nanoparticles was also observed. NP-KF-P and NP-KF-blanks were successfully synthesized by the ionic gelation method. The diameters of NP-KF-P and NP-KF-blanks were 153.9 ± 1.3 and 129.0 ± 3.4 nm, respectively. Propolis encapsulation efficiency in NP-KF-P was 30.37–73.36%, and 90% of the propolis could be released at pH 4.

**Keywords** Nanoparticles · Chitosan · Folic acid · Propolis

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## 10.1 Introduction

Nanoparticle technology had recently become an innovation in the drug delivery system development. Nanoparticles as drug delivery systems assure that drugs encapsulated in nanoparticles can reach the correct target cells (Vllasaliu et al., 2013). Nanoparticles can be targeted to a particular tissue/cell by a passive or active targeting system. In an active drug delivery system, the nanoparticle is conjugated with a specific ligand, which can help the nanoparticle to recognize the targeted receptor in the target area. In passive drug delivery systems, the nanoparticle reaches its destination by utilizing the target tissue's physiological condition, without any specific ligand (Vllasaliu et al., 2013). Moreover, it is possible to adjust the nanoparticle size, as well as the surface properties of nanoparticles and the release of active substances from nanoparticles. Due to their diminutive size, nanoparticles could easily pass through blood vessels and enter target cells (Mohanraj & Chen, 2006). Therefore, the use of nanoparticles can maximize the effect of drugs on target cells and minimize drug accumulation in healthy tissues.

## 10.2 Principle

Nanoparticles can be synthesized from various components, including lipids, polymers, and inorganic substances (Zhang et al., 2006). For biomedical purposes, nanoparticles must be biocompatible and non-toxic (Wilczewska et al., 2012). Chitosan is one of the components that can be used as nanoparticles. Chitosan has a low level of cytotoxicity and is degradable (Kean & Thanou, 2010). Therefore, chitosan is very suitable to use as a nanoparticle component for biomedical purposes. There are various kinds of chitosan with different molecular weights and degrees of deacetylation. Both of these features affect the biodegradable properties of chitosan (Kean & Thanou, 2010).

Chitosan (β(1–4) 2-amino 2-deoxy β-d glucan) is a polysaccharide obtained by chitin deacetylation (Fig. 10.1; Schmitz et al., 2019). Chitosan is composed of b(1–4)-linked D-glucosamine monomer and N-acetyl-D-glucosamine monomer (Riva et al., 2011). Chitin and chitosan support components of various organisms such as the exoskeletons of crustaceans and insects and parts of the fungal cell wall (Riva et al., 2011).

Chitosan is soluble in an acidic solution (pH below 6.5) due to its amine groups becoming protonated (Riva et al., 2011). Chitosan solubility in acidic conditions is beneficial in drug delivery systems, because these characteristics can be applied to regulate drug release from chitosan nanoparticles (Cheng et al., 2017; Viviek et al., 2013; Wang et al., 2017).

Chitosan nanoparticles can be formed with the help of tripolyphosphate (TPP), which has phosphate groups. This phosphate group (negative charge) from TPP will be cross-linked to the amine group from chitosan (positive charge) (Fig. 10.2; Chávez de Paz et al., 2011; Cho et al., 2010).

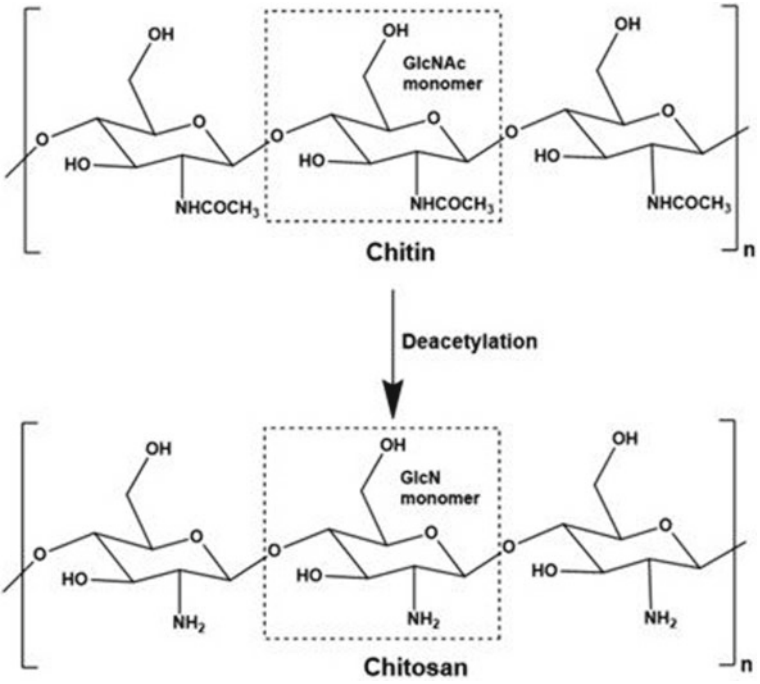


**Fig. 10.1** Deacetylation of chitin to form chitosan (Schmitz et al., 2019)

The size of chitosan nanoparticles can be very small. Hence, chitosan nanoparticles can cross biological boundaries, such as tissue, thereby improving the effectiveness of the drug (Wang et al., 2011). Moreover, chitosan nanoparticles under 400 nm will be able to deliver specific drugs to cancerous tissues using a passive drug delivery system, specifically through leaky vasculature surrounding the cancer tissues (Danhier et al., 2010).

In the active drug delivery system, nanoparticles are conjugated with ligands to direct the nanoparticles to the desired cell. Folic acid is one of the ligands that is often used to designate nanoparticles in an active drug delivery system. Folic acid will bind to folic acid receptors, which are found abundantly in various types of cancer cells, such as the breast, brain, kidneys, breasts, lungs, or retinoblastoma cancer cells. Folic acid receptor expression is 100–300 times higher in cancer cells than in healthy cells (Parveen & Sahoo, 2010). Furthermore, Vllasaliu et al. (2013) reported that due to carboxyl groups in folic acid, it can be easily conjugated with other macromolecules, such as chitosan. As a potential ligand, folic acid can potentially enhance the endocytosis of chitosan nanoparticles into the targeted cells (Jin et al., 2016; Parveen & Sahoo, 2010). Yang et al. (2010) also reported that folic acid conjugated to chitosan nanoparticles could increase the accumulation of protoporphyrin IX in colorectal cancer cells. Vllasaliu et al. (2013) stated that folic acid is a

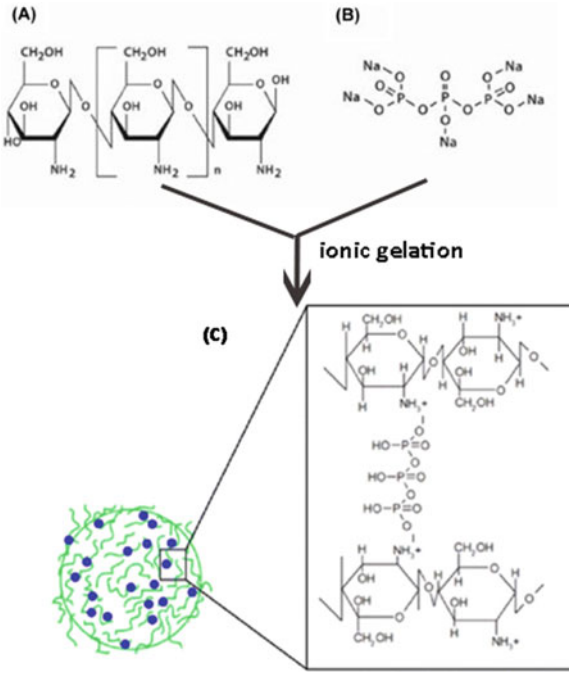


**Fig. 10.2** Structure of (**a**) chitosan, (**b**) sodium tripolyphosphate (TPP), (**c**) crosslink between chitosan and TPP (Chávez de Paz et al., 2011; Cho et al., 2010)

more stable and inexpensive ligand compared to other ligands, such as monoclonal antibodies.

Based on the explanation above, chitosan nanoparticles can be used to deliver a particular drug. In this research, bioactive compounds from propolis were encapsulated in chitosan nanoparticles. Propolis has antibacterial, anti-inflammatory, and anticancer activities. However, propolis has low bioavailability and is easily degraded in the body, especially in the digestive tract, so that this condition can reduce the effectiveness of propolis in the body (Elbaz et al., 2016). In order to increase the efficiency of the propolis effect, it is crucial to increase the bioavailability of propolis in the body, i.e. via folic acid conjugated chitosan nanoparticles, as a drug delivery system.

The essential parameters for nanoparticles are particle size and surface charge of particles. Generally, nanoparticle sizes must be constructed in such a way that the particles can pass through the endothelium of abnormal blood vessels in tumor tissue (Haley & Frenkel, 2008). The particle surface charge is revealed by its zeta potential value. A good zeta potential value should be more than 30 mV. The higher the zeta potential value of a particle, the greater the repulsion force between nanoparticles so that they do not quickly aggregate (Honary & Zahir, 2013).

The internalization of nanoparticles by cells occurs through various endocytic pathways. Cells can phagocytose nanoparticles bigger than 250 nm. Nanoparticles with a size of about 100 nm will be pinocytosed, whereas nanoparticles less than 100 nm will be internalized by clathrin or caveolin proteins. Generally, nanoparticles

that bind to ligands from specific receptors on cancer cells will be internalized into cells by both proteins (Rajabi & Mousa, 2016).

In addition to the size of the nanoparticles, the surface charge of the nanoparticles also affects the internalization of the nanoparticles. Positively charged nanoparticles can enter the cell faster because of the strong electrostatic interaction between the nanoparticle and the negatively charged cell membrane (Rajabi & Mousa, 2016). Bannunah et al. (2014) showed that positively charged nanoparticles have been internalized better than negatively charged nanoparticles.

Synthesis of chitosan nanoparticles generally uses the ionic gelation method with polyanion compounds. The amine group in chitosan is ionized in a weak acid environment, allowing the polymer to stick to negatively charged surfaces (Loh et al., 2010). Sodium tripolyphosphate (Na-TPP) is one of the most widely used cross-linkers in the synthesis of chitosan nanoparticles because the polyanion is non-toxic and has multivalent properties (Fan et al., 2012). When the chitosan solution is mixed with the TPP solution, nanoparticles are formed by the formation of molecular bonds between phosphate in TPP and amine groups in chitosan (Fig. 10.2, Chávez de Paz et al., 2011; Cho et al., 2010).

## 10.3 Materials and Methods

### *10.3.1 Materials*

For synthesizing folic acid conjugated chitosan nanoparticles containing propolis, several reagents are needed such as low molecular weight chitosan (Sigma-Aldrich), sodium tripolyphosphate (TPP; Sigma-Aldrich), folic acid, propolis. Propolis was isolated from Trigona bees (Rahmi Propolis). Other reagents used for preparing nanoparticles include EDC (1-(3-Dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride) (Sigma-Aldrich), and trehalose.

### *10.3.2 Extraction of Propolis*

Propolis extraction was carried out to facilitate and optimize the encapsulation of bioactive propolis compounds into nanoparticles. The method used was as follows:

1. 40 mg of propolis was dissolved in 80 mL ethanol 70% (1:2) by stirring with a blender.
2. The Erlenmeyer flask containing this propolis solution was wrapped with aluminum foil, then stirred on a shaker at 150 rpm.
3. Every 24 h, the propolis solution was filtered and 80 mL of 70% ethanol was added to the propolis. This was repeated for three weeks until the propolis filtrate was clear.

4. In order to obtain a concentrated propolis extract, the propolis filtrate was evaporated with a rotary evaporator. This concentrated propolis extract contained various compounds, such as phenolic compounds.
5. The concentrated extract of propolis was then placed in an evaporating dish and dried in an oven at 40 °C until it became a propolis powder.

### *10.3.3 Conjugation of Folic Acid with Chitosan*

Folic acid needed to be bound to chitosan before the synthesis of nanoparticles. The conjugation procedure was modified from the method developed by Yang et al. (2010), which is as follows.

1. 1.178 mg of folic acid was dissolved in 2 mL 100% DMSO. This folic acid solution was then added to 2 mL DMSO containing 1.56 mg EDC. This solution was added to 100 mL 0.5% chitosan.
2. The solution was stirred at 400 rpm for 16 h.
3. The pH of the mixture was adjusted to pH 9.0, centrifuged at 4500 rpm for 10 min, at 25 °C and the supernatant was discarded.
4. The pellet was suspended in a small volume of 2% acetic acid. The conjugated nanoparticles were purified by dialysis with a cutoff between 12,000 and 14,000 Dalton.
5. Dialysis was first carried out for one day against PBS pH 7.4, followed by another day against distilled water.
6. The conjugate was then freeze-dried.

### *10.3.4 Preparation of Chitosan-Folic Acid Nanoparticles*

The chitosan-folic acid nanoparticles (NP-KP-P) were synthesized by modifying the method by Yang et al. (2010).

1. Chitosan-folic acid (0.05%) was dissolved in 100 mL of 2% acetic acid (pH 4.7), and stirred overnight. The chitosan-folic acid solution was then sonicated for 30 min.
2. For the process of propolis encapsulation, 0.48 mL propolis was mixed with 12 mL chitosan-folic acid solution.
3. The mixture of chitosan solution with propolis was stirred for 20 min, then 4.8 mL TPP was added dropwise to the chitosan-folic acid-propolis solution or a chitosan-folic acid solution so that nanoparticles would form.
4. The nanoparticle suspension was then centrifuged at 11,000 rpm for 1 h.
5. The pellet was then resuspended with 1 mL milli-Q water and sonicated for 30 min, while the supernatant was stored to be used later to determine the concentration of the encapsulated propolis.

6. Chitosan-folic acid (NP-KF-Blanks) and chitosan-folic acid-propolis nanoparticles (NP-KF-P) were characterized, which included the diameter, zeta potential, morphology, and chemical bonds of the nanoparticles. The morphology of the nanoparticles was examined using a scanning electron microscope. The chemical bonds in the conjugate were observed using FTIR (Fourier Transform Infrared Spectrophotometer). The diameter and zeta potential of the nanoparticles were measured using a Particle Size Analyzer (Malvern). Afterward, the nanoparticle suspension was mixed with trehalose 20% and freeze-dried. After freeze-drying, the nanoparticle suspension turned into nanoparticle powder.

### *10.3.5 Characterization of Nanoparticles*

The encapsulation efficiency (EE) of the drug in chitosan-propolis nanoparticle (NP-KP-P) was measured using an indirect method, namely by measuring the concentration of the drug that remained in the supernatant (Bahreini et al., 2014; Xue et al., 2015). The amount of drug encapsulated in the nanoparticle was the total number of added drugs minus the amount of drug present in the supernatant (Rampino et al., 2013). The remaining drug concentration could be determined by measuring the absorbance of the supernatant at the appropriate wavelength (Xue et al., 2015) with a UV–Vis spectrophotometer. The absorbance values obtained were then compared to the standard curves of the drug. Equation (10.1) is used to calculate the efficiency of the encapsulation (EE).

$$EE(\%) = \frac{\text{Total drug concentration} - \text{residual drug concentration in the supernatant}}{\text{total drug concentration}} \times 100\% \tag{10.1}$$

The absorbance of NP-KF-P supernatant was measured using the Folin-Ciocalteu assay at a wavelength of 750 nm. The Folin-Ciocalteu assay was used to determine the total phenolic compounds found in propolis extract (Kubiliene et al., 2015). Supernatants from empty nanoparticles (NP-KF-Blanks) were used as blank when measuring drug-containing nanoparticle supernatants (Rampino et al., 2013).

### *10.3.6* In vitro *Study of the Release of Propolis from Nanoparticles*

This analysis was conducted by modifying the method developed by Viviek et al. (2013). A total of 5 mg of chitosan-folic acid nanoparticles containing propolis (NP-KF-P) were dissolved in 2 mL milli-Q water and placed in a dialysis tubing. The dialysis tubing containing a nanoparticle suspension was then placed in a beaker glass containing 30 mL PBS with various pH (pH 4.0; 6.0; 7.4). Propolis released from nanoparticles will pass through the dialysis membrane into the PBS solution.

The concentration of the released propolis was measured at 0, 3, 6, 9, 12, 24, 48 h and each time, the dialysis tubing was placed in new PBS. The concentration of released propolis was measured using a spectrophotometer using the Folin-Ciocalteu assay at a wavelength of 750 nm, primarily to determine the total phenolic compounds found in propolis extracts.

## 10.4 Result and Discussion

### *10.4.1 Folic Acid-Chitosan Conjugate*

Carboxyl groups in folic acid would bind to the amine group in chitosan (Vllasaliu et al., 2013), which would effectively reduce the amount of free amine in chitosan. This free amine in chitosan would bind to TPP during the ionic gelation process. Hence, excessive folic acid could inhibit the formation of chitosan-folic acid nanoparticles. The result of chitosan-folic acid nanoparticle synthesis (NP-KF-blanks) and chitosan-folic acid-containing propolis nanoparticle synthesis (NP-KF-P) is presented in Fig. 10.3.

NP-KF-P was about 80–110 nm, whereas NP-KF-blanks was around 100–120 nm (Fig. 10.3). The structure of NP-KF-blanks and NP-KF-P were round, compact, and not aggregated. The zeta potential of NP-KF-blanks and NP-KF-P confirmed these results. The zeta potential of the nanoparticles was higher than 25 mV (Table 10.1), which revealed that the nanoparticles would be stable and would not easily aggregate.

TPP regulated the diameter of folic acid nanoparticles as well as the Polydispersity Index (PDI) of NP-KF-blanks. PDI is an indicator of nanoparticle distribution based on the diameter of nanoparticles in suspension. Table 10.1 shows that the PDI

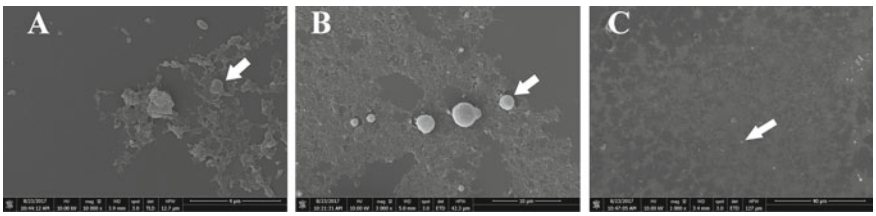


**Fig. 10.3** Observation of folic acid-chitosan conjugate nanoparticle morphology using SEM. (**A**) chitosan-folic acid conjugate nanoparticles (NP-KF-blanks) (arrow); (**B**) chitosan-folic acid-containing propolis nanoparticles (NP-KF-P) (arrow); (**C**) chitosan-folic acid powder (arrow)

**Table 10.1** Nanoparticle characteristics

| Nanoparticle | Diameter of nanoparticle | PDI | Zeta potensial ± SD (mV) |
|---|---|---|---|
| NP-KF-blanks | 129 nm | 0.369 | 30.5 ± 1.04 |

of chitosan-folic acid nanoparticles (NP-KF-blanks) was 0.369. PDI (below 0.5) of NP-KF-blanks showed that the distribution of nanoparticles based on the diameter of nanoparticles was homogeneous. Rampino et al. (2013) explained that nanoparticles with PDI below 0.5 indicated that, based on the diameter of the nanoparticles, the nanoparticles were evenly distributed. Zeta potential shows the charge on the surface of nanoparticles, reflecting the stability of nanoparticles (Pan et al., 2012). Based on Table 10.1, the zeta potential of NP-KF-blanks was 30.5 mV. These results indicated that NP-KF-blanks were successfully synthesized and would not be rapidly aggregated. Jin et al. (2016) explained that a zeta potential greater than 25 mV could increase the stability of nanoparticles in nanoparticle suspensions. The positive charge of nanoparticles would repel each other so that the nanoparticles would not be aggregated (Fonte et al., 2012).

Chitosan nanoparticles (NP-K) and chitosan-folic acid nanoparticles (NP-KF-blanks) have a spherical shape with a diameter below 200 nm (Fig. 10.4). The addition of 10% ethanol containing 10% propolis (Fig. 10.4c) showed that the NP-KF was spherical, and had a diameter of less than 200 nm.

Sonication affects the formation of nanoparticles after the synthesis process. Pradhan et al. (2016) explained that sonication could distribute nanoparticles, which were likely to aggregate after synthesis. In this study, sonication was performed twice, before and after nanoparticle synthesis. Sonication of the chitosan-folic acid solution was performed to shorten the chitosan chain. The short chitosan chain would allow TPP to bind to the short-chain and form small spheres during ionic gelation. Optimization results of nanoparticle synthesis (Table 10.2) showed that without sonication, the diameter of the nanoparticles (bigger than 200 nm) was larger than that of sonication.

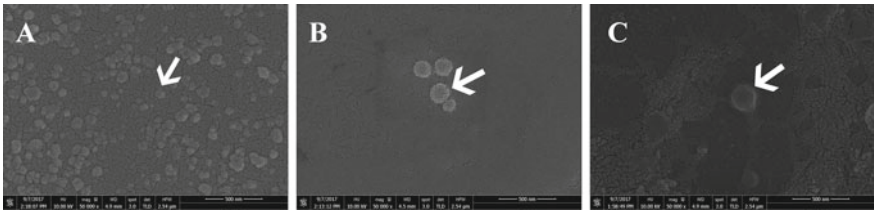


**Fig. 10.4** Morphology of folic acid-chitosan conjugate nanoparticles using SEM. (**A**) chitosan nanoparticles (NP-K); (**B**) NP-KF-blanks; (**C**) chitosan-folic acid nanoparticles containing 10% propolis (NP-KF-P) (white arrow)

**Table 10.2** Sonification effect on nanoparticle diameter

| Optimasi | Type of nanopartikle | Diameter of nanoparticles |
|---|---|---|
| Chitosan-folic acid without sonification | NP-KF-blanks | 160 nm |
| | NP-KF-P | 239 nm |
| Chitosan-folic acid with sonification 30 min | NP-KF-blanks | 153 nm; 129 nm |
| | NP-KF-P | 176 nm; 153 nm |

### 10.4.2 Encapsulation Efficiency of Propolis in Nanoparticles

The encapsulation efficiency of a drug was influenced by how many groups were successfully bound to the nanoparticles. Hydrogen bonds will be formed between functional groups in propolis and chitosan (Elbaz et al., 2016; Franca et al., 2014). Table 10.3 revealed that the encapsulation efficiency of propolis in NP-KF-P varied between 35.1 ± 2.3% and 76.4 ± 2.4%, with the concentration of propolis in the NP-KF-P between 3.87 ± 0.27 μg/mL and 8.73 ± 0.29 μg/mL.

The attainment of propolis encapsulation in nanoparticles was confirmed by the encapsulation efficiency and the NP-KF-P diameter. The results of the synthesis of chitosan-folic acid nanoparticles in this study are revealed in Table 10.4. NP-KF and NP-KF-propolis were successfully synthesized with an average diameter of 129 ± 3.4 nm and 153.9 ± 1.3 nm, respectively. The difference in diameter indicated that propolis was successfully encapsulated in chitosan-folic acid nanoparticles, thereby increasing the diameter of the nanoparticles.

The addition of propolis to chitosan-folic acid nanoparticles also did not significantly alter PDI compared to NP-KF. NP-KF-P had a PDI value of 0.380. The PDI value (< 0.5) of the NP-KF-P also showed that the diameter of the nanoparticles was relatively homogeneous. The zeta potential of the NF-KF-P was 29.7 mV (Table 10.4), indicating that NP-KF-P had been successfully synthesized and will not easily aggregate. This result was compatible with the SEM results of the NP-KF-P (Fig. 10.3b).

**Table 10.3** Propolis encapsulation efficiency in NP-KF-P

| Propolis encapsulation efficiency (%) | Propolis concentration in NP-KF-P (μg/mL) |
|---|---|
| 76.4 ± 2.4 | 8.73 ± 0.29 |
| 60. 3 ± 5.3 | 6.93 ± 0.62 |
| 50.2 ± 0.2 | 5.73 ± 0,03 |
| 40.3 ± 0.6 | 4.6 ± 0.07 |
| 35.1 ± 2.3 | 3.87 ± 0.27 |

**Table 10.4** Diameter, distribution and zeta potensial of chitosan-folic acid nanoparticles

| Nanoparticle | Diameter of nanoparticle ± SD (nm) | PDI | Zeta potensial ± SD (mV) |
|---|---|---|---|
| Chitosan-folic acid nanoparticle blanks (NP-KF-blanks) | 129 ± 3.4 | 0.369 | 30.5 ± 1.04 |
| Chitosan-folic acid-propolis nanoparticles (NP-KF-P) | 153.9 ± 1.3 | 0.380 | 29.7 ± 0.82 |

PDI = Polydispersity Index

### *10.4.3 FTIR Analysis*

FTIR analysis was used to distinguish among folic acid conjugated chitosan, folic acid conjugated chitosan nanoparticles, and folic acid chitosan nanoparticles containing propolis. Figure 10.4 showed that chitosan had a broad absorption area for O–H and N–H groups. The conjugation of chitosan with folic acid narrowed the absorption area of the O–H and N–H (Fig. 10.2b-1), which was probably caused by the appearance of a suspected bond between the N–H group in chitosan with the carboxyl group in folic acid. Additionally, there was an amide group [C(=O)N] at the wavenumber of 1656.85 $cm^{-1}$, which was a unique bond between the amine (in chitosan) and the carboxyl group (in folic acid). These FTIR results showed that chitosan was conjugated with folic acid.

The FTIR analysis on chitosan-folic acid blank (NP-KF-blanks) nanoparticles showed that the absorption area of O–H and N–H groups of this nanoparticle was smaller than (Fig. 10.5c-1) chitosan-folic acid powder (10.5b-1). This feature was

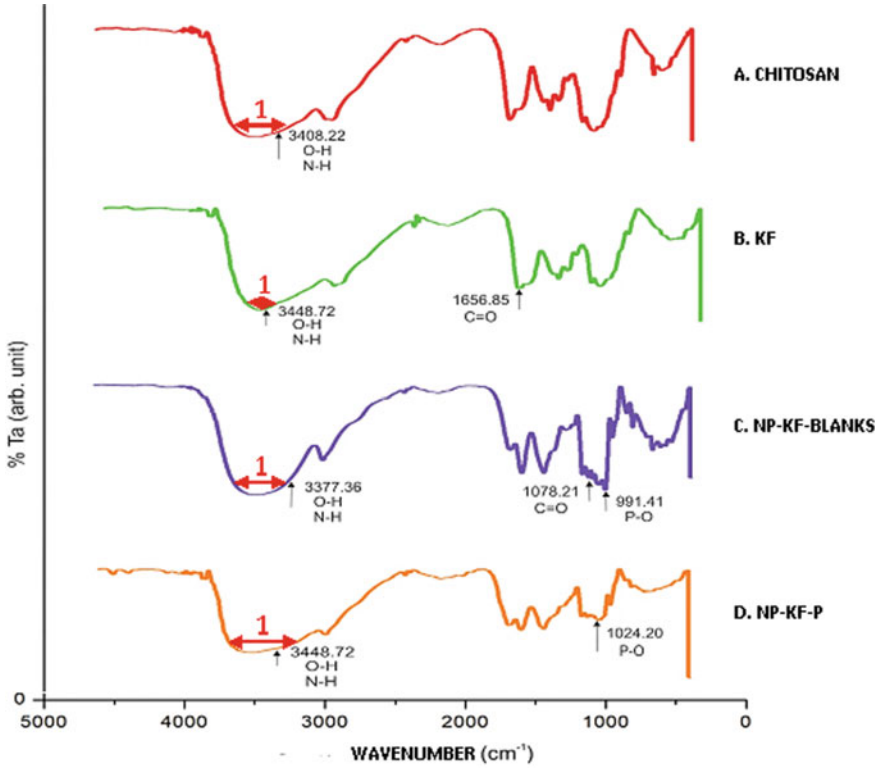


**Fig. 10.5** FTIR results of chitosan, KF, NP-KF-blanks, NP-KF-P. KF = chitosan folic acid; NP-KF = chitosan-folic acid nanoparticles; NP-KF-P = NP-KF containing propolis. (1) broad absorption area of O–H and N–H groups

probably affected by a bond between the N–H group from chitosan and the phosphate group from TPP. According to Mattu et al. (2013, the phosphate group in TPP would bind to the amine group in chitosan), in the formation of nanoparticles. The FTIR result of NP-KF-P also showed the presence of phosphate groups (P-O) from TPP at a wavenumber of 991.41. According to Pramanik et al. (2009), the phosphate group (P-O) from TPP bound to chitosan absorbed the infrared light in the wavenumber range 493–1047.

FTIR of chitosan-folic acid nanoparticles containing propolis (NP-KF-P) showed that propolis had been encapsulated in chitosan-folic acid nanoparticles. The addition of propolis in the chitosan-folic acid nanoparticles broadens the absorption area of the O–H and N–H groups (Fig. 10.5d-1) compared to the chitosan-folic acid nanoparticles blanks (Fig. 10.5c-1). The O–H group of propolis might affect the widening of the O–H and N–H groups' absorption area in chitosan-folic acid nanoparticles. Huang et al. (2014) stated that propolis contains various flavonoid compounds that have O–H groups. Hasan et al. (2013) also explained that the FTIR analysis of propolis compounds showed a broad O–H group at a wavenumber of 3267 due to its phenolic compounds, especially flavonoids.

### *10.4.4* In vitro *Study of Propolis Released from NP-KF-P*

As shown in Fig. 10.6, the release of propolis from NP-KF-P occurred quickly in an acidic environment (pH 4 and 6). After 48 h in an acidic environment, 89% of propolis was released from NP-KF-P at pH 4 and 60% was released at pH 6. In contrast, only a small percentage of propolis (13%) was released from NP-KF-P at pH 7. This condition would be beneficial for the propolis delivery system because the

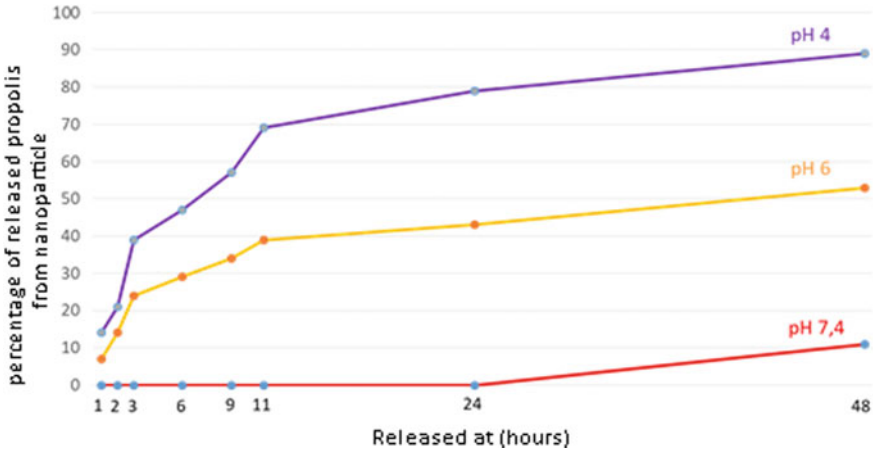


**Fig. 10.6** In vitro study of propolis released from NP-KF-P at three different pH variations

nanoparticle could be delivered into the cells, and then inside the cell, propolis could be released inside the lysosome, which has an acidic environment. Alternatively, the NP-KF-P could be transported safely through the blood (pH 7) to the tumor tissue. The propolis would be released around the tumor tissue due to its acid environment.

## 10.5 Conclusion

The synthesis of folic acid conjugated chitosan nanoparticles that encapsulated propolis is regulated by several factors, including folic acid:chitosan ratio, the molecular weight of chitosan, the proportion of chitosan-folic acid to TPP during ionic gelation, the concentration of propolis used, and the duration of sonication. Spherical propolis conjugated chitosan-folic acid nanoparticles were successfully synthesized with nanoparticles with a diameter of 129 ± 3.4 nm (NP-KF-blanks) and 153.9 ± 1.3 nm (NP-KF-P). Sonication of the chitosan-folic acid solution before synthesis for 30 min reduced the diameter of chitosan-folic acid-propolis nanoparticles, with a propolis encapsulation efficiency between 35.1 ± 2.3% and 76.4 ± 2.4%. Due to the solubility of chitosan in an acidic environment, the percentage of drug released was highest at pH 4 (about 90%).

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