PREPARATION OF NANOPARICLE OF PAPAYA *(Carica papaya* L. var. Callina*)* FRUIT SHAKES BY USING IONIC GELATION METHODS

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Abstract. Nanoparticles are drug delivery systems that can increase the effectiveness of active ingredients. Papaya fruit sap *(Carica papaya* L. var. Callina) is known to contain active compounds such as papain, flavonoids, and saponins that have antibacterial potential. This study aims to make and characterize nanoparticles from papaya fruit sap powder using the ionic gelation method. Papaya gum powder was obtained through a drying process with the addition of sodium metabisulfite, then formulated into nanoparticles using chitosan and sodium tripolyphosphate (NaTPP). Characterization tests included percent transmittance (UV-Vis), *particle size* and zeta potential (PSA), and morphology (SEM). The results showed that the nanoparticles had an average *particle size* of 58.3 nm, a polydispersity index of 0.429, and a zeta potential value of +26.61 mV. The particle morphology showed an irregular shape and agglomeration. This study proves that papaya fruit gum powder nanoparticles can be prepared by the ionic gelation method and show good physicochemical characteristics.

Key words: [Papaya fruit sap, nanoparticles, ionic gelation, chitosan, characterization]

INTRODUCTION

Indonesia is an agricultural country rich in biological resources, including medicinal plants that have the potential to be developed as active ingredients in pharmaceutical preparations. One local plant that has great potential is papaya *(Carica papaya* L.), especially the California variety (var. *Callina*). Papaya fruit contains white sap that is rich in proteolytic enzymes such as papain, as well as other bioactive compounds such as flavonoids, saponins, and alkaloids (Malle *et al*., 2015; Milind, 2011). These compounds are known to have various biological activities, such as antibacterial, anti-inflammatory, anticancer, and accelerating wound healing (Fitria *et al*., 2014).

Papain in papaya gum acts as an *enzymatic debridement* agent, by liquefying necrotic tissue (*eschar*) and facilitating cell migration to the wound area, thus accelerating the healing process (Fuadah *et al*., 2023). In addition, carpain exhibits antibacterial activity by inhibiting the growth of pathogenic bacteria, such as *Staphylococcus aureus* (Fitria *et al*., 2014). However, conventional formulations of papaya gum, such as granulation, show lower antibacterial effectiveness than standard antibiotics (Fuadah *et al*., 2023).

The development of new dosage forms, such as nanoparticles, is one alternative to increase the effectiveness of active compounds in papaya gum. Nanoparticle technology is able to increase the solubility, stability, bioavailability, and penetration ability of active substances to the therapeutic target (Abdassah, 2017; Jamkhande *et al*., 2019). Nanoparticles are defined as particles of 1-100 nm in size that have a high surface area, are able to penetrate tissues efficiently, and modify drug release profiles (Mohanraj & Chen, 2006). In addition, this technology enables more selective delivery of active substances and reduces systemic side effects (Kawashima *et al*., 2000).

In nanoparticle formulations, the use of natural polymers such as chitosan is widely developed due to its biocompatible, biodegradable, non-toxic, and positive charge at low pH, so that it easily interacts with anions (Pertiwi *et al*., 2018). To form nanoparticles, cross-linking agents such as sodium tripolyphosphate (NaTPP) are needed, which acts as a polyanion in the ionic gelation process. The ionic gelation method is a technique for forming nanoparticles through electrostatic interactions between chitosan amine groups and NaTPP phosphate groups without the use of organic solvents (Setiawan *et al*., 2015; Giri *et al*., 2021).

This method is considered simple, efficient, and safe because the process takes place at room temperature and uses environmentally friendly materials (Fucinõs *et al*., 2014). To maintain stability and prevent particle agglomeration during the formulation process, surfactants such as Tween 80 are also added (Rahmi & Sari, 2014). Stable nanoparticles generally have a zeta potential above ±25 mV, which reflects the repulsive force between particles, thus preventing agglomeration (Mohanraj & Chen, 2006). In addition, nanoparticle characterization is essential to evaluate the success of the formulation. Parameters that are often used include *particle size* and polydispersity index (PdI) which indicate *particle size* homogeneity (Sreeram & Nair, 2008), percent transmittance to measure solution clarity (Maharani *et al*., 2022), and surface morphology via Scanning Electron Microscopy (SEM) to see particle shape and the possibility of agglomeration (Abdelwahed *et al*., 2006).

METHODS

This research is a laboratory experiment. Papaya fruit sap was dried into powder with the addition of sodium metabisulfite. Nanoparticles were prepared through ionic gelation using chitosan as polymer and NaTPP as crosslinking agent. Evaluation of nanoparticles included: percent transmittance (UV-Vis), *particle size* and zeta potential (PSA), and particle morphology (SEM).

RESULT AND DISCUSSION

1. Results of Papaya Gummy Powder Preparation

Papaya fruit sap taken from Regaloh Village, Tlogowungu Pati was selected fruit that is still on the tree trunk, unripe fruit aged 2.5-3 months, green, fresh, and healthy, incised at the time (05.30-08.00 WIB) to avoid environmental influences that can damage the enzyme content in the sap. Nainggolan (2003) direct sunlight, dust, and high temperatures can reduce the proteolytic level of the sap. The incision is done lengthwise from the base to the tip of the fruit using a sharp knife with a depth of 2 mm and a distance between incisions of 1-2 cm. The resulting sap is collected in a plastic cup container and stored at 10°C to avoid unpleasant odors (Hidayat *et al*., 2023).

The sap collected in the container was then weighed as much as 30 grams. After that, the sap was mixed with 0.84 grams of sodium metabisulfite, which was then dissolved in 120 ml of distilled water. The purpose of adding sodium metabisulfite is to maintain proteolytic effectiveness and prevent damage to enzymes (Anggraini *et al*., 2020). Then the mixture was stirred evenly with a mortar, and this mixture formed a milky white sap emulsion. Then the sap emulsion was oven dried at ± 55 °C for 5 hours. After drying, the sap was taken and then crushed with a mortar and sieved with a 40 mesh sieve and stored in a container (Fuadah *et al*., 2023).

Table 1. Results of Papaya Fruit Gum Powder Preparation

Wet weight of sap (gram)	Wet sap color	Dry weight of sap (gram)	Color of dried sap	Drying shrinkage (%)
30	Yellow-white	3,35	Yellow-white	89%

Source: Processed Primary Data (2025)

Table 1 shows that the color stability of the sap in wet and dry conditions can be caused by the addition of sodium metabisulfite, which can prevent oxidation reactions, so that it will stabilize the clear color of the fruit sap and maintain the degree of whiteness in the fruit sap (Zainal, 2003). Drying shrinkage is one of the non-specific parameters whose purpose is to provide a maximum limit (range) on the amount of compounds lost in the drying process (Djulfikri *et al*., 2023). The drying results showed a weight loss of 89%, which means that most of the water content in the sap has evaporated. Drying at 55°C is done so that water can evaporate properly without damaging enzymes or other active substances present in papaya sap. The drying process is strongly influenced by the temperature and the length of drying time. However, drying using a temperature that is too high can result in uneven drying (Martunis, 2012).

2. Preparation of Papaya Fruit Gum *(Carica papaya* L. var. Callina) Nanoparticle Solution

Papaya sap powder was weighed at 50 mg and then dissolved in 0.5 mL of DMSO, and then dissolved in 0.1% NATPP. Next, the mixture of extract and NATPP was poured slowly into a 0.2% chitosan solution in 1% acetic acid. The mixing of chitosan and sodium tripolyphosphate polymers will result in an interaction between the positive charge on the amino group of chitosan and the negative charge of tripolyphosphate, forming a colloid with a size in the nanometer range. Then, 0.5 ml of Tween 80 was added using a *magnetic stirrer* for 20 minutes until all the sodium tripolyphosphate solution ran out and a nanoparticle suspension was formed. The addition of tween 80 aims to make the surfactant produce a uniform nano *particle size* so that it serves to minimize the occurrence of clumping (agglomeration) between particles, so that the nanoparticle formation process will be perfect. The hydrophobic and hydrophilic properties possessed by surfactants will maintain the final *particle size* so that the resulting nanoparticles are more stable (Rahma *et al*., 2021).

3. Percent Transmittance Test Results

Table 3. Results of Percent Transmittance of Getah Powder Nanoparticles

Value (%T)	Results
90,6%	Clear

Source: Processed Primary Data (2025)

Transmittance testing aims to determine the clarity of papaya fruit sap nanoparticle preparations (Wahyu *et al*., 2021). The percent transmittance test was carried out by inserting ± 3.5 mL of papaya gum nanoparticle suspension into a cuvette and measuring at a wavelength of 650 nm in the red spectrum region that has minimal absorbance of compounds, making it suitable for measuring turbidity and clarity (Maharani *et al*., 2022). The blank used was aquadestilata. The results show a transmittance value of 90.6%, which indicates high clarity. This is by Huda & Wahyuningsih (2016), who stated that a transmittance value of close to 100% indicates water-like clarity. This clarity indicates that nanometer-sized particles are well dispersed and not visible to the naked eye.

4. PSA *(Particle size analyzer)* and Polydispersity Index results

Table 4. Results of *Particle Size* and Polydispersity Index of Getah Nanoparticles

Particle Size	Value
Average size (nm)	58.3 nm
Polydispersity index (PI)	0.46 PI

Source: Processed Primary Data (2025)

A Particle Size Analyzer was used to measure the polydispersion index and *particle size* of *papaya* fruit gum *(Carica papaya* L. var. Callina) nanoparticles. From the particle diameter measurement results of papaya fruit gum nanocitosan, Table 4. The average *particle size* formed shows that most of the particles are in the nanometer size range (<100 nm). These characterization results indicate that papaya gum nanocitosan particles still meet the nano*particle size* category, which is 1-100 nm (Jamkhande *et al*., 2019), although there is a small number of inhomogeneous particles.

The polydispersion index value is used to estimate the range of *particle size* distribution present in a sample and determine the presence or absence of aggregation Napsah *et al,* 2014). The smaller the polydispersity index value, the more homogeneous the *particle size*. The polydispersion index value has three ranges, namely monodispersion (≤ 0.3), polydispersion (0.3-0.7), and superdispersion (≥ 0.7). The PdI value of papaya gum nanoparticles is 0.465. The polydispersity index value is in the range of 0.3-0.7, which means that it is in the polydispersion range, indicating that the sample has poor homogeneity, sufficient variability, and has a tendency to aggregate (Nurul *et al*., 2025). The results of this study show that there is no relationship between *particle size* and polydispersion index, because the polydispersion index shows the homogeneity of *particle size*. These results are following the research of Ningsih *et al* (2017) and Mardliyati *et al*, (2012) that there is no relationship between the value of the polydispersion index and the magnitude of the *particle size*, but it is related to the homogeneity of the *particle size* indicated by the number of peaks in the *particle size* analyser measurement results (Mizana, 2020).

5. SEM *(Scanning electron microscope)* results

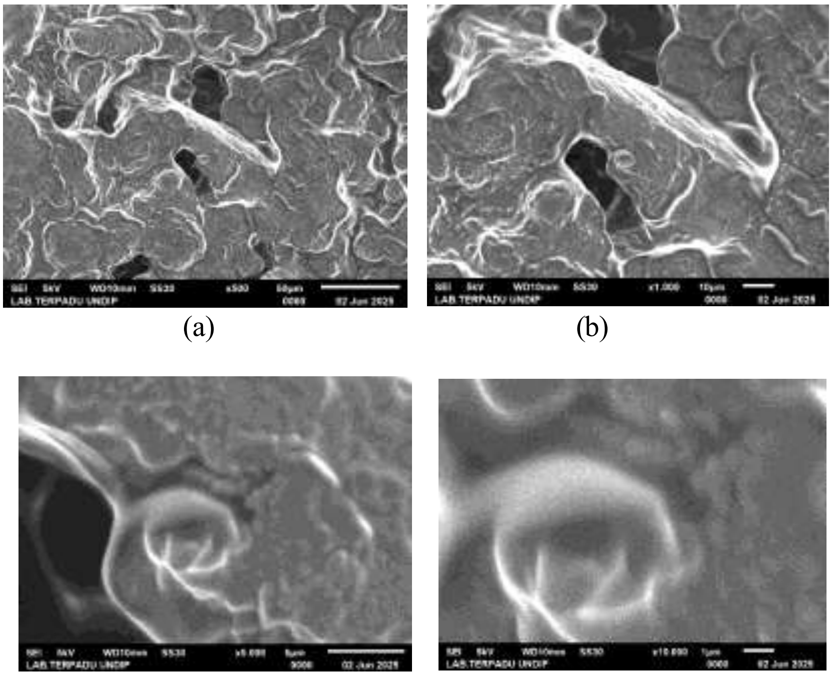

Figure 5. *Scanning Electron Microscopy* Results of Papaya Fruit Sap Chitosan Nanoparticles with (a) 500x magnification, (b) 1000x magnification, (c) 5000x magnification, (d) 10,000x magnification

Physical characterization of particles was carried out by *Scanning Electron Microscopy* (SEM), which was used to observe morphology. This method is an efficient way to obtain images of the specimen surface. The data obtained from SEM is in the form of two-dimensional photographs displaying the specimen surface and *particle size*. SEM analysis was performed with magnifications of 500x, 1000x, and 5000x. 10000x. The morphology of papaya fruit sap nanoparticles can be seen in Figure 5.

The results of SEM analysis of nano chitosan samples show an uneven surface and agglomeration or accumulation at several points, so that the shape cannot be predicted because the gaps between nanoparticles are not visible (Yunita *et al*., 2020). This agglomeration can also occur due to the lack of stable velocity between particles in a sample. The particles in the sample have a nano size, which results in collisions between the particles so that clumps form in the sample. Agglomeration is what makes the microstructure of the sample uneven, but agglomeration does not affect the content of a sample (Daulay *et al*., 2022). Another factor that can affect SEM observation results is the condition of the sample surface. Uneven or too soft surfaces can complicate the focusing process, especially at high magnification, so that the resulting image becomes less clear and the details of particle morphology cannot be observed optimally. In addition, contamination or condensation can also occur if the sample is not completely dry, which can cause artifacts in the SEM image. Therefore, good sample preparation, such as complete drying, is very important to obtain accurate visualization results.

CONCLUSION

Papaya fruit gum powder nanoparticles were successfully formulated by the ionic gelation method. Characterization showed nano *particle size*, fairly homogeneous distribution, and moderate stability. However, further optimization is needed to obtain a more uniform particle morphology and prevent agglomeration.

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