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Optimization of Guava Leaf Extract Loading in Chitosan-Based Nanoencapsulation for Oxidation Inhibition

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Abstract

Guava leaves contain various active compounds, including tannins, phenolics, and flavonoids, with potential as antioxidants, antimicrobials, and anti-inflammatory agents. Guava leaf extract has been used in various fields, including manufacturing, as a corrosion inhibitor. Nanoencapsulation is a method for maintaining the stability and durability of guava leaf extract under various environmental conditions, including changes in pH and temperature, as well as chemical degradation. In this study, guava leaf extract was obtained by ultrasonication. Nanoencapsulation of guava leaf extract at varying concentrations was performed using the ionic gelation method with the natural polymer chitosan and the cross-linking agent STPP. Sample characteristics were identified using X-ray Fluorescence Spectroscopy, Fourier Transform Infrared Spectroscopy, Particle Size Analysis, and Inductively Coupled Plasma–Optical Emission Spectroscopy. The optimal concentration of guava leaf extract in this encapsulation study, 2000–3500 ppm, resulted in particle sizes of 315–390 nm and a polydispersity index below 0.5, indicating well-dispersed particles. The smaller, more dispersed particles will increase the electrostatic bonding between particles, thereby strengthening the interactions involved in the formation of the complex layer. This layer reduces pyrite oxidation by decreasing the release of sulfur and iron into the leaching environment. Nanoencapsulation of guava leaf extract using the ionic gelation method with chitosan is quite effective.

1. Introduction

Guava leaves have long been known for their numerous benefits, particularly in health. Traditional medicine uses guava leaves to make tea for treating digestive problems, as a remedy for intestinal parasites, and for conditions such as diabetes and metabolic disorders. Further research on the composition of guava leaves has revealed the presence of active compounds, including flavonoids (quercetin, kaempferol, and myricetin), tannins, phenolics (gallic acid, ferulic acid, and caffeic acid), carotenoids, and triterpenoids [1]. The various bioactive compounds in guava leaves have potential as antioxidants, antimicrobials, and anti-inflammatories, which are highly valuable in the fields of health, food, and the pharmaceutical industry [2], and the manufacturing sector.

Guava leaves extract has been widely applied in the health sector [3] and used in food processing [4]. Recent studies have shown that guava leaf extract is highly effective at inhibiting oxidation reactions during the corrosion of iron and steel alloys. In addition to its role as a corrosion inhibitor, guava leaf extract also shows potential to inhibit oxidation reactions in pyrite (FeS_2), suggesting a surface-protective or passivation effect. Oxidized pyrite minerals cause acid mine drainage, which can degrade water quality, increase the mobilization of dissolved metals, and result in long-term environmental impacts on aquatic and soil ecosystems in mining areas. The use of environmentally friendly natural materials has the potential to reduce acid mine drainage caused by pyrite oxidation. Guava leaves are a natural material with potential as an inhibitor, proven effective at inhibiting corrosion of metals and steel exposed to acidic conditions

[5]. Various compounds in guava leaves, such as polyphenols and tannins, play a role in forming a protective layer that inhibits oxidation. The hydroxyl (-OH) groups contribute free electrons to electrostatically bind metal ions, thereby forming a stable, passivating complex layer [5]. This layer acts as an inhibitor, reducing the interaction between oxygen and water with pyrite minerals.

Various extraction methods have been developed to produce optimal guava leaf extracts. Guava leaf extraction using the maceration method with 30–70% ethanol [6] and water [7], as well as the Soxhlet method [8]. Ultrasound-Assisted Extraction (UAE) is an extraction method that uses ultrasonic waves to enhance extraction efficiency in a shorter time. The use of ultrasonication improves extraction efficiency by accelerating the process up to 40 times, saving 30–85% in energy, and reducing production costs by up to 69% [9]. Probe-based ultrasonication has the advantage of concentrating energy on localized samples, thereby making cavitation more efficient during the extraction process [10].

Nanoencapsulation is a method used to enhance the stability and shelf life of guava leaf extract under various environmental conditions. One simple method is ionic gelation using natural polymers such as chitosan and the cross-linking agent TPP (tripolyphosphate). Guava leaf nanoencapsulation has been widely utilized, particularly in the medical field, including as a natural textile product for cotton gauze and wound-healing bandages [11]. As a natural polymer, chitosan has been widely used because it has proven quite effective as a matrix in the encapsulation process, including enhancing the stability and antimicrobial activity of mango extracts [12] and the stability of mefenamic acid against the effects of temperature and light [13].

The encapsulation of active substances using chitosan nanostructures begins when the negative charge of the active compound (e.g., the hydroxyl group -OH) binds to the positive charge of chitosan, forming hydrogen bonds. Subsequently, the addition of a cross-linking agent (such as TPP) causes it to bind with chitosan, forming a three-dimensional network (cross-linked). The active substance is trapped (encapsulated) within the formed chitosan nanoparticle structure. This is because the electrostatic bonds between chitosan and TPP are stronger than hydrogen bonds [14]. Various developments in guava leaf extracts have prompted the author to conduct research on the nanoencapsulation of guava leaves using simple methods such as ionic gelation. In this work, guava leaf extract was obtained through ultrasonication using an ultrasonic homogenizer. Variations in the concentration of guava leaf extract during nanoencapsulation were analyzed to determine their effects on the characteristics of the resulting microcapsules, including particle size.

2. Experimental

2.1. Materials and Equipment

The materials used were guava leaves, distilled water, 96% ethanol, chitosan, glacial acetic acid (Sigma Aldrich), sodium tripolyphosphate (STPP), NaOH (Merck), and filter paper. The equipment used in this study included a set of glassware, oven, magnetic stirrer, coffee grinder, sieve shaker, digital balance (Ohaus), ultrasonic homogenizer, vacuum evaporator, pH meter, Particle Size Analyzer (PSA), (FTIR – Fourier Transform Infrared Spectroscopy), X-ray Fluorescence (XRF), and Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP-OES).

2.2. Guava Leaves Extraction

The extraction process began with washing the guava leaves using distilled water, followed by drying in a low-temperature oven. The dried leaves were then ground into powder using a grinder and sieved to obtain a uniform particle size. The resulting leaf powder was macerated in 96% ethanol to facilitate the extraction of bioactive compounds.

The guava leaf–ethanol mixture was subsequently subjected to ultrasonic-assisted extraction using an ultrasonic homogenizer to disrupt plant cell walls and enhance the diffusion of bioactive constituents into the solvent. Extraction was carried out at a frequency of 20– 25 kHz using a 6 mm probe diameter, with an output power of 60% (250 W) for 60 s. The extraction temperature was maintained at -5°C using a temperature-controlled probe.

Following sonication, the extract was concentrated using a vacuum evaporator to remove the solvent, yielding a concentrated guava leaf extract. The extract produced in this study was a crude extract and not standardized for any specific active compounds.

2.3. Nanoencapsulation of Guava Leaf Extract with Chitosan–STPP

Nanoencapsulation of guava leaf extract was carried out using the ionic gelation method with chitosan as the polymer matrix and sodium tripolyphosphate (STPP) as the cross-linking agent. Guava leaf extract was weighed according to the desired concentration and dissolved in 10 mL of an ethanol–distilled water mixture to prepare extract solutions with concentrations of 500, 1000, 2000, 3500, 5000, and 8000 ppm (mg/L).

Chitosan solution was prepared by dissolving 320 mg of chitosan in 160 mL of 1% (v/v) acetic acid under magnetic stirring at 60°C for 1 hour. The diluted guava leaf extract was then added dropwise to the chitosan solution and stirred continuously for an additional 1 h to ensure homogeneous mixing. Subsequently, 40 mL of STPP solution (2 mg/L) was added gradually to the extract–chitosan mixture while maintaining the temperature at 60°C. The mixture was stirred for 1 hour to facilitate ionic cross-linking and nanoparticle formation. Afterward, 1 M NaOH was added dropwise until the pH reached 6. The resulting suspension was filtered through filter paper and then allowed to stand for 24 hours to facilitate the separation of the non-encapsulated extract from the nanoencapsulated fraction.

2.4. Leaching Test

Guava leaf extract (PGL) and its nanoencapsulated form were evaluated for their effectiveness as oxidation inhibitors on pyrite surfaces. The test specimens consisted of pure pyrite (FeS_2) stones with uniform dimensions of 1 × 1 × 1 cm to ensure consistent surface area and exposure conditions. Prior to treatment, all samples were cleaned to remove surface impurities and dried under controlled conditions.

The coating process was performed using the dip-coating method. Pyrite samples were immersed in either the guava leaf extract solution or the nanoencapsulated extract suspension for a predetermined period to facilitate the adsorption of active compounds onto the mineral surface. After immersion, the samples were withdrawn at a controlled rate and dried to form a stable protective layer. The coating procedure was repeated as necessary to obtain a uniform and adequate coating thickness.

To evaluate coating performance, both coated and uncoated pyrite samples were subjected to leaching tests. The samples were immersed in 1 M HCl solution for 48 hours (2 × 24 hours) under controlled conditions to accelerate pyrite dissolution and simulate an aggressive acidic environment. During the leaching process, the release of iron (Fe) and sulfur (S) from the pyrite matrix into the solution was monitored as an indicator of pyrite oxidation and degradation.

Following the leaching period, the concentrations of Fe and S in the leachate were determined using Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP–OES), a sensitive and reliable technique for quantitative multi-element analysis. The inhibitory performance of the coatings was assessed by comparing the concentrations of released Fe and S ions between coated and uncoated pyrite samples.

3. Results and Discussion

3.1. Characteristics of Guava Leaf Extract

The guava leaf extract obtained was a viscous extract with a dark greenish-black appearance. The extraction process yielded approximately 10% extract relative to the dry weight of the guava leaves used, corresponding to a yield of 1 part extract per 10 parts raw material. XRF analysis was conducted to identify the inorganic constituents present in the guava leaves, as shown in Figure 1. The analysis revealed a low ash fraction of approximately 2.4 wt%, consisting predominantly of potassium (K), chlorine (Cl), sulfur (S), and phosphorus (P) compounds. The remaining fraction was not quantified by XRF, as the technique has limited sensitivity toward light elements and organic components.

Therefore, the unquantified portion is presumed to consist primarily of organic matter, including bioactive phytochemicals such as polyphenols, tannins, flavonoids, and other secondary metabolites commonly found in guava leaves. The low inorganic content and predominance of organic constituents suggest that the extract is rich in phytochemical compounds that may contribute to its antioxidant and metal-chelating properties, making it a potential candidate for pyrite oxidation inhibition.

As shown in Table 1, the inorganic constituents detected by XRF were reported in their oxide-equivalent forms. The dominant inorganic component was potassium, expressed as K_2O (1.30%). Potassium is an essential macronutrient in plants and plays important roles in enzyme activation, osmotic regulation, photosynthesis, and overall plant metabolism [15]. Other detected elements included chlorine (Cl, 0.413%), sulfur expressed as SO_3 (0.236%), and phosphorus expressed as P_2O_5 (0.146%), which are involved in various physiological and metabolic processes in plants [16, 17, 18].

Table 1. Mineral oxides of the guava leaves extract by XRF

No.	Element	% Mass
1.	MgO	0.0638
2.	SiO_2	0.0801
3.	P_2O_5	0.146
4.	SO_3	0.236
5.	Cl	0.413
6.	K_2O	1.30
7.	CaO	0.0635
8.	Fe_2O_3	0.0087
9.	CuO	0.0041
10.	ZnO	0.0111
11.	Rb_2O	0.0062
12.	Ag_2O	0.107
13.	Balance	97.6

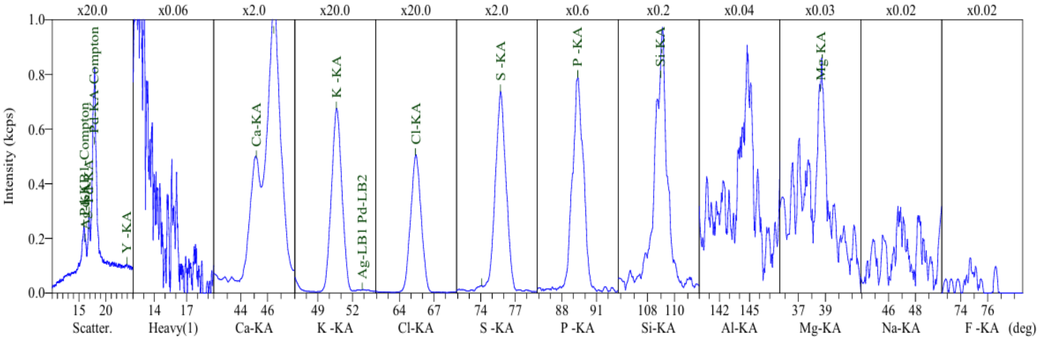

Figure 1. Mineral composition of guava leaves extract by XRF

Several trace elements were also detected at relatively low concentrations, including Fe_2O_3 (0.0087%), CuO (0.0041%), and ZnO (0.0111%). In addition, silver (Ag) was detected at 0.107%. Since silver is not commonly reported as a major constituent of guava leaves, its presence may be associated with environmental contamination, sample handling, or instrumental factors. Further analysis would be required to confirm its origin.

No detectable lead (Pb) was observed in the sample, indicating the absence of this heavy metal within the detection limits of the XRF analysis. Overall, the results suggest that the inorganic fraction of the guava leaf extract is composed primarily of essential plant nutrients and trace elements, with no detectable Pb contamination.

3.2. FTIR Analysis of Nanoencapsulated Guava Leaves

FTIR spectroscopy was used to identify the functional groups present in the guava leaf extract and its nanoencapsulated form, and to evaluate interactions during the encapsulation process [19]. Based on Figure 2, the spectra of both samples showed several characteristic absorption bands, indicating the presence of similar functional groups before and after nanoencapsulation. The most prominent band, observed at approximately 3307 cm^{-1}, corresponded to O–H stretching vibrations. This broad band may be attributed to hydroxyl groups in the phenolic compounds of the guava leaf extract, as well as hydroxyl and amino groups in the chitosan matrix. Absorption bands in the 3307–2940 cm^{-1} region are characteristic of hydroxyl-containing compounds and are commonly associated with phenolic constituents [20].

The absorption band in the 1630–1650 cm^{-1} region is generally interpreted as the C=O functional group (amide) or the C=C functional group of aromatic compounds [21], taking into account the possibility of band overlap between chitosan and the extract during the encapsulation process. The C=O bond appears at 1560.11 cm^{-1} as a catechin compound, as well as the amide group of chitosan as the matrix. At this peak, there is likely an overlap of several vibrational contributions from the compounds. In the nanoencapsulation, absorption was observed in the low wavenumber regions of 470 cm^{-1} and 480 cm^{-1}. However, the appearance of these bands cannot be directly interpreted as a shift from the C–H bond, due to the excessive distance between them. These absorption bands are likely related to vibrational contributions in the low wavenumber region, such as interactions between the chitosan–STPP matrix structure and/or contributions from phosphate groups.

The peak at 1486.25 cm^{-1} corresponds to C–H vibrations of the CH_2 group, while the peak at 1087.08 cm^{-1} is attributed to C–O stretching of primary alcohols. The peak at 1383.08 cm^{-1} represents O–H deformation vibrations, and the peak at 1017.19 cm^{-1} corresponds to C– O stretching of secondary alcohols. In the guava leaf extract, the 1017.19 cm^{-1} peak appears with relatively high intensity but is absent in the nanoencapsulated sample, which is suspected to have bound to other compounds during the encapsulation process. The absorption band around 2100–2200 cm^{-1} indicates the presence of C≡C functional groups. These absorption bands are associated with phenolic compounds, including tannins and flavonoids, which are major constituents of guava leaf extract [20]. The FTIR results show that the nanoencapsulation process of the guava leaf extract does not alter the main structure of the compounds present in the extract.

3.3. Characteristics of Guava Leaf Nanoencapsulation

Nanoencapsulation of active compounds is one approach to enhance the stability and durability of bioactive substances under various conditions. The nanoencapsulation process of tannins begins with the initial interaction of active compounds present in guava leaf extract, as shown in Figure 3. Electrostatic interactions lead to the formation of an initial complex between the active compounds—containing carboxyl (COOH) and hydroxyl (OH) functional groups—and the amine (NH_3^+) groups of the chitosan solution. After the active compounds are adsorbed onto the chitosan chains, STPP is added as a crosslinking agent. Strong electrostatic interactions then occur between the phosphate groups of STPP and the amine groups of chitosan, resulting in the formation of a polymeric network. In this process, the guava leaf extract becomes entrapped (encapsulated) within the chitosan–STPP matrix formed through the crosslinking mechanism.

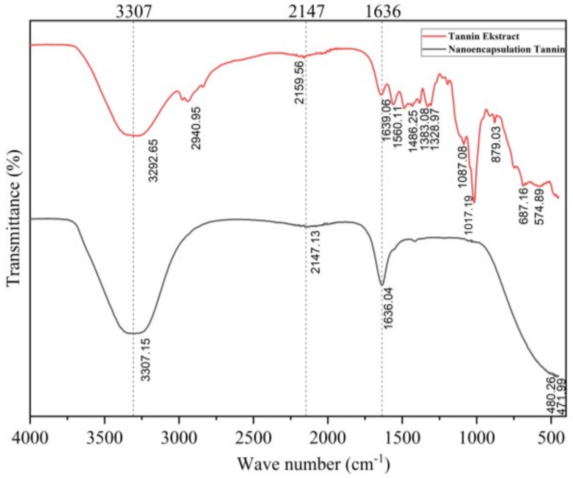

Figure 2. FTIR spectra of nanoencapsulated guava leaves

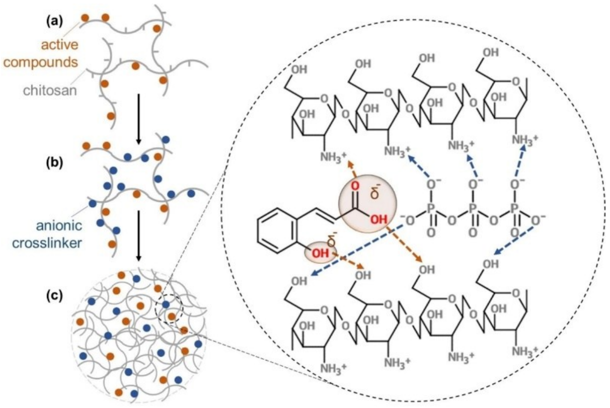

Figure 3. Diagram of active compound nanoencapsulation in chitosan [14]

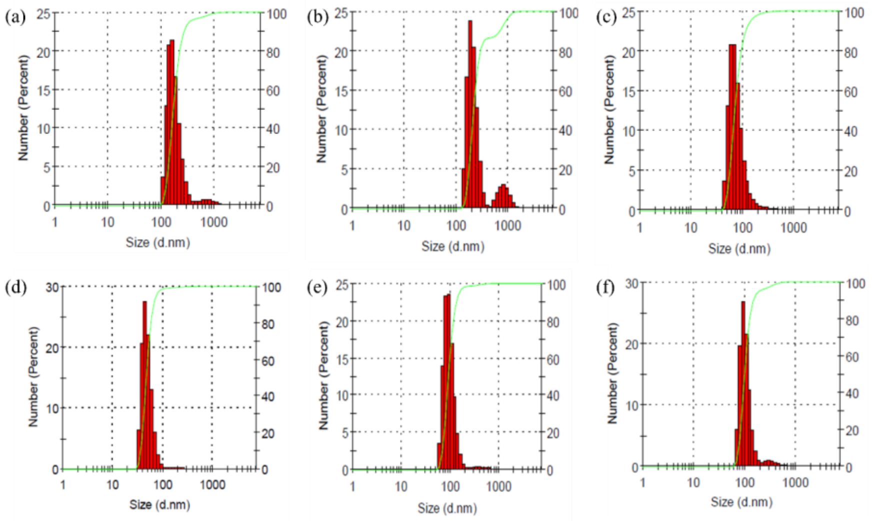

Figure 4. Particle size distribution of guava leaf nanoencapsulation in concentration (a) 500 ppm, (b) 1000 ppm, (c) 2500 ppm, (d) 3500 ppm, (e) 5000 ppm, and (d) 8000 ppm

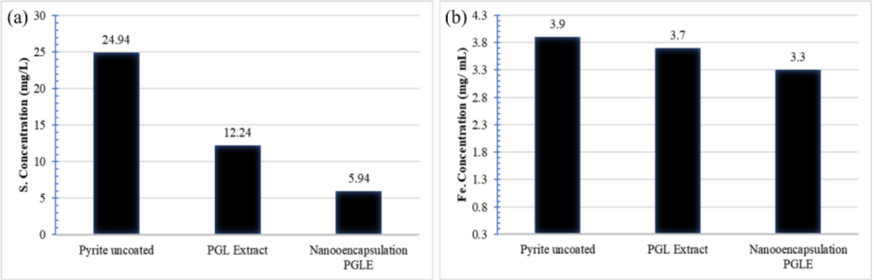

Figure 5. Concentration of (a) sulfur and (b) iron released for different fossil fuels that contain pyrite (FeS_2)

Table 2. Particle size of guava leaf nanoencapsulation

Guava leaf extract concentration (ppm)	Particle size (nm)	Pd Index
500	665.8	0.908
1000	1119	0.682
2000	315	0.448
3500	390.8	0.438
5000	462	0.617
8000	687	0.621

Particle size is an important parameter that determines the properties and characteristics of a material. The results of the PSA analysis showed that the particle sizes obtained from the nanoencapsulation process, based on the concentration of guava leaf extract, are presented in Table 2. As shown in the table, nanoencapsulation of guava leaf extract using the ion-exchange method with chitosan polymer resulted in a wide range of particle sizes. This is consistent with previous studies, where grape extract produced particles ranging from 418 to 853 nm [22], dragon fruit extract from 234.49 to 977.50 nm [23], and temu kunci extract from 389 to 877 nm [24]. At a guava leaf extract concentration of 500 ppm, the resulting particle size was 665.8 nm with a PDI value of 0.908. A PDI value close to 1 indicates that the particle size distribution in the nanoencapsulation process at an extract concentration of 500 ppm suggests that the particles have not yet dispersed properly. This indicates that the encapsulation process is not yet stable and the resulting particles exhibit highly variable sizes. At an extract concentration of 1000 ppm, the particle size increased to 1119 nm with a PDI of 0.682, suggesting particle aggregation or clumping during the nanoencapsulation process.

However, at an extract concentration of 2000 ppm, the particle size decreased significantly to 315 nm with a PDI of 0.448. At this concentration, the particles exhibit a narrower particle size distribution and appear dispersed. Furthermore, the particle size increased as the extract concentration increased. Based on particle size and the PDI, a concentration of 2000–3500 ppm is optimal under the test conditions, minimizing particle size distribution and maintaining a PDI <0.5.

At extract concentrations below 2000 ppm, an increase in PDI was observed, likely due to the amount of active compounds being too low compared to the amounts of chitosan and STPP. This resulted in suboptimal interaction between the active compounds and the chitosan matrix. Meanwhile, an increase in PDI also occurs at high concentrations above 2000 ppm, likely due to an excessively high amount of extract during the encapsulation process. An excessively high concentration of active compounds disrupts the balance of the bonds between the amine groups of chitosan and the phosphate groups of STPP, causing the particles to fail to disperse properly and form aggregates.

3.4. Leaching Test Analysis

In this study, the leaching test was employed to evaluate the effectiveness of the protective layer formed by guava leaf extract in inhibiting oxidation reactions on pyrite surfaces. The measurements were carried out using the ICP-OES method, which is used to identify and quantify the concentrations of various metals simultaneously. Figure 4 shows the total concentrations of sulfur (S) and iron (Fe) from coated and uncoated fossil samples dissolved in a leaching solution such as HCl acid solution.

Based on Figure 5(a), the uncoated pyrite mineral exhibits the highest sulfur release, reaching 24.94 mg/L, indicating that oxidation proceeds readily in the absence of any protective layer. The application of guava leaf extract significantly reduces sulfur release to 12.54 mg/L, demonstrating its ability to act as an oxidation inhibitor. Furthermore, the use of nanoencapsulated guava leaf extract results in a more pronounced reduction, lowering sulfur release to 5.94 mg/L. This substantial decrease suggests that nanoencapsulation enhances the stability and controlled release of active compounds, leading to the formation of a more uniform and durable protective layer on the pyrite surface. As a result, the interaction between the mineral surface and oxidizing agents such as oxygen and water is effectively minimized.

In addition, Figure 5(b) shows that both guava leaf extract and its nanoencapsulated form contribute to a gradual reduction in iron release. The iron concentration without coating was 3.9 mg/L; it then decreased to 3.7 mg/L with guava leaf extract coating, and further decreased to 3.3 mg/L with PGLE nanoencapsulation coating. The release of FeS_2 in the leaching solution indicates that sulfur release has a higher concentration compared to iron. This is because in the FeS_2 mineral bond, the S-S chemical bond has a weaker bond strength compared to the Fe-S bond. Thus, during the leaching process, the S-S bond breaks first and more easily than the Fe-S bond.

The reduction in sulfur and iron leaching indicates the effectiveness of the protective layer in inhibiting oxidation reactions in pyrite minerals in the leaching medium under test conditions. These results are consistent with the possibility of a surface protection or passivation mechanism, although not directly prove the inhibition of oxidation reactions. The mechanism of protective layer formation occurs when -OH groups and phenolic groups from guava leaf extract and PGLE nanoencapsulation interact with iron (Fe) derived from FeS_2 minerals. This interaction forms a polyphenol-iron chelate complex, which is water-insoluble and stable. This layer can limit direct contact between the pyrite surface and the leaching medium, including HCl acid solution, thereby reducing the release of Fe and S into the solution.

The encapsulation process enhances the protective layer, as chitosan, acting as the matrix, possesses adhesive properties toward the surface and the ability to form a polymer network through ionic interactions with STPP. This structure allows the active compounds from the extract to be distributed more evenly and persist longer on the mineral surface compared to the unencapsulated extract. Nanoencapsulation of guava leaf extract (PGLE) offers a potential approach as a safe and environmentally friendly protective agent against oxidation reactions in pyrite minerals.

4. Conclusion

The guava leaf extract obtained through the extraction method demonstrated a good content of bioactive compounds and no detection of hazardous metals, such as Pb, in the extract. Characterization of the nanoencapsulation of the guava leaf extract indicated the presence of functional groups associated with the main compounds, namely phenolics, polyphenols, and flavonoids. The optimal extract concentration was obtained in the range of 2000–3500 ppm, as indicated by a relatively small particle size with a homogeneous distribution. Solubility tests showed that nanoencapsulation of guava leaf extract effectively inhibits oxidation reactions in pyrite by forming a protective layer. The nanoencapsulation of guava leaf extract demonstrates an increase in the extract's effectiveness in forming a protective layer. However, this method still requires further research when applied on an industrial scale, such as for controlling acidic conditions caused by pyrite oxidation in mining. The consistency of particle size and distribution remains a challenge that needs to be optimized to maintain its stability and effectiveness in large-scale applications.

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