Green Synthesis of Zinc Oxide Nanoparticles using Brown Algae on Oil Palm Empty Fruit Bunch Pulp and Paper Substrates: Effect of pH 6 to 8

Zakiah Sobri,[a] Ainun Zuriyati Mohamed @ Asa'ari,[a,*] Muhammad Azri Mohd Azib,[b] Muhammad Irdin Mohd Nazri,[b] Nur Hanani Zainal Abedin,[c] and Edi Syams Zainudin,[a,b,*]

Green synthesis in producing zinc oxide nanoparticles is well known for its ecofriendly nature and acceptable cost. This study determined the effect of pH 6 to 8 on the green synthesis of zinc oxide nanoparticles using brown algae on oil palm empty fruit bunch pulp (OPEFB) and paper substrates. Ten samples including blank samples were prepared. Brown algae extract was prepared followed by preparation of pulp and paper from commercial OPEFB sheets. The brown algae were set at 3 pH levels, 6 (blank sample), 7 and 8. The PU samples underwent handsheet making based on TAPPI Standard T205, while the PA samples were air-dried prior to testing. All samples were analyzed *via* FESEM, EDX, and XRD, which confirmed that zinc oxide nanoparticles were successfully synthesized on pulp and paper surfaces. It was shown that higher pH levels enhanced the synthesis performance of zinc oxide nanoparticles, and 70 °C was the optimal temperature. The nanoparticles size obtained in this study were 0.27 to 0.54 nm and 0.51 to 1.05 nm for green-synthesized PU and PA samples respectively. Green synthesis was observed to operate better on pulp fiber surfaces rather than paper surfaces.

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Contact information: a: Laboratory of Biopolymer and Derivatives, Institute of Tropical Forestry and Forest Products (INTROP), Universiti Putra Malaysia, 43400, UPM Serdang, Selangor, Malaysia; b: Department of Mechanical Engineering and Materials, Faculty of Engineering, Universiti Putra Malaysia, 43400, UPM Serdang, Selangor, Malaysia; c: Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400, UPM Serdang, Selangor, Malaysia;
** Corresponding authors: ainunzuriyati@upm.edu.my; edisyam@upm.edu.my*

INTRODUCTION

Various approaches can be applied to the synthesis of nanoparticles. For instance, chemical, physical, and green methodologies can be used (Ijaz *et al.* 2020). Green synthesis is becoming a popular approach due to its ecofriendly nature and acceptable cost. It is a subset of green chemistry that promotes sustainable processes in chemical industries based on basic principles such as prevention or reduction of waste, usage of non-toxic materials, and reducing environmental pollution (Ahmed *et al.* 2022). Additionally, green synthesis methods have been used as solutions in environmental issues (Khan 2020), pharmaceutical (Kar *et al*. 2022), and nanotechnologies (Huston *et al*. 2021). The synthesis involves biological agents including algae, fungi, yeast, enzymes, polysaccharides (Gour and Jain

2019), fruit extracts (Chakravarty *et al*. 2022), and microorganisms (Bahrulolum *et al*. 2021). For instance, the extracts may permit biological production of environmentally friendly metallic particles with well-defined size and form (Radulescu *et al.* 2023). Brown algae contain a high concentration of phenolic compounds that have been recognized as a natural source of antioxidants. Brown algae is known as macroalgae compared to other algae. It has high photon conversion efficiency, which is beneficial during synthesis (Song *et al.* 2015). Hence, brown algae is considered an excellent potential in green synthesis based on its abundance (Irianto *et al*. 2024).

The conditions during green synthesis are crucial because the formation of nucleation centers is dependent on pH, temperature, reaction time, and reactant concentration (Vijayaram *et al.* 2024). For instance, higher pH increases the reduction of metal ions (Radulesca *et al.* 2023). El Ouardy *et al.* (2023), who focused on the formation of silver nanoparticles, stated that alkaline pH improves the nanoparticle formation by amplifying positive charged silver ions and negative charged functional groups from microalgae. The average size of 30 nm silver nanoparticles had high crystallinity, as shown by XRD analysis. The work of Tamang *et al.* (2024) showed that nanoparticle growth can be influenced by pH, thus affecting the final size. A polydispersity and smaller size of zinc oxide nanoparticles was shown as a smaller wavelength absorption in UV as pH adjusted from 8 to 9.

Zinc oxide nanoparticles can be regarded as environmentally friendly. They are the most widely produced nanomaterials due to their utilization in huge range of industries including pharmaceutical (sunscreen, acne treatment, shampoo to treat itchy scalp and dandruff), medical (heal wounds due to antimicrobial and anti-inflammatory properties (Farouk *et al*. 2024), cosmetic, construction (paints), plant growth and development, and more. Zinc oxide is also very popular in food technology as a means to resist food spoilage bacteria, human pathogen bacteria, and yeast. The particles have shown considerable bacterial activities against multi-resistant strains, except that the particles are largely insoluble and less concentrated, which made it dependent on species and strains (Buter *et al.* 2023). Hence, antimicrobial activities of zinc oxide nanoparticles reported by Modafer (2024) have shown about 22 to 27 mm inhibition zones measured against various microorganisms. The biggest inhibition zone, 27 m, was found against *Enterococcus faecalis* and *Klebsiella pneumonia*, multi-resistant bacteria. The study also reported promising antiviral activity. The antimicrobial efficiency of nanocellulose hydrogels is influenced by concentration of zinc oxide nanoparticles, as the higher concentration led to effective antimicrobial activity (Huang *et al.* 2019).

Nanoparticles of zinc oxide can be green synthesized using microorganisms, plants, and algae. Abdelhady *et al*. (2024) green-synthesized zinc oxide nanoparticles from fungus as a biocontrol causative agent for potatoes. The synthesis was conducted by adding zinc acetate into mycelium filtrates until the brown colour changed to white precipitate, which was centrifuged and dried at 300 °C. A similar colour shift was observed in zinc oxide nanoparticles synthesized from macroalgae *Hypnea pannosa* (Modafer 2024). Zinc oxide nanoparticles were analyzed *via* EDX and XRD to determine the composition of zinc and oxygen along with crystallinity property of nanoparticles formed. The nanoparticles were in the range of 14.2 nm to 107.5 nm (Modafer 2024). The wavelength absorption and the element bonding spectrum were measured by using UV and FTIR. They absorbed at 281.5 to 380.0 nm, while the Zn-O bond can be found at 615.1 and 471.5 cm^{-1} (Kalaba *et al.* 2021; Abdelhady *et al.* 2024; Modafer 2024).

Oil palm tree is a tropical cultivated plant and a significant economic crop in Malaysia that produces cooking oil, food products, and biofuels. Waste products of oil palm such as palm kernel shell can be utilized as animal feedstock, while oil palm empty fruit bunch (OPEFB) can be used as compost and other products of biomass such as paper. Prior to papermaking, the OPEFB needs to be pulped by a pulping process. Common pulping methods that can be used to process OPEFB are mechanical, chemical, or hybrid. Alkaline pre-treatment with sodium hydroxide is introduced to OPEFB before mechanical pulping to soften the fibers. Hence, the fibers are easily separated into individual fibers during the process. The potential of OPEFB for papermaking has been explored since the 1990s as an alternative raw material that has almost similar properties to wood fiber. It consists of 43% to 65% cellulose and 17% to 33% hemicellulose. Fibers having this composition are suitable in papermaking manufacturing for printing, writing, and corrugated board while minimizing imported recycled fiber. Such fiber has renewable and economic values, which lead to global sustainability *via* commercial applications (Mohd Ali *et al.* 2020). Studies have shown OPEFB contributes to good mechanical properties in paper and composite with the application of low temperature of pulping process and can be applied at a higher percentage for blending process during the papermaking (Mohd Hassan *et al*. 2020; Nanna *et al.* 2020). Practically, chemical pulping such as kraft and sulfite pulping methods are preferred to produce high quality and strong fibers for printing and packaging applications (Mohd Ali *et al.* 2020).

Most studies using green synthesis to produce zinc oxide nanoparticles either have solely produced the particles or grown them on textile surfaces. In this study, the authors carried out the green synthesis directly on pulp and paper. Therefore, this method may avoid the need to add or incorporate zinc oxide in a separate step. Rather, the nanoparticles are directly embedded or attached on the pulp and paper surfaces *via* a green synthesis approach. This paper is targeting to fill the gap and contribute to finding out more about green zinc oxide nanoparticles on different substrates, as well as the effects of pH and other identified parameters such as temperature. In summary, the relationship of pH of algae in generating the green zinc oxide particles on pulp and paper substrates of oil palm empty fruit bunch was analyzed. The morphological properties, composition, and crystallinity of green synthesized zinc oxide particles were analyzed by field emission scanning electron microscopy with energy dispersive X-ray (FESEM-EDX), as well as with X-ray diffraction (XRD).

EXPERIMENTAL

Materials and Chemical

The air-dried brown algae were purchased from Tawau, Sabah, Malaysia. Commercial pulp from oil palm EFB was received from Eco Palm Paper Sdn Bhd, Pekan, Pahang, Malaysia. Zinc chloride ($ZnCl_2$), ACS grade 98% and sodium chloride (NaOH), ACS grade 99% were purchased from R&M Chemicals.

Preparation of Brown Algae Extract

Brown algae was procured in air-dried form, which then were cleaned with distilled water 5 times in order to remove the impurities such as salt. The algae were then oven-dried to achieve less than 10% moisture content for 24 h, and later was ground into powder. An amount of 2 g brown algae powder was heated in 100 mL of distilled water at 60 °C in order to extract an algae solution. The heated brown algae solution was filtered using filter paper and stored at 4 °C in a laboratory refrigerator.

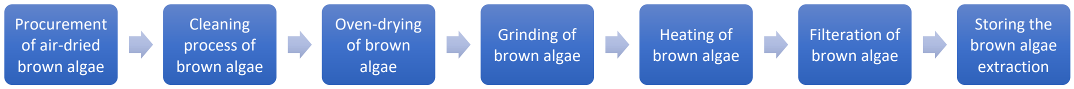


Fig. 1. Preparation of brown algae extract

Preparation of Pulp and Paper Substrates

The commercial oil palm EFB paper (A4 size) was separated into two parts of preparation, namely oil palm EFB (i) paper and (ii) pulp. The paper preparation involved the cutting of the commercial paper into 16 small parts of 6 cm x 4 cm. In preparation of oil palm EFB pulp, the paper is cut into small pieces of 1 cm x 1 cm and then soaked in water for 24 h. A British disintegrator was operated at 3,000 rev in order to disperse the soaked papers into individual fibres of oil palm EFB pulp.

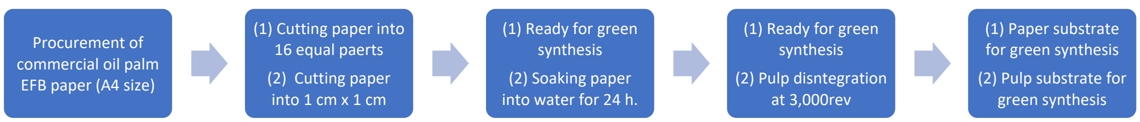


Fig. 2. Preparation of pulp and paper substrates

Green Synthesis of Zinc Oxide Particles on Pulp and Paper Substrates

The zinc oxide particles were green synthesized with 100 mL of brown algae extract and 100 mL of 0.5 M of zinc chloride at 60, 70, and 80 °C for 4 h. The pH level of brown algae extract was measured as pH 6, which was then adjusted with 2% of NaOH solution to pH 7 and 8. Table 1 shows the parameters and codes for all prepared samples. There were 10 samples that had been green synthesized, for which the emphasis was to carry out the process at pH 8 and temperature of 70 °C. The selection was analyzed based on previous study (Sobri *et al*. 2021). Green synthesized pulp was subjected to handsheet making to obtain paper sheet, while green synthesized paper needed to be air-dried prior to paper testing. Both types of papers were tested according to TAPPI Standard T205 (2006).

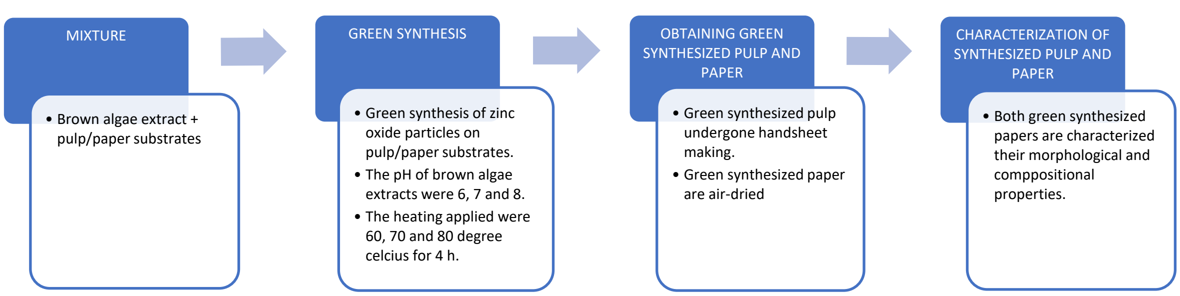


Fig. 3. Green synthesis of zinc oxide particles on pulp and paper substrates

Characterization of Green Synthesized Pulp and Paper

Both types of green synthesized samples were analyzed in order to determine their morphological, compositional and physical properties *via* Field Emission Scanning

Electron Microscopy (FESEM: FEI, NOVA NANOSEM 230 model), Energy Dispersive X-ray (EDX: Oxford Instruments, Max 20 model), and X-ray Diffraction (XRD: Phillips, PW 3040/60 MPD X'PERT HIGH PRO PANALYTICAL model) analysis, respectively.

The morphological observation was carried out using FESEM to observe the distribution of zinc oxide particles, while the compositional such as elemental property was examined using EDX. The crystallinity property of zinc oxide particles on samples was measured with XRD.

Table 1. Codes of Samples and their Parameters

Code of Sample	pH	Temperature	Type of Substrate
670 PU	6	70 °C	Pulp
670 PA			Paper
770 PU	7		Pulp
770 PA			Paper
860 PU	8	60 °C	Pulp
860 PA			Paper
870 PU	8	70 °C	Pulp
870 PA			Paper
880 PU	8	80 °C	Pulp
880 PA			Paper

RESULTS AND DISCUSSION

Morphological Observation of Green Synthesized Pulp and Paper

Based on the FESEM micrographs in Fig. 4, the zinc oxide particles formed on the surfaces were distributed in the form of spherical-shaped clumps. The generation and distribution of zinc oxide particles were more detected on PU (green synthesized samples prepared from pulp substrates) compared to PA (green synthesized samples prepared from paper substrates) samples, as shown in Fig. 4 and illustrated in Fig. 5.

To be specific, this condition was clearly evident in samples 670 PU, 770 PU, and 870 PU. In addition, pH 8 was found to be the best condition to provide green synthesis to both pulp and paper substrates in generating zinc oxide particles. This is supported by Mohammadi and Ghasemi (2018), who mentioned that such a condition is good for the growth of nanoparticles.

Vijayaram *et al.* (2024) also stated that the formation of nanoparticles can be enhanced through higher pH levels. Besides that, a heating temperature of 70 °C provided the greatest impact of green synthesis to occur, as shown for 870 PU.

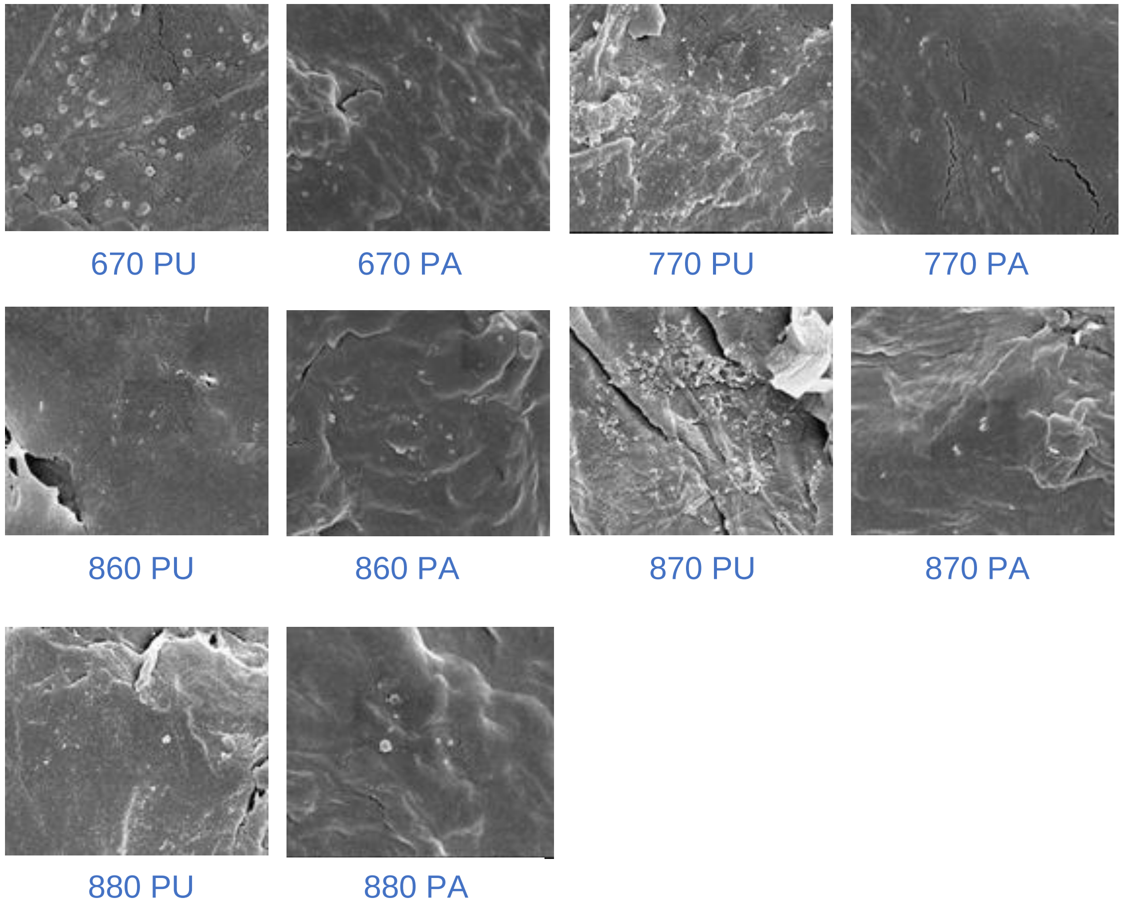
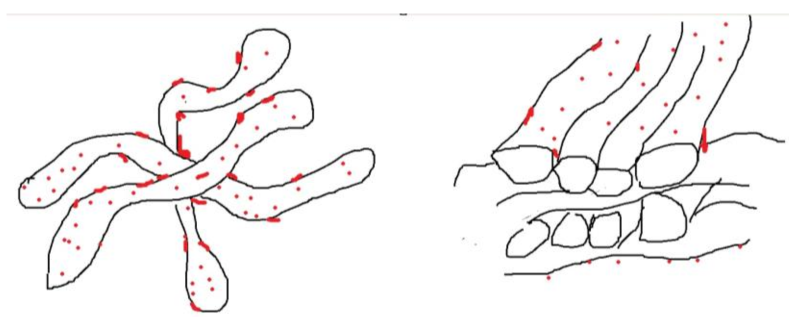


Fig. 4. Green synthesized papers prepared from pulp (left) and paper (right) substrates at 50K magnification



(a) (b)

Fig. 5. Illustration of generation and distribution of zinc oxide particles during green synthesis occurred on (a) pulp and (b) paper substrates

Compositional Analysis of Green Synthesized Pulp and Paper via EDX

The particles detected on the surface of all samples were analyzed for their elemental composition by using EDX. Main element captured was carbon, followed by zinc and oxygen, as shown in Fig. 6. High zinc weight percentage was mostly found in PU samples such as 670 PU, 770 PU, and 870 PU with increments of 4%, beginning with 670 PU. That specimen had a value of 6.13%, 770 PU had a value of 10.65%, and 870 PU had a value of 14.65%. However, in general, PA samples exhibited from 5.45% to 7.63% of zinc weight percentage.

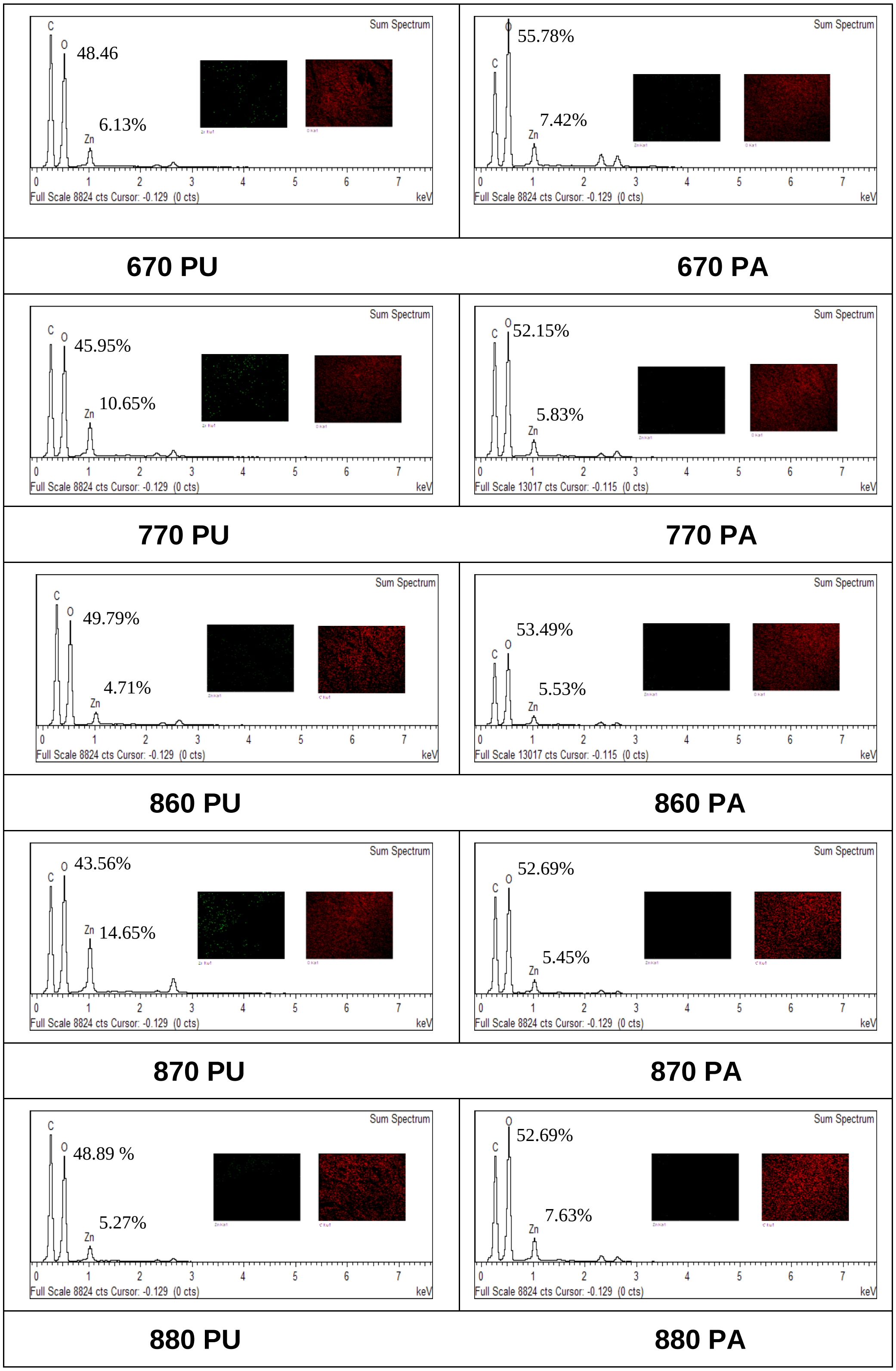


Fig. 6. Composition of zinc and oxygen in green synthesized samples with mapping distribution of the elements

It is clear that green synthesis could be carried out better by using pulp substrates rather than paper samples. In addition, a temperature of 70 °C was observed having the best temperature for green synthesis in this study which 60 °C is not enough to accommodate more green synthesis to happen, while 80° C may degenerate the synthesis mechanism.

According to Bhattacharjee *et al.* (2022), phytochemicals structure in algae extract could deteriorate and become unstable at high temperature, which then leads to reduction of metal ions and uncontrolled aggregation of nanoparticles. Additionally, temperature controls formation of nanoparticles, such that at higher temperature, less nanoparticles may be formed, as was measured by decreasing photocatalytic activity (Abdullah *et al*. 2020). The results of zinc weight percentage are clearer when looking at the mapping area for zinc distribution on pulp and paper samples. More area was covered by zinc in PU samples compared to PA samples.

Physical Analysis of Green Synthesized Pulp and Paper via XRD

The crystallinities of zinc oxide particles of all samples were measured by XRD. The sharp peak indicates crystallinity of the particles formed starting from high intensity with a curve pattern that indicates amorphous materials. The peaks of PA samples were smoother than PU, as shown in Fig. 7. In contrast to PA samples, PU samples exhibited more sharp and clear peaks that indicated the presence of more crystallinity particles. Regardless, the peaks at $2\theta = 21.71^o$ to 22.39^o indicate crystallinity of zinc oxide particles in PU samples, while $2\theta = 21.67^o$ to 22.89^o represent peaks for zinc oxide particles in PA samples. To be specific, the peaks shown in PU samples are sharper and broader with a range of average counts 251.24 cts - 1407.43 cts, which means that the particles formed were small in size. The 870 PU showed the highest peak. This supported the previous findings explained earlier; in the alkaline state of pH 8, temperature of 70°C is an optimum temperature to generate the green synthesis of zinc oxide particles that are in crystalline form.

To determine the performance of PU and PA samples under different pH but same temperature, samples 670, 770, and 870 were compared. The peaks shown in samples 670, 770, and 870 are increasing in intensity and have more narrow peaks, which indicated bigger crystalline zinc oxide particles formed when pH was increased (Babayevska *et al.* 2022) especially for the PU samples. A study conducted by Wang *et al.* (2020), has shown that zinc oxide nanoparticles increased in size and aggregated when the alkaline solution was applied. Besides the changes in particle size, coagulation also occurs at alkaline conditions, which affect the zinc oxide's morphology and particle properties (Doan Thi *et al.* 2020).

Meanwhile, the crystallinity of particles in all paper samples are represented by higher peaks ranging from 1823.32 cts to 2373.78 cts with similar broadness. Such peaks were found in the XRD pattern. At 70 °C, the highest peak is shown by 770 PU at $2\theta = 22.14^o$ followed by 670 and 870 PU. The PA samples do not seem to be influenced by pH parameter after perusing the peaks. Crystallinity of particles is more affected by temperature because high crystal growth is formed at higher temperature which results in bigger particle size (Bandeira *et al.* 2020).

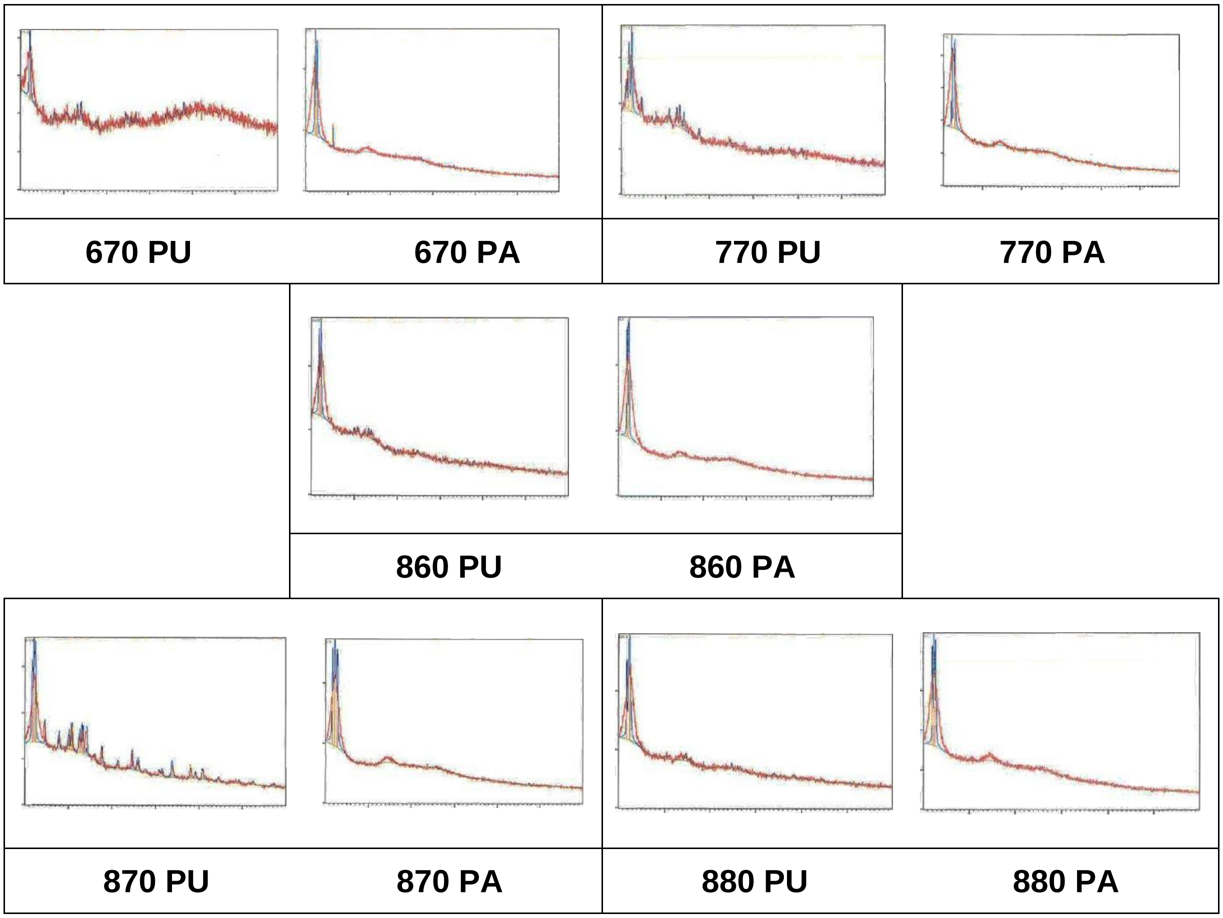


Fig. 7. Crystallinity of zinc oxide particles of PU and PA samples

Crystalline Size of Zinc Oxide Particles on Green Synthesized PU and PA Samples

Based on peak analysis data from XRD, Scherrer's equation is applied to calculate crystallite size of zinc oxide particles formed on green synthesized PU and PA samples as tabulated in Table 2 and Table 3. The equation is as stated below.

$$D = \frac{K\lambda}{\beta \cos \theta} \tag{1}$$

where D is the crystallite size, K is the shape factor (0.89), λ is 0.1541 nm, β is the FHWM, and θ is the angle.

As the pH during green synthesis was increased and at the same time the heating level was kept constant, in instance 670 PU, 770 PU, and 870 PU, the crystallite size became higher. This increasing trend can be seen by comparing samples 670 PA and 770 PA, which showed 0.78 nm to 1.05 nm respectively. This is parallel to the finding by Babayevska *et al.* (2022), who mentioned that the higher pH that can generate higher crystallite size. However, increasing temperature but at the same pH of green synthesize, both PU and PA samples exhibited almost similar trends. Samples 860 PU, 870 PU, and 880 PU showed a decreasing trend. The same was found for samples 860 PA and 880 PA, excluding 870 PA. This finding is in conflict with Bandeira *et al.* (2020), who found that crystallinity of particles is more affected by temperature due to high growth of crystal at higher temperature, which results in bigger particle size.

Table 2. Calculated Crystallite Sizes of Zinc Oxide Particles Green Synthesized PU and PA Samples

Samples	Crystallite Size (nm)	
670	**670 PU**	**670 PA**
	0.44	0.78
770	**770 PU**	**770 PA**
	0.54	1.05
860	**860 PU**	**860 PA**
	0.54	0.82
870	**870 PU**	**870 PA**
	0.54	0.51
880	**880 PU**	**880 PA**
	0.27	0.77

The crystallite size depends on the ionic charges present in the solution, in which the number of hydroxyl ions (OH^-) plays an important role in forming zinc oxide with zinc ions (Zn^{2+}). For instance, at neutral pH, equal amounts of positive and negative charged ions are present and have no influence of pH on zinc oxide crystal growth (Mohammadi and Ghasemi 2018).

The formation of crystallite particles may be bigger or smaller depending on the pH, temperature, and biosynthesis method as well (Shaba *et al.* 2021). The chemical equations of zinc oxide particle formation from algae extract is shown in Equations 2 and 3 and describe the reactions involved (Alprol *et al.* 2023).

Overall: $$ZnCl_{2\,(aq.)} + (C_6H_5OH)_n + H_2O \longrightarrow ZnO + 2HCl \quad (2)$$

Basic equation: $$Zn^{2+} + 2OH^- \longrightarrow Zn(OH)_2 \longrightarrow ZnO + H_2O \quad (3)$$

In short, crystallite particles of zinc oxide was formed at 0.27 to 0.54 nm and 0.51 to 1.05 nm for green synthesized PU and PA samples accordingly. The ANOVA test was conducted using SAS to preset the relationship between the substrates (pulp or paper) and the parameters (pH and temperature).

The ANOVA test was conducted by using SAS to show the relationship between substrates and parameters of pH and temperatures. Based on Table 3, the p-values showed significant differences between substrates and parameters with ($p < 0.001$). Comparing interaction pH and temperature with both substrates, the pH showed a more significant 53.15% mean square difference than temperature.

Table 3. ANOVA Test Exhibiting the Relationship between Substrates and Parameter of pH and Temperature

Parameters	Mean Square	Sum of Squares	p-value
pH	4.115	125.414	< 0.001
Pulp x Paper	50.401		< 0.001
pH x Pulp x Paper	33.391		< 0.001
Temperature	3.106	93.785	< 0.001
Pulp x Paper	56.286		< 0.001
Temp. x Pulp x Paper	15.643		< 0.001

CONCLUSIONS

1. Zinc oxide nanoparticles were successfully synthesized on pulp (PU) and paper (PA) surfaces *via* a green synthesis approach using brown algae.
2. The pH influenced the performance of green synthesis of zinc nanoparticles which pH 8 showed better results in terms of morphological, compositional, and physical analysis *via* field emission scanning electron microscopy (FESEM), energy dispersive X-ray spectrometry (EDX), and X-ray diffraction (XRD). Besides that, temperature also is one of the factor that initiate the effectiveness in green synthesis, for which 70 °C was found to be an acceptable temperature in this study.
3. The nanoparticles sizes obtained in this study were 0.27 to 0.54 nm and 0.51 to 1.05 nm for green synthesized PU and PA samples respectively. Green synthesis is observed to operate better on pulp form rather than paper form.

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