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Green synthesis of ZnO nanoparticles usingguava leaf extract and potential for biomedical applications

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Abstract—The conventional ways of creating metal oxide nanoparticles in biomedicine and pharmaceuticals have been replaced by environmentally friendly alternatives that do not require the use of chemical stabilizing and reducing agents. ZnO nanoparticles were synthesized in this study using *Psidium guajava*, a valuable herbal plant with medicinal potential that is commonly known as guava. In pharmaceutical formulations, bioactive components derived from medicinal plants are essential because they function as capping and reducing agents for metal ions during the creation of nanoparticles. Zinc oxide nanoparticles are produced when the phytochemicals in guava leaf extract undergo reduction/oxidation. The experiment was repeated with different precursor concentrations (3g, 4g, 5g, and 6g) to examine the effect of precursor molarity on the physical properties of the nanoparticles. Furthermore, a comparison was made between the average particle size of ZnO nanoparticles produced chemically and those produced through biosynthesis. X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS), and ultraviolet-visible spectroscopy (UV-Vis) were used to analyze the produced nanoparticles.

Keywords: *Zinc oxide; Guava leaf; XRD; Nanoparticles; SEM; Biosynthesis*

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1. Introduction

Nanotechnology refers to the technology that enables the production of materials of an incredibly small size [1]. The crucial aspect of nanotechnology is the development of environmentally friendly methods for creating nanoparticles. These methods should allow for control over the size, shape, and composition of the particles [2]. According to sources, zinc oxide is a type of inorganic compound with diverse properties. It can act as a catalytic, semiconducting, piezoelectric, and optoelectronic device [3]. Furthermore, ZnO nanoparticles possess distinct characteristics. These include a superior ability to absorb light, heightened catalytic efficiency due to their significant surface area relative to their volume, and a wide difference between their ability to conduct electricity and the energy levels of their electrons. Various methods can be used for the synthesis of metal oxide nanoparticles; these techniques encompass both physical and chemical approaches, such as sol-gel synthesis, chemical precipitation methods, microwave methods, etc. [3]. The biosynthesis of metal oxide nanoparticles, especially using botanical extracts, has become an evolving field in nanotechnology. The present study demonstrates an eco-friendly synthesis of ZnO nanoparticles using the leaf extract of Psidium guajava, commonly known as guava.The plant species was identified by a botanical specialist, and after the manuscript was a plant authenticity certificate with information on the identified plant.The effect of precursor molarity on the structure, size, optical properties, and chemical composition of ZnO nanoparticles has been studied. Here, we used zinc acetate as a precursor and sodium hydroxide as a reducing agent. By changing the molarity of the precursor, we repeated the experiment and studied how it affected the physical properties of the produced nanoparticles [4]. Particles were characterized by Scanning Electron Microscopy, Energy Dispersive X-ray spectroscopy (SEM-EDAX), Ultraviolet-visible spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD) analysis. All the characterization techniques used in this study revealed the crystallinity, size, optical properties, and composition of ZnO nanoparticles. The average particle size as measured from the X-ray pattern using the Debye-Scherrer formula was found to be in the range of 20–40 nm for precursor molarities of 3g,4g, 5, and 6g, respectively. The results revealed that average particle size increases with increasing precursor molarity.

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2. Materials and methods

Fresh and healthy leaves of guava were collected from the surrounding area and it was authenticated by Dr. Ratheesh Narayanan M.K, Taxonomist, Head of the PG Department of Botany & Research Guide, Payyanur College, Payyannur on 28.02.2024., zinc acetate, and sodium hydroxide. The authentication certificate is attached at the end of the manuscript.

2.1 Preparation of leaf extract

Guava leaves (30 g) were combined with 50 ml of deionized water and heated to 60°C for 30 minutes. During this process, the solution acquired a yellow color, and this yellow extract was filtered using Whatman No. 1 filter paper and stored in a cool place.

2.2 Synthesis of ZnO nanoparticles

Zinc acetate (5g) was mixed with 50 ml of double-distilled water and stirred for 30 minutes. Then, 10 ml of guava leaf extract was added slowly. The solution turned a pale-yellow color, and after that, sodium hydroxide (2M) was added drop by drop while stirring for 30 minutes. A gelatinous white precipitate formed and was stirred continuously for 2 hours. The solution was left at room temperature overnight. The formed white precipitate was washed several times with distilled water and dried in an oven at 600 °C for 2 hours. The whole experiment was repeated under 3 different molarities(6g,4g, and 3g) of precursor. Fig. 1 represents the flowchart of biosynthesis.

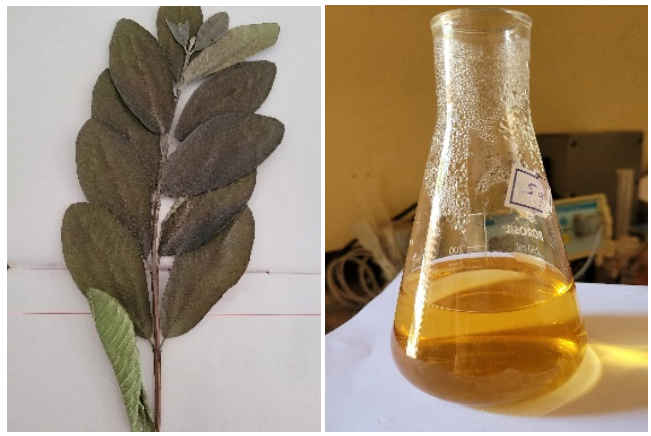
2.3 Chemicalsynthesis of ZnO nanoparticles

In 100 milliliters of distilled water, 5grams of zinc acetate and 1.6 grams of NaOH were combined. The NaOH solution was then gradually added to the zinc acetate solution at room temperature and vigorously stirred to create a white suspension. This white precipitate is filtered, thoroughly cleaned with distilled water, and allowed to dry for two hours at 150oC in an oven for 2 hours. To compare the results, the experiment was repeated under different molarities (6g,4g, and 3g) of zinc acetate.

3. Results and discussion

3.1 Morphology and size analysis (rigaku, japan)

We used X-ray diffraction to determine the crystal structure and growth orientation of ZnO nanoparticles [5]. The chart below (Fig. 2) displays the XRD pattern of ZnO nanoparticles made through biological methods with different amounts of precursors. Our findings show that the prepared samples have a hexagonal wurtzite structure, and they only show peaks characteristic of ZnO, confirming the creation of pure ZnO



(a) Guava leaf (b) Guava leaf extract

(c) ZnO nanoparticles (d) ZnO nanoparticles under different molarities

Figure 1: Representation of the flow chart of biosynthesis.

nanoparticles without impurities. The X-ray diffraction patterns of the green-synthesized nanoparticles, using guava leaf extract, exhibit various peaks at 2θ values like 31.81, 34.48, 36.3, 47.56, 56.64, 62.39, and 67.8 for each amount of precursor. All these peaks match with the (100), (002), (101), (102), (110), (103), (200), (112), and (201) planes, as per the standard JCPDS card with reference code 00-036-1451. We calculated the average particle size using the Debye-Scherrer formula, ranging from 20 to 40 nm for zinc salt concentrations of 3g, 4g, 5g, and 6g. These findings indicate that the quantity of precursor strongly affects the nanoparticles' crystallinity and size.Table 1, Table. 2, Table. 3, and Table. 4 below show the values of 2θ, d-spacing, and Full Width at Half Maximum (FWHM) obtained from X-ray Diffraction (XRD) characterization of ZnO nanoparticles with a precursor molarities of 3g,4g, 5g. and 6g.

3.1.1 Average particle size using chemical method

Fig. 3 a, b, c, and d show the XRD patterns of ZnO nanoparticles created with precursor concentrations of 3g, 4g, 5g, and 6g utilizing chemical reducing agents. At particular 2theta values, these patterns exhibit nine distinct peaks, which are 31.705, 34.37, 36.20, 47.49, 56.56, 62.82, 66.51, 67.92, and 69. As evidenced by Figure 1.3 these peaks, which correspond

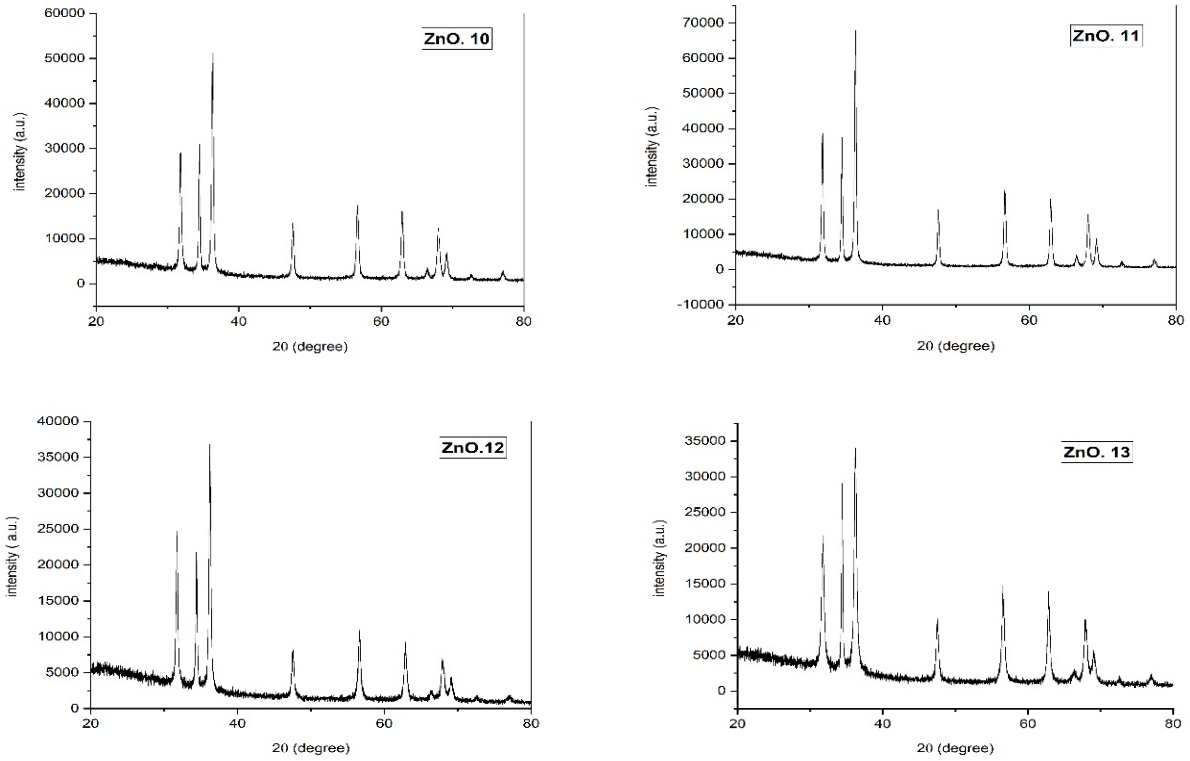


Figure 2: XRD graphs of ZnO nanoparticles under different precursor molarities

Table 1: The table presents the values of 2θ, d-spacing, and Full Width at Half Maximum (FWHM) obtained from X-ray Diffraction (XRD) characterization of ZnO nanoparticles with a precursor molarity of 3g

PEAK	2θ	d-spacing	FWHM
1	31.823	2.8098	0.279
2	34.465	2.6002	0.181
3	36.292	2.4734	0.289
4	47.579	1.9096	0.271
5	56.636	1.6238	0.341
6	62.924	1.47586	0.261
7	66.44	1.4061	0.38
8	67.986	1.37776	0.337
9	69.123	1.35786	0.335

Table 2: The table provides measurements of 2θ angles, d-spacing values, and Full Width at Half Maximum (FWHM) extracted from X-ray Diffraction (XRD) analysis of ZnO nanoparticles synthesized using a precursor molarity of 4g

PEAK	2θ	d-spacing	FWHM
1	31.81	2.8109	0.24
2	34.48	2.599	0.191
3	36.296	2.4731	0.245
4	47.569	1.91	0.249
5	56.642	1.6237	0.273
6	62.897	1.47643	0.252
7	66.39	1.407	0.332
8	67.966	1.37813	0.28
9	69.117	1.35795	9.281

to the (100), (002), (101), (102), (110), (103), (200), (112), and (201) planes, respectively, show a hexagonal phase with a wurtzite structure (JCPDS NO: 36-1451).

Crucially, the creation of pure ZnO nanoparticles is confirmed by the absence of diffraction signals linked to contaminants. The results of the analysis show that the average particle size for precursor concentrations of 3g, 4g, 5g, and 6g is, respectively, 35 nm, 40.2 nm, 44.9 nm, and 46 nm. This shows that the importance of precursor molarity on nucleation and crystal formation is highlighted by the suggestion that particle size rises with increased precursor concentrations. The results also show that ZnO nanoparticles created by the chemical approach have much larger average particle sizes than those prepared by the biological method. The Debye-Sherrer formula is used to calculate the average particle size, and the results show a significant difference in particle size. Table. 5 below shows

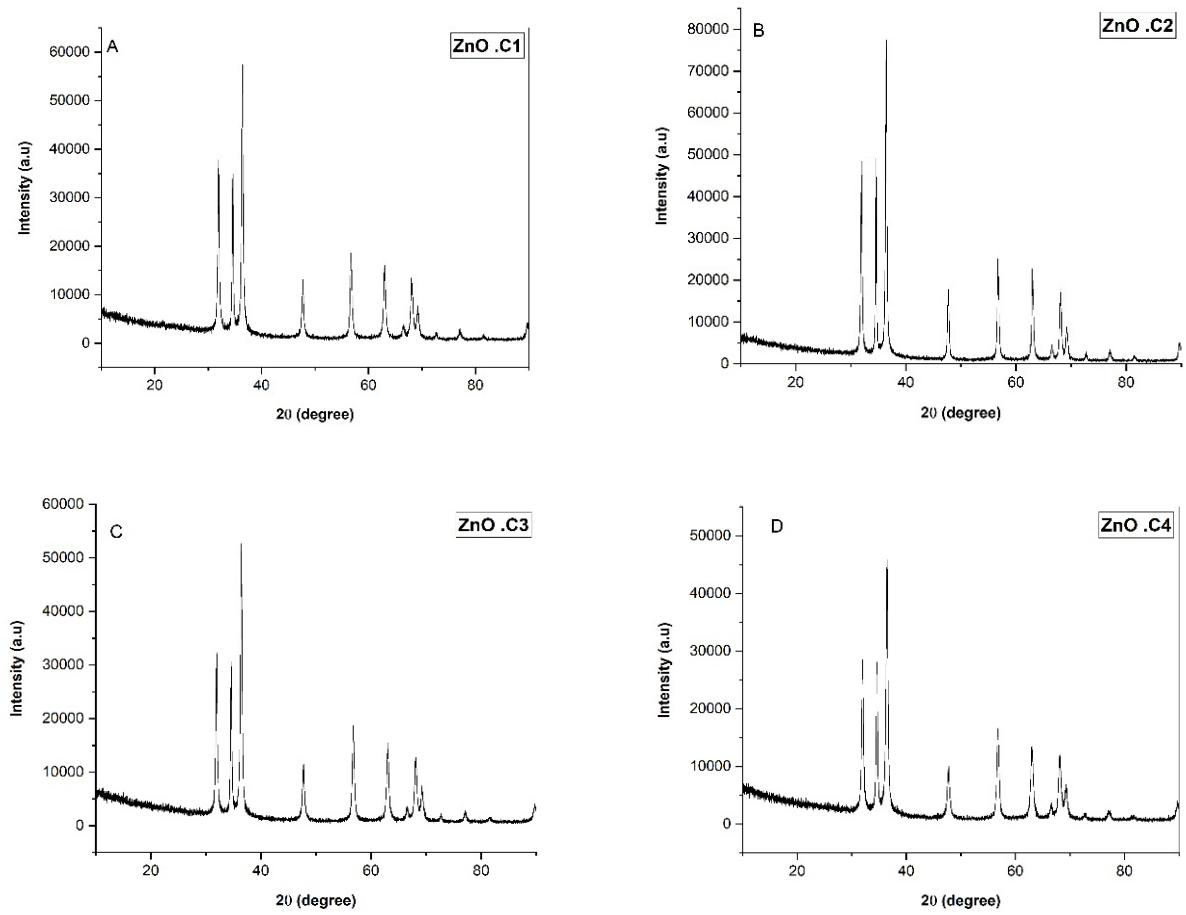


Figure 3: XRD patterns of chemically synthesized ZnO nanoparticles under 4 precursor molarities

the comparison in average particle size of ZnO nanoparticles synthesized using chemical and biological methods.

3.2 Optical study (Perkin Elmer Lambda 365, America)

The UV-visible spectroscopy analysis was conducted to evaluate the optical properties and gain insights into the electronic characteristics of the synthesized nanoparticles [6]. Fig. 4 above illustrates the UV-visible spectra, depicting variations in zinc salt concentrations. Notably, zinc nanoparticles display absorption within the 300–400 nm range. An observable trend indicates that higher precursor concentrations result in an elevated absorption peak. This phenomenon is attributed to exceeding a critical threshold value for metal ion concentration, leading to a subsequent decrease in the synthesized nanoparticle's yield. This occurrence is attributed to exceeding a critical concentration threshold for metal ions, resulting in a subsequent reduction in the overall yield of the synthesized nanoparticles [7]. Observations reveal a correlation between the band gap of zinc oxide nanoparticles and the concentration of the precursor. Notably, the band gap exhibits a decreasing trend with increased precursor concentration. The Kubelka-Munk/Tauc method of determining the energy band gap of the synthesized ZnO nanoparticles at varying precursor concentrations is depicted in Fig. 5 below. The energy band gap of ZnO nanoparticles for 3g, 4g, 5g, and 6g is found to be 3.23 eV, 3.2 eV, 3.17 eV, and 3.16 eV, respectively, using the Tauc equation. The findings demonstrate that when precursor molarity increases, the energy band gap of the ZnO nanoparticle reduces.

3.3 FTIR Analysis (Thermo Nicolet is50, America)

The FTIR transmission spectra of the newly synthesized ZnO nanoparticles prepared using varying concentrations of zinc acetate precursor are presented in Fig. 6 These infrared analyses were conducted to validate the purity and properties of the metal nanoparticles [8]. The distinctive peak observed in the 350–390 cm range signifies the Zn-O stretching of ZnO nanoparticles. Typically, metal oxide nanoparticles exhibit

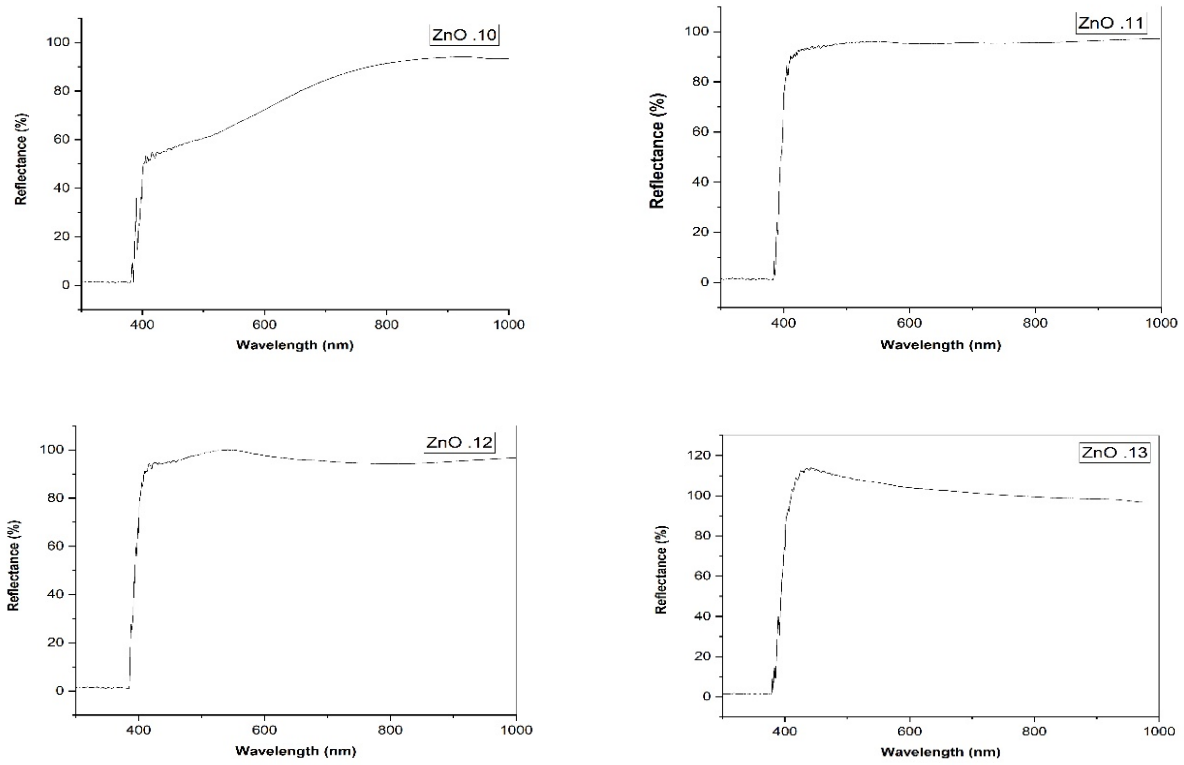


Figure 4: DRS spectra of ZnO nanoparticles with different precursor molarities 3g, 4g,5g, and 6 g respectively

Table 3: The table displays data on 2θ angles, d-spacing, and Full Width at Half Maximum (FWHM) acquired through X-ray Diffraction (XRD) analysis of ZnO nanoparticles produced with a 5g precursor molarity

PEAK	2θ	d- spacing	FWHM
1	31.736	2.8172	0.264
2	34.338	2.6058	0.19
3	36.233	2.4772	0.274
4	47.508	1.9123	0.31
5	56.578	1.6254	0.344
6	62.812	1.4782	0.394
7	66.35	1.4078	0.37
8	67.897	1.3793	0.39
9	69.039	1.3593	0.33

Table 4: The table presents information obtained from the X-ray Diffraction (XRD) study of ZnO nanoparticles prepared with a 6g precursor molarity concerning 2θ angles, d-spacing, and Full Width at Half Maximum (FWHM)

PEAK	2θ	d- spacing	FWHM
1	31.74	2.8169	0.411
2	34.414	2.6039	0.172
3	36.216	2.4784	0.375
4	47.488	1.9131	0.391
5	56.562	1.6258	0.361
6	62.827	1.4779	0.332
7	66.39	1.4069	0.46
8	67.891	1.3795	0.43
9	69.033	1.3594	0.46

absorption bands in fingerprint regions, specifically below 1000 cm, arising from interatomic vibrations. The outcomes reveal a discernible trend: the concentration of the precursor decreases, and the sharpness of the absorbance peak associated with the ZnO stretch diminishes. This observation suggests a correlation between the concentration of the zinc acetate precursor and the intensity of the Zn-O stretching vibration in the synthesized ZnO nanoparticles, providing valuable insights into the synthesis process and nanoparticle characteristics.

3.4 SEM-EDS Analysis (Jeol 630Ola/oxfordXMXN)

Through SEM analysis, we discovered that the samples we created exist in the nanometer size range. Additionally, the surface appearance changes depending on the concentration of the precursor used. When we increase the molarity of

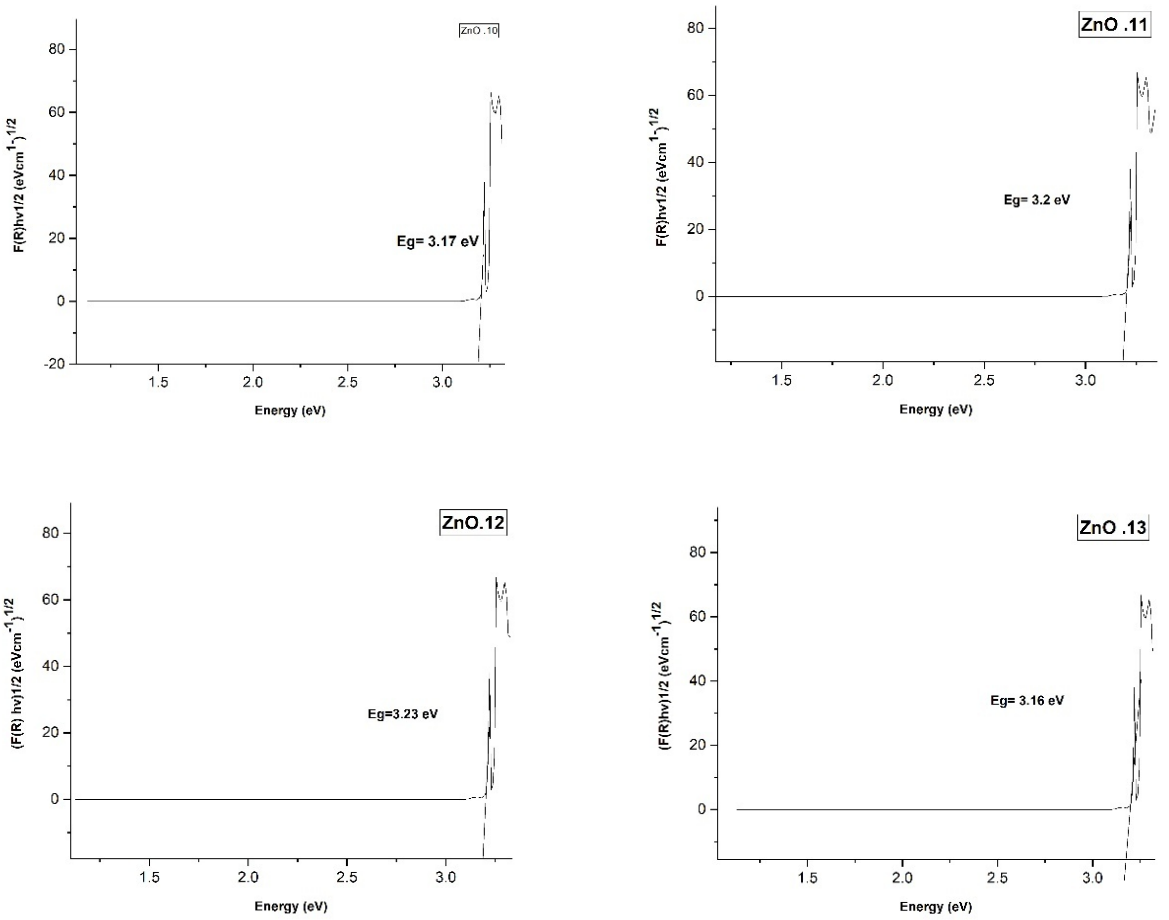


Figure 5: Energy band gap of ZnO nanoparticles

Table 5: Comparison between chemical and method

Precursor	Average Particle Size	
Molarity (g)	Bio method	Chemical Method
3	21.8	35
4	31.9	40.2
5	35	44.9
6	38	46

the precursor, the size of the particle also increases. EDS spectroscopy relies on the unique atomic structure of elements, generating peaks in the X-ray spectrum for each element [9]. Examining the spectra, we determined that all our prepared samples exclusively contain zinc (Zn) and oxygen (O) elements, with no detection of any other elements. Consequently, we can confidently state that the synthesized samples are impurity-free. The SEM-EDS graph below (Fig. 7, Fig. 8, Fig. 9, and Fig. 10) highlights variations in zinc oxide nanoparticle formation under different precursor molarity conditions. Table. 6 presents a detailed account of the amounts of zinc (Zn) and oxygen (O) generated at each specific precursor concentration throughout the synthesis process.

Table 6: Elemental composition of Zn and O

Zinc Acetate Concentrations	Element	Weight (%)	Atomic (%)
5g	Zn	80.47	50.21
	O	19.53	49.79
4g	Zn	89.59	67.82
	O	10.41	32.18
3g	Zn	79.39	48.53
	O	20.61	51.47
6g	Zn	77.63	45.92
	O	22.37	54.08

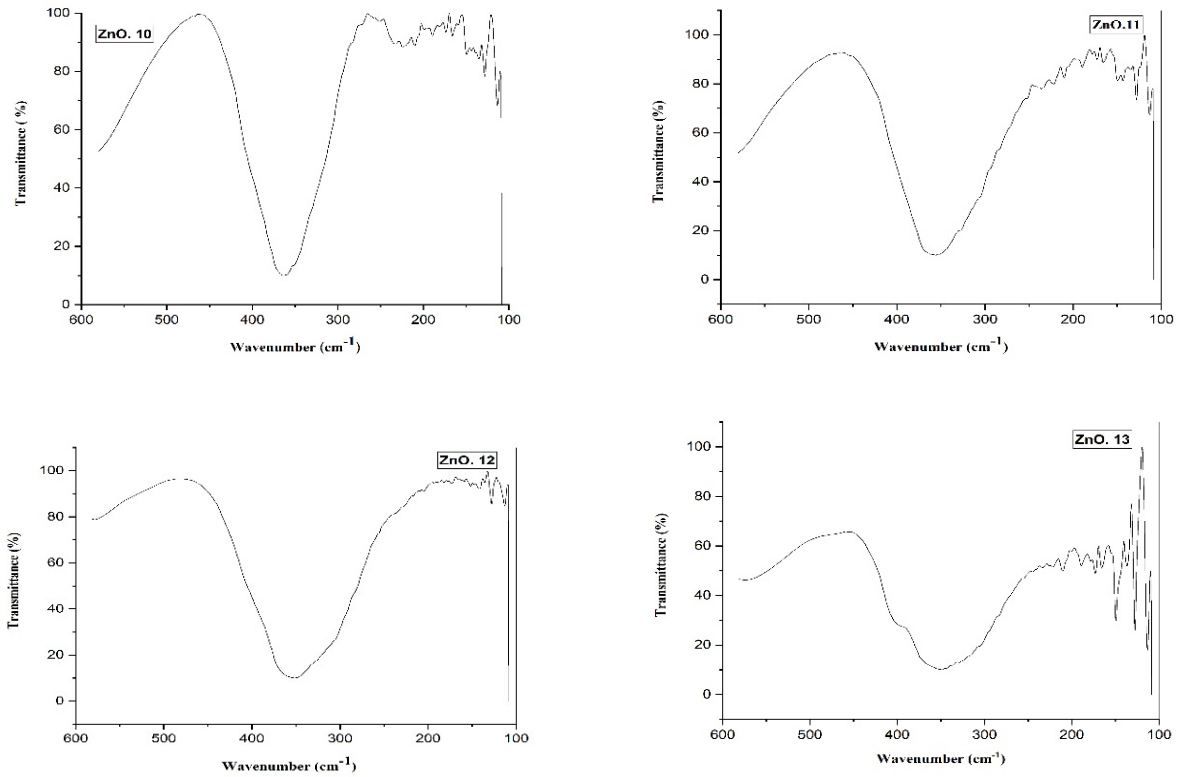


Figure 6: FTIR spectra of biologically synthesized ZnO nanoparticles under different precursor concentrations

4. Conclusion

This paper outlines a green synthesis method for ZnO nanoparticles using guava leaf extract, emphasizing its simplicity and reduced toxicity, leading to minimizing waste and contaminants in the ZnO production process. The crystallite size and homogeneity of the nanoparticles are dependent on the amount of precursor used. Chemical synthesis is also used for comparison purposes. Guava leaf extract contains molecules that serve as capping, reducing, and stabilizing agents, contributing to the formation mechanism of ZnO nanoparticles. FTIR analysis confirms the Zn-O bonding in all samples, providing insight into the chemical structure. XRD results reveal hexagonal wurtzite-shaped crystalline particles with sizes ranging from 20 nm to 40 nm. For chemical synthesis, particle size ranges between 30-50 nm. Furthermore, an increase in molarity concentrations correlates with an observed growth in grain size. EDS analysis confirms the successful synthesis of ZnO nanoparticles in this study. The energy band gap of ZnO is found to be in the range of 3-3.3 eV and a decrease in band gap was found to be at higher precursor concentrations. Overall, the study showcases a sustainable and efficient approach to obtaining ZnO nanoparticles with desirable characteristics, laying the groundwork for environmentally friendly nanomaterial synthesis. This study underscores the significant advantages of biosynthesis over chemical methods in producing small-sized nanoparticles. It emphasizes that, in contrast to conventional chemical methods, biosynthesis makes manufacturing small-sized nanoparticles more easily possible. This is further highlighted by the observable variation in nanoparticle size within the XRD pattern. Furthermore, the vast range of applications for ultrasmall-sized nanoparticles in nanoparticle production is highlighted by the necessity of using biosynthetic techniques in various sectors.

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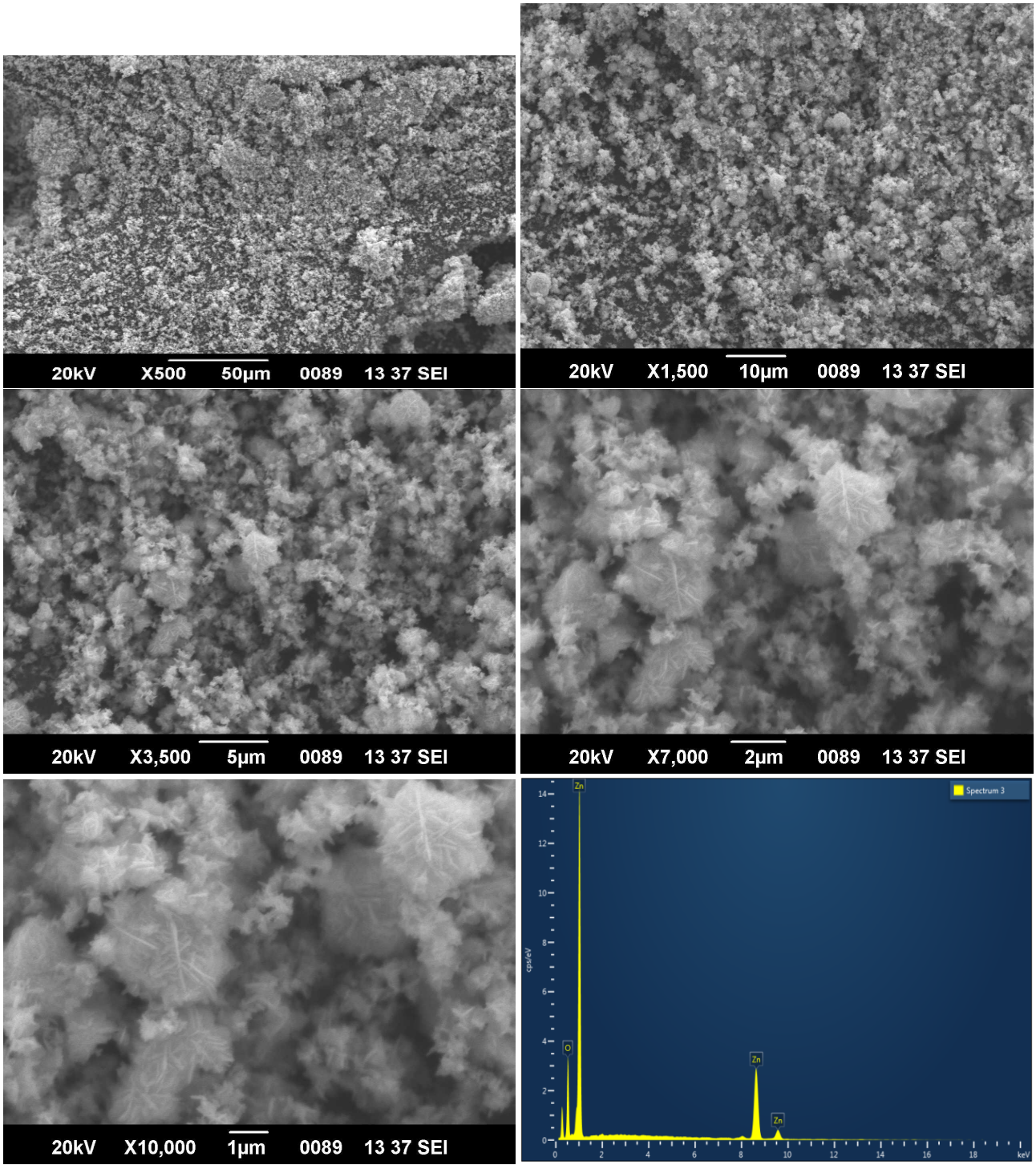

Figure 7: SEM-EDS graph of ZnO nanoparticles with precursor molarity 3g.

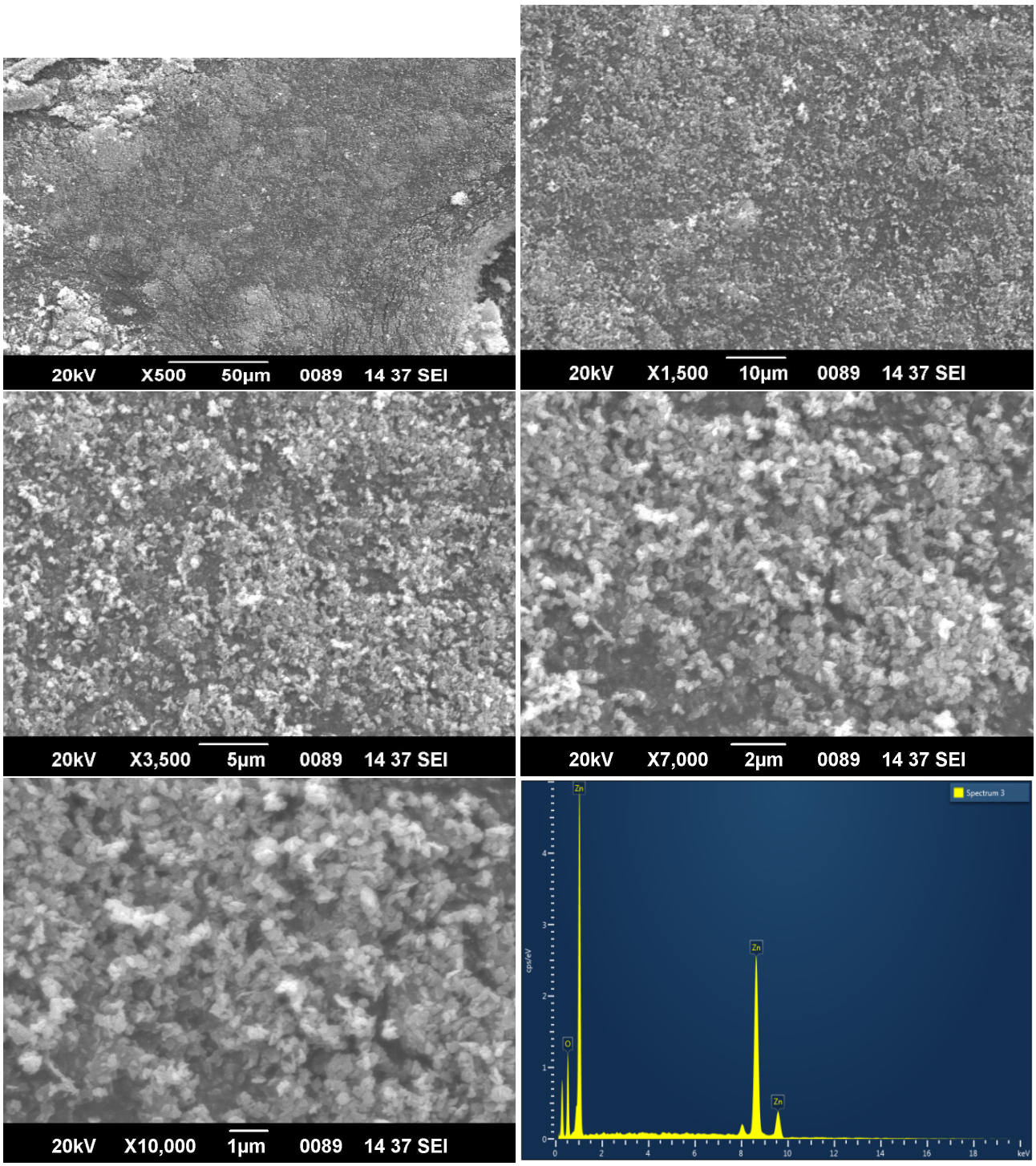

Figure 8: SEM-EDS graph of ZnO nanoparticles with precursor molarity 4g.

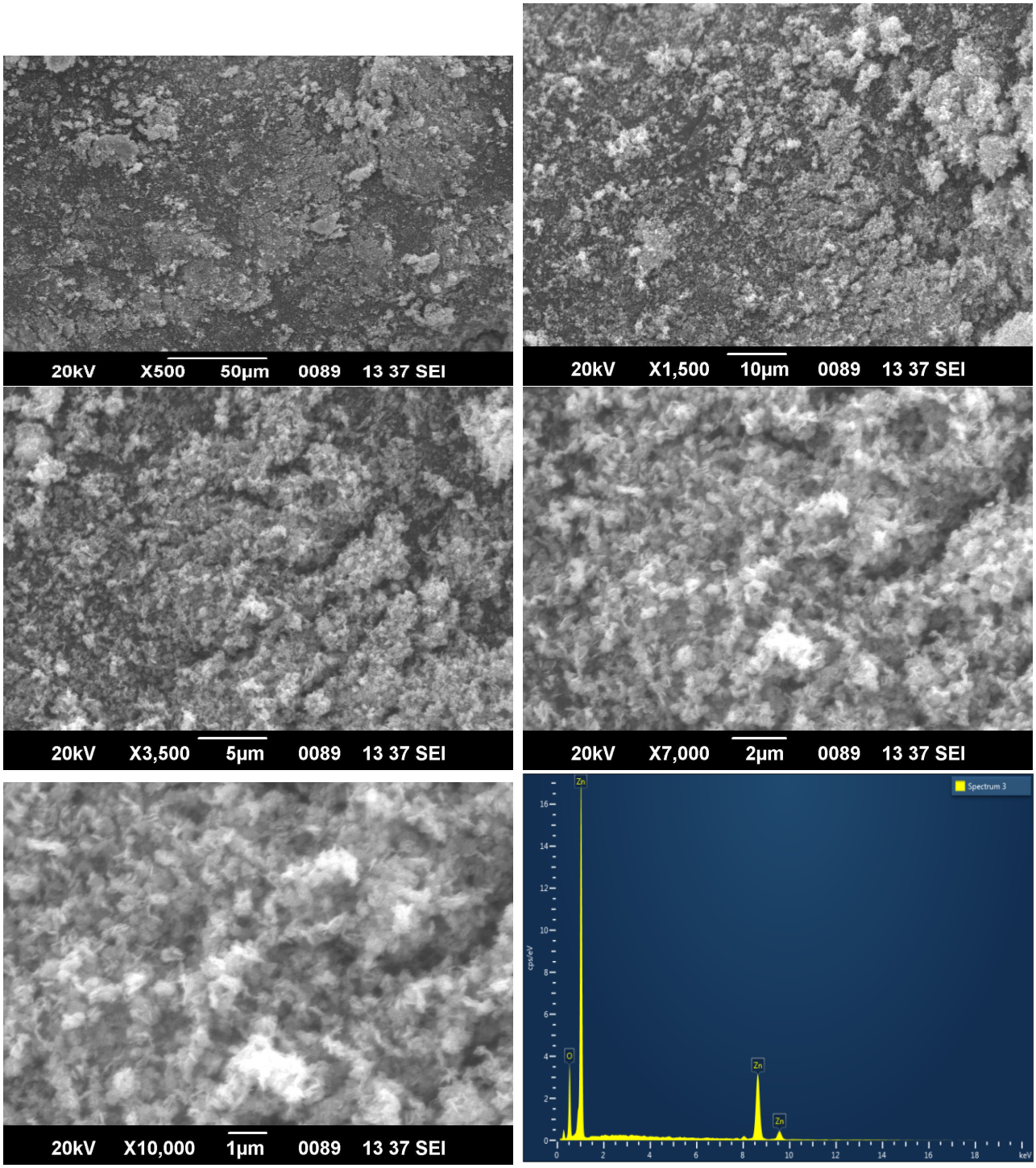

Figure 9: SEM-EDS graph of ZnO nanoparticles with precursor molarity 5g.

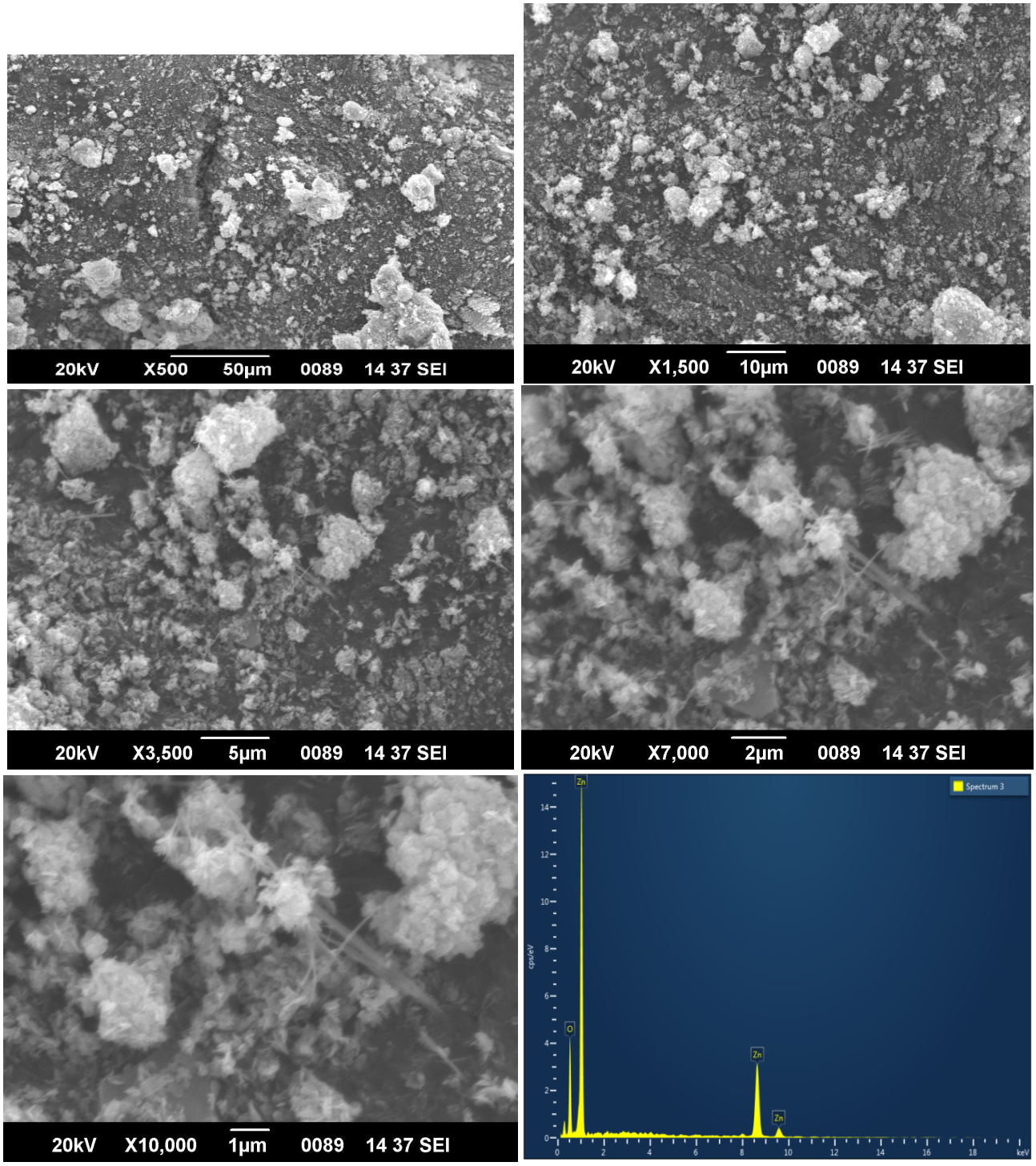

Figure 10: SEM-EDS graph of ZnO nanoparticles with precursor molarity 6g.

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