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The potential of electrochemical pesticide analyses by green synthesized, waste olive leaves-based, silver nanoparticles

EZGI ADAK[1,2], MERVE KESKIN[3] and HALIT ARSLAN[1]*

[1]*Department of Chemistry, Faculty of Science, Gazi University, 06500, Ankara, Turkiye,* [2]*Gazi University Graduate School of Natural and Applied Science, 06500, Ankara, Turkiye and* [3]*Vocational School of Health Services, Bilecik Seyh Edebali University, 11210, Bilecik, Turkiye*

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Abstract: Pesticides are chemicals that negatively affect human health and the environment. Unconscious use of pesticides creates residues in foods or agricultural products and it also cause water and soil pollution. Quick and easy determination of pesticide residues is important for environmental inspections. For this reason, it is necessary to develop alternative methods to traditional methods such as chromatography for the determination of pesticide residues. Electrochemical sensors are new generation analytical devices developed for the determination of pesticides that enable rapid and practical analysis. Carbon paste electrodes used in the design of electrochemical sensors are modified using different materials such as silver nanoparticles. Silver nanoparticles used for modification could be synthesized using chemical methods, but these methods are harmful to the environment because toxic chemicals are used in the synthesis procedure. For this reason, the green synthesis technique was developed as an alternative to chemical techniques. In this study, waste olive leaves were used in green synthesis of silver nanoparticle as an electron precursor, the nanoparticles (OL-AgNPs) were characterized and modified carbon paste electrodes were prepared. The prepared modified carbon paste electrodes were used in the determination of thiocholine (as a product of hydrolysis of acetyl thiocholine by enzymatic reactions), H_2O_2 (as a product of many oxireductase enzyme reactions) and the widely used pesticides cyprodinil and mepanipyrim. The results showed that the modified carbon paste electrodes were more sensitive than the carbon paste electrodes for all analytes. It was clear that the modified carbon paste electrodes could be used in pesticide determinations.

Keywords: biological synthesis; modified carbon paste electrode; nanoparticle; sustainability; voltammetry; agricultural waste.

* Corresponding author. E-mail: halit@gazi.edu.tr
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INTRODUCTION

Pesticides are chemicals used to increase agricultural products' yield and quality and protect forests and plantations.[1] Cyprodinil and mepanipyrim are the active ingredients of some commonly used pesticides.[2] Residues of these pesticides are found in agricultural products.[3] Unconscious and excessive use of these pesticides affects the environment and human health negatively.[2] Excessive pesticide residues cause many diseases and their determination in trace amounts is crucial.[4] Also, pesticides inhibit the important enzymes such as acetylcholinesterase (AChE) which is an key enzyme for the functioning of the central nervous system and muscle.[5] Thiocholine and acetate are the products of acetylthiocholine ester (ATCh) hydrolysis with acetylcholinesterase (AChE).[6] In addition to determination of thiocholine, H_2O_2 monitoring could be provide the indirectly measurement of choline which react with chloline oxidase, an oxireductase enzyme is important for central nervous system, in the pesticide determinations.[7] The irreversible inhibition of the activity of cholinesterases and acetylcholinesterase could have an important role to develop novel biosensors to determine pesticides.[7] Therefore, thiocholine and H_2O_2 are two important analytes for the determinations of pesticides by biosensors.

Traditional chromatographic methods such as mass spectrometry, capillary electrophoresis and high-performance liquid chromatography are used for the analysis of pesticides in the environment.[8] However, they have several drawbacks, including complexity, labor-intensive sample preparation and the need for expensive equipment and skilled personnel.[8] In addition, electrochemical methods are used for the determination of pesticides due to their high sensitivity and selectivity and short analysis time compared to other methods. The use of modified electrodes in electrochemical studies is becoming widespread.[9,10] Modified electrodes increase the selectivity and sensitivity of the electrodes could be prepared by adding conductive substances to the electrode material.[11–15] The range of electrochemical methods is expanded by this variety. Nanoparticles could be used when modifying electrodes.[16] Nanoparticles with sizes between 1–100 nm show unique physical and chemical properties.[17,18] They are widely used in many fields, especially in medicine, biotechnology, biomedical, textile, agriculture, defense industry, cosmetics and chemical sectors.[19,20] The most feasible technique for producing nanoparticles in a cost-effective, straightforward, safe and ecologically friendly manner without using hazardous chemicals, high pressure, heat, or energy is called green synthesis.[21,22] Agricultural and domestic wastes are increasing by the time as a result of population growth. The agricultural wastes such as olive, grape seeds, pine sawdust, almond, nut, hazelnut, pecan, corn, peach and many more can be used in green synthesis of nanoparticles.[23] Olive leaves are found in large quantities as residue in olive oil industries in Türkiye. Therefore, olive leaves can be evaluated as a cheap raw material that can be used as a useful resource for high

value-added products.[24] Phytochemicals present in plant extracts could be used as a precursor to reduce metal ions to metallic nanoparticles in the green synthesis process.[25,26] Due to their easy accessibility, using plant extracts in green synthesis of nanoparticles is an important research topic in the field of bionanotechnology today.[27–29] Factors such as the pH, temperature, reaction time, concentration of metal salt, dose of the plant extract and pressure affect the synthesis and application of metal nanoparticles through green synthesis from plant extract.[30] For example, it has been observed that as the pH value of the plant extract increases, the nanoparticle formation rate increases and the nanoparticle size decreases.[31] After green synthesis, characterization studies of nanoparticles should be carried out. Nanoparticles can be separated by their size, surface area, and distribution pattern. UV–Vis absorption spectroscopy, scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX), X-ray diffraction (XRD), Fourier transform spectroscopy (FTIR), *etc*., techniques are commonly used in the characterization of nanoparticles.[22,32] Metal nanoparticles are used in various industrial and biological applications.[19] They are widely used in nanotechnology fields due to their properties such as electrical conductivity.[33]

In this study, silver nanoparticles were synthesized and characterized. Their application in the determination of pesticides, H_2O_2 and thiocholine using electrochemical techniques was also examined. The synthesized silver nanoparticles were used to modify the carbon paste electrode to the voltammetric determination. The resulting silver nanoparticles modified carbon paste electrode showed a better performance than the carbon paste electrode. This study revealed that trace amounts of various pesticides and materials can be determined voltammetrically using a modified carbon paste electrode with silver nanoparticles.

EXPERIMENTAL

Apparatus and reagents

For electrochemical studies, CH Instruments, model 1230B, electrochemical analyzer was used.

Three electrode cell systems were used in experimental studies. Platinum wire (counter electrode) and Ag/AgCl (3 M KCl) reference electrode were employed, and as the working electrode, green synthesized waste olive leaves based-silver nanoparticles modified carbon paste electrode (OL-AgNPs-MCPE) was used in the electrooxidation studies.

Acetylthiocholine chloride (ATCh), acetylcholinesterase (AChE), mepanipyrim, cyprodinil, hydrogen peroxide, silver nitrate and all other chemicals were obtained from Sigma Aldrich.

Green synthesis and characterization of silver nanoparticles

To prepare olive leaves extract, waste olive leaves were obtained from Bodrum district of Muğla province during the olive harvest period. The supplied leaves were cleaned. It was then dried at 45 °C. After the dried plant leaves were ground, a certain amount was weighed, boiled pure water was added and kept for 60 min, and after cooling, they were filtered with filter paper and stored in a dark bottle at 4 °C until use. Then, the extract was mixed with 0.05 M $AgNO_3$

and stirred at 60 °C for 45 min. Finally, the obtained solution was centrifuged at 10000 rpm for 15 min to acquire, olive leaves-based, silver nanoparticles.[26]

The synthesized nanoparticles (OL-AgNPs) were characterized by UV absorption spectroscopy (T80+ high-performance double-beam spectrophotometer from PG Instruments), scanning electron microscopy (SEM, Zeiss/Supra 40 VP), energy dispersive X-ray (EDX), zeta potential (Malvern/Nano-ZS), Fourier transform infrared spectroscopy (FTIR, Thermo Fisher) and X-Ray diffraction analysis.

Preparation of electrodes

To prepare working electrode, a carbon paste electrode (CPE) was prepared by mixing carbon powder with mineral oil and the paste was filled into the electrode's hollow.[34] The mixture was dried for 24 h. After that, the prepared electrode was cleaned, polished and sanded to be used in electrochemical analyses (Fig. 1 a).[34]

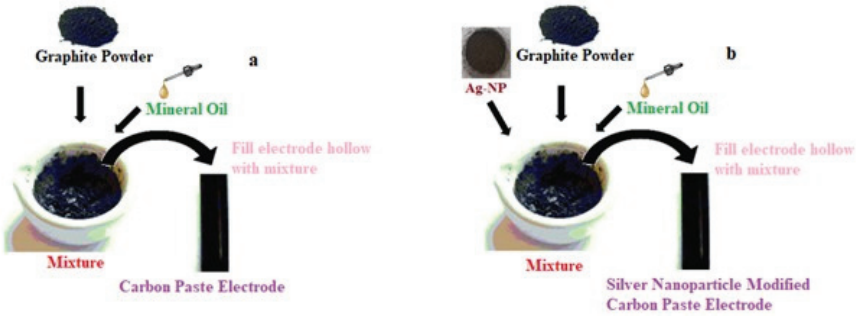

Fig. 1. Preparation of electrodes; a) carbon paste electrode (CPE) and b) silver nanoparticles modified carbon paste electrode (OL-AgNPs-MCPE).

A green synthesized waste olive leaves-based silver nanoparticles modified carbon paste electrode (OL-AgNPs-MCPE) was prepared by mixing carbon powder with mineral oil and 8 mg silver nanoparticles, respectively.[35] The pastes were filled into the hollow electrodes. The mixtures were dried for 24 h. The prepared electrode was polished and sanded to use in electrochemical analyses (Fig. 1 b).

Electrochemical measurements

All electrochemical measurements were performed by using square wave voltammetry. To determine H_2O_2, square wave voltammograms were applied, both carbon paste (CP) and modified carbon paste (MCP) electrodes in pH 2.0 Britton–Robinson (BR) buffer solution (f = 15 Hz; ΔE = 25 mV). Increasing concentrations of H_2O_2 solutions were added to the solution medium, voltammograms were taken and current changes were observed against increasing concentration. The analyses were performed, both CPE and OL-AgNPs-MCPE and the current changes were compared.

To determine the thiocholine, voltammograms were applied to both CP and MCP electrodes in 10 mL pH 7.5 BR buffer solution and 100 µL acetylcholine esterase (AChE) enzyme (f =15 Hz; ΔE = 25 mV). Increasing concentrations of acetylthiocholine solutions (ATCh) were added to the solution medium, voltammograms were taken and current changes were observed against increasing concentration. The analyses were performed both CPE and OL-AgNPs--MCPE and the current changes were compared. The catalyzes of acetylthiocholine to thiocholine by acetylcholine esterase is presented (Fig. 2). Acetylthiocholine is hydrolyzed by the enz-

yme acetylcholinesterase into thiocholine and acetic acid. The thiocholine is oxidized by dimerization *via* $2H^+$ loss. The oxidation reaction of thiocholine has been thoroughly investigated.

$$\text{Acetylthiocholine} + H_2O \xrightarrow{\text{AChE}} \text{Thiocholine} + \text{Acetic acid}$$

$$2\text{Thiocholine}_{(red)} \rightarrow \text{Thiocholine}_{(ox)}(\text{dimeric}) + 2H^+ + 2e^-$$

Fig. 2. Reaction mechanism of hydrolyzing acetylthiocholine by AChE.

To determine cyprodinil and mepanipyrim (Fig. 3), square wave voltammograms were applied to both CP and MCP electrodes in pH 2.0 BR buffer solution (f = 15 Hz; ΔE = 25 mV). Increasing concentrations of cyprodinil and mepanipyrim solutions were added to the solution medium, voltammograms were taken and current changes observed against increasing concentration. The analyses were performed both CPE and OL-AgNPs-MCPE and the current changes compared. All analyses were performed for cyprodinil and mepanipyrim separately.

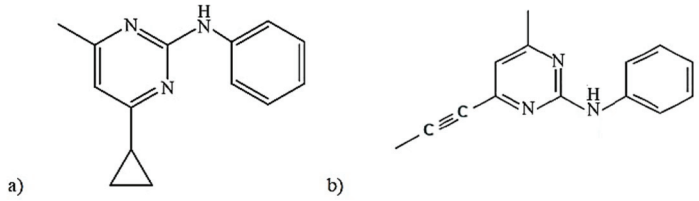

Fig 3. Chemical structre of cyprodinil (a) and mepanipyrim (b).

RESULTS AND DISCUSSION

UV–Vis spectroscopy can be used as an easy and dependable way to check the stability of the nanoparticles.[36] The progress of the reaction following its plasmon absorption bands at λ_{max} values was monitored for Ag nanoparticle evolution. Maximum absorbance at 415 nm was seen for silver nanoparticles by UV–Vis absorption spectroscopy technique. The literature search revealed that, a peak around 410 nm stands for silver nanoparticles.[37] The UV–Vis spectra at different pH, temperature, time and $AgNO_3$ concentration were recorded during the evolution of the Ag nanoparticles (Fig. 4).

The formation of silver nanoparticles can be observed through UV–Vis spectroscopy, with peak formation occurring between 395 and 425 nm. The pH of the plant extract used in the formation of silver nanoparticles has been found to be effective. As the pH of the plant extract increases, the amount of synthesized silver nanoparticles increases, and the size of the synthesized nanoparticles decreases.[38,39] To investigate the effect of the pH value of the olive leaf extract on nanoparticle formation, nanoparticles were synthesized using solutions with pH values ranging from 5.0 to 11.0, and measurements were taken using UV–Vis spectroscopy. It was observed that as the pH of the plant extract increased, the absorbance at 410 nm also increased, with the highest absorbance value measured at pH

10 (Fig. 5a). The obtained results were compatible with alternative methods of Ag nanoparticle synthesis in the literature.[40,41]

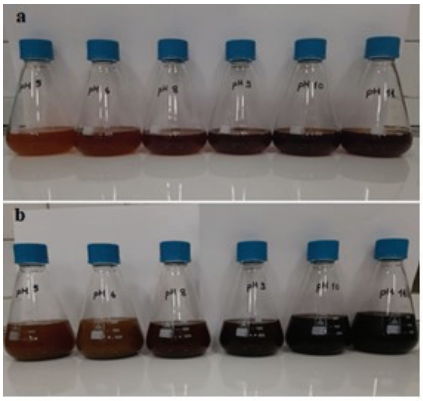

Fig. 4. a) The change in the color with changing pH of olive leaves extract in the pH range of 5.0––11.0 and b) silver nanoparticles synthesized using olive leaves extract in the pH range of 5.0––11.0 (60 min at 60 °C).

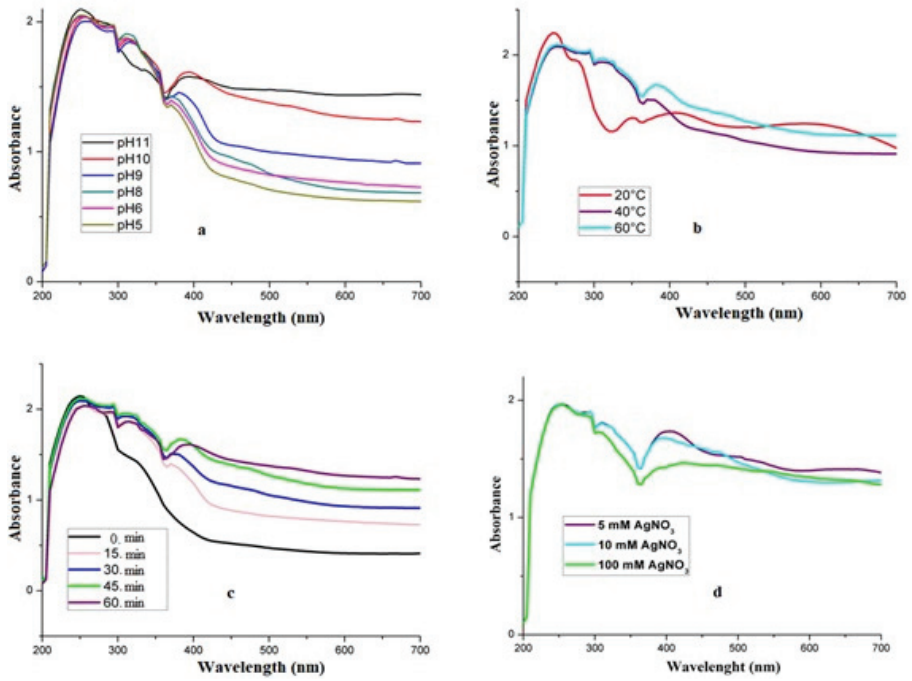

Fig. 5. UV–Vis spectra of silver nanoparticles for determination of optimal; a) pH, b) temperature, c) time and d) $AgNO_3$ concentration.

Another parameter affecting nanoparticle formation is temperature. It has been observed that as the temperature increases, the formation of nanoparticles also increases.[42] The formation of nanoparticles synthesized using extracts at temperatures ranging from 20 to 60 °C was monitored using UV–Vis spectroscopy. It was

observed that as the temperature increased, the absorbance peak at 410 nm also increased. Higher temperatures were not used in the experiments due to the potential degradation of the plant extract at elevated temperatures. The optimal value has been determined to be 60 °C (Fig. 5b).

The time parameter also affects nanoparticle formation.[43] The absorbance of the peak observed at 410 nm increased until the 45th min. At 60 °C, a decrease in absorbance was observed at the 60th min, which was thought to be due to aggregation and/or agglomeration. As a consequence, the optimal time for synthesizing silver nanoparticles using olive leaves extract was selected as 45 min (Fig. 5c). Studies have shown that higher $AgNO_3$ concentrations result in larger particle sizes.[44] High $AgNO_3$ concentrations negatively affect nanoparticle formation due to aggregation and/or agglomeration. Therefore, at lower $AgNO_3$ concentrations, more silver nanoparticles were formed, and higher absorbance values were measured at 410 nm. Finally, the optimization of synthesis was completed after determining the optimum $AgNO_3$ concentration (Fig 5d). A summary of the optimal parameters for synthesizing silver nanoparticles using olive leaves extract is given in Table I.

TABLE I. Optimum parameters for the green synthesized waste olive leaves-based silver nanoparticles

Parameter	Optimum value
pH	10
Temperature, °C	60
Time, min	45
$AgNO_3$ concentration, mM	5

A review of the literature reveals numerous studies in which silver nanoparticles are synthesized using aqueous extracts. The fluctuations observed in the UV peaks are not believed to be due to the use of aqueous extracts. Instead, these fluctuations are thought to be caused by the heterogeneity of the solution.

XRD was used to analyze the structure of OL-AgNPs. The five separate diffraction peaks of the 2θ values of 38.12, 44.28, 64.43, 77.48 and 81.54° can be assigned the planes of (111), (200), (220), (311) and (222), respectively (Fig. 6), indicating that the silver nanoparticles are crystalline and face-centered cubic (fcc) structure in nature.

FT-IR spectra of olive leaves extract and silver nanoparticles synthesized using olive leaves extract are given in Fig. 7, and corresponding data in Table II.

The spectra obtained as a result of the FT-IR analysis determined the presence of phytochemicals properties found in the essence of the olive leaves used in the synthesis of OL-AgNPs, and responsible for the reduction of silver nanoparticles. The band observed at 3309.81 cm^{-1} in the olive leaves extract, 3276.99 cm^{-1} in the synthesized OL-AgNPs, the bands observed at 1634.04, 1629.30, 666.83 and

644.56 cm^{-1} in the olive leaves extract and synthesized OL-AgNPs, respectively. Additionally, a band at 2358.35 cm^{-1} was detected in the FT-IR spectrum of OL--AgNPs. These functional molecules can be found with silver nanoparticles. The band observed at 3309.81 cm^{-1} indicate O–H stretching properties. These band indicates the presence of alcohols and phenols (O–H).[45] The band at 1634.04 cm^{-1} corresponded to the C=O stretching of alcohols.[46] And, the band at 666.83 cm^{-1} indicated chloroalkanes (C–X) stretching properties.[47] FT-IR results show that green synthesis of silver nanoparticles with plant extracts, primary and secondary metabolites such as sugars, phenolics, flavonoids, terpenes, etc. were functionally activated in the process.[48]

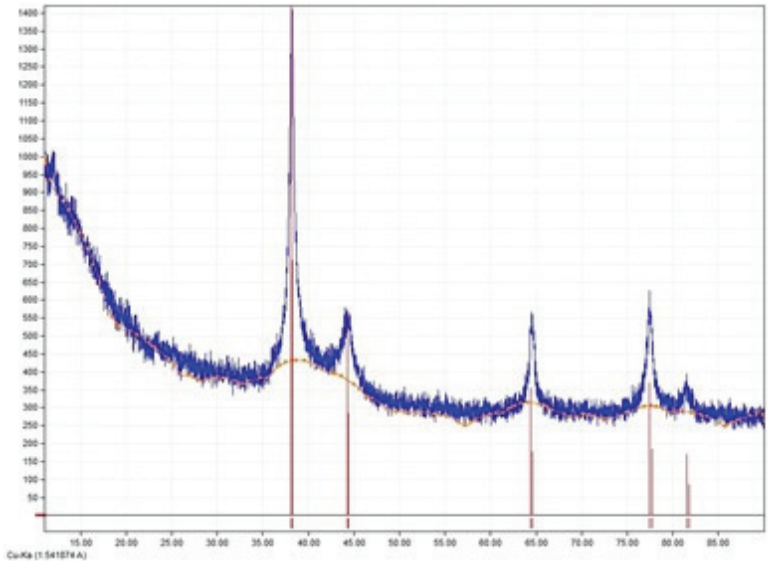

Fig. 6. XRD pattern of OL-AgNPs synthesized using olive leaves extract.

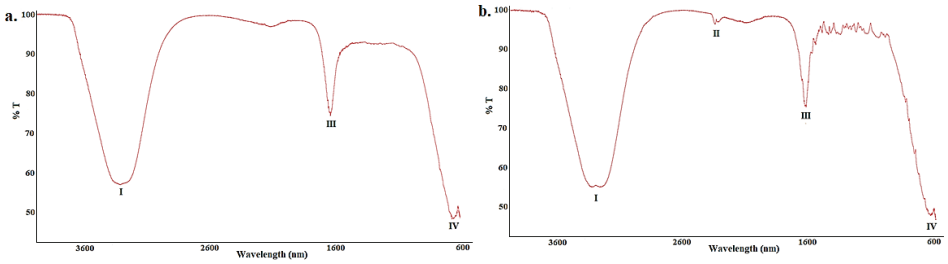

Fig. 7. FTIR spectra of: a) olive leaves extract and b) silver nanoparticles synthesized using olive leaves extract.

The zeta potential of OL-AgNPs was determined –26.7 mV (Fig. 8).

TABLE II. The peak list (cm^{-1}) of olive leaves extract and OL-AgNPs

Sample	I	II	III	IV
a. Olive leaves extract	3309.81	–	1634.04	666.83
b. Silver nanoparticles synthesized using olive leaves extract	3276.99	2358.35	1629.30	644.56

Zeta potential is the electrical charge on the surface of the surrounding material. For the high stability and stability of the AgNP colloid, the zeta potential should be in high negative value, which prevents the particles from sticking together or aggregating. Additionally, nanoparticles with increased negative charge values can enter the cell more easily.[49]

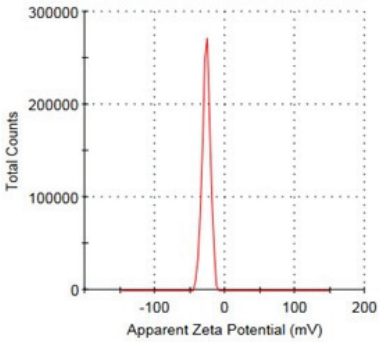

Fig 8. Zeta potential of OL-AgNPs.

Energy diffraction X-ray (EDX) is carried out to analyze the dispersed size distribution of nanoparticle components.[50] The elemental composition of silver in OL-AgNPs was determined as 86.25 % for the normalized atomic value. OL--AgNPs showed a characteristic optical absorption peak at nearly 2.8 keV due to surface plasmon resonance (Fig. 9).

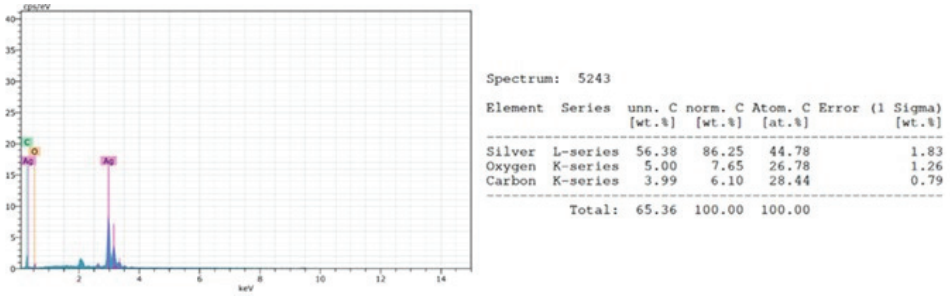

Fig. 9. Elemental composition of OL-AgNPs by EDX analysis.

Identification peaks for the main silver (Ag) emission energies were shown and these peaks match the spectrum, indicating that silver had been accurately

determined. The average particle sizes were found 85 nm (Fig. 10). The results obtained in this study were compatible with the literature (Table III).

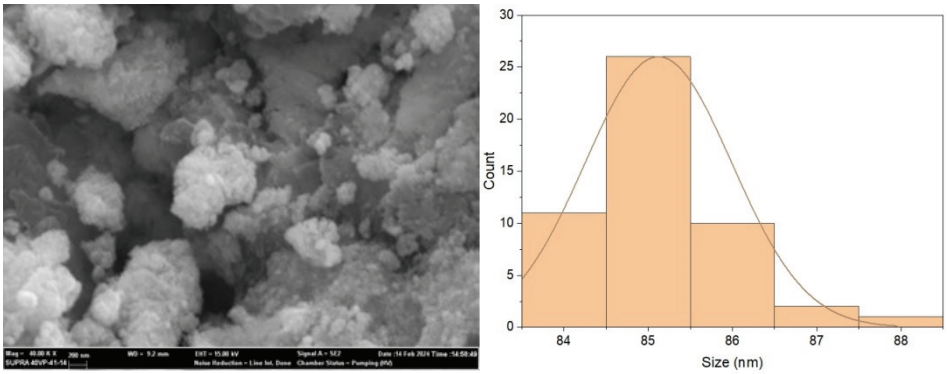

Fig. 10. SEM images and histogram of OL-AgNPs.

TABLE III. Comparison of optimum parameters for the green synthesized silver nanoparticles

Natural source of reducing materials	pH	Temperature °C	Time min	Size of silver particles, nm	Reference
Eucalyptus oleosa	–	25	960	45	51
Lemon	7	80	240	50	52
Hibiscus rosasinensis leaves	6	70	30	43	53
Olive leaves	8	22	52	50	54
Moringa oleifera leaves	–	Direct sunlight	60	57	55
Olive leaves	10	60	45	85	This study

Table IV provides the limit of detection (*LOD*) values for cyprodinil and mepanipyrim pesticides determined using different methods. It is anticipated that the electrodes prepared with the synthesized nanoparticles will enable the detection of these pesticides with lower *LOD* values.

TABLE IV. Comparison of *LOD*s of some methods developed for the determination of pesticides

Method used	Pesticide	*LOD*	Reference
Gas chromatographic determination	Cyprodinil	0.05 mg/kg	56
High-performance liquid chromatography–diode array detection	Cyprodinil	42.9 µg/L	57
QuEChERS method coupled with UPLC-MS/MS	Cyprodinil	below 0.4 µg/kg	58
Square wave stripping voltammetric determination	Cyprodinil	0.076 mg/L	59
QuEChERS method coupled with UPLC-MS/MS	Mepanipyrim	below 0.4 µg/kg	58
Single drop microextraction and gas chromatography––mass spectrometry	Mepanipyrim	0.03 µg L^{-1}	60
Liquid chromatography-tandem mass spectrometry	Mepanipyrim	0.10 µg /kg	61

1.0×10^{-3} M H_2O_2 stock solution was used to compare the sensitivity of CPE and OL-AgNPs-MCPE to H_2O_2. Fig. 11 shows that OL-AgNPs-MCPE is more than 50 times more sensitive to H_2O_2 than CPE.

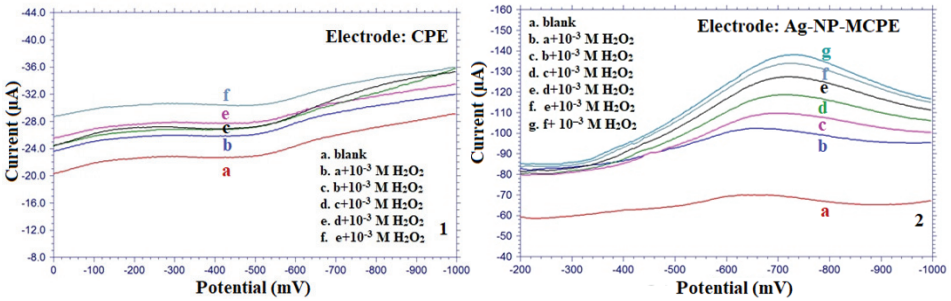

Fig. 11. Square wave voltammograms for H_2O_2 on: 1) CPE and 2) OL-AgNPs-MCPE (pH 2.0 BR buffer solution; f = 15 Hz; ΔE = 25 mV).

It was clear that the prepared OL-AgNPs-MCPE had a good performance in detection of H_2O_2. Different biosensors, which are based on measuring H_2O_2, could be designed by using OL-AgNPs-MCPE. 2.0×10^{-3} M mepanipyrim stock solution was used to compare the sensitivity of CPE and OL-AgNPs-MCPE to mepanipyrim. Fig. 11 shows that OL-AgNPs-MCPE was more than 4 times sensitive to mepanipyrim than CPE (Fig. 12).

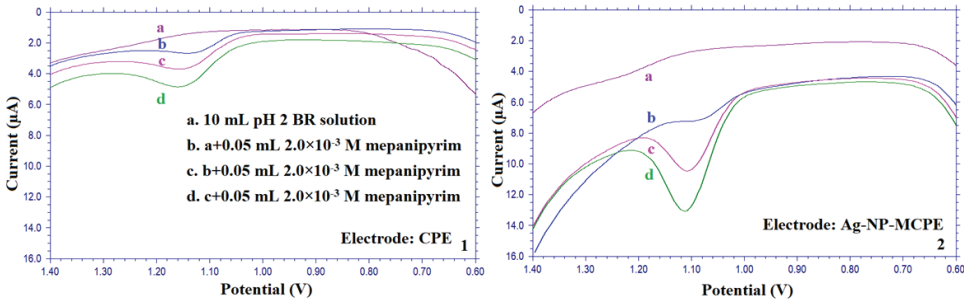

Fig. 12. Square wave voltammograms for mepanipyrim on: 1) CPE and 2) OL-AgNPs-MCPE (pH 2.0 BR buffer solution; f = 15 Hz; ΔE = 25 mV).

1.0×10^{-3} M cyprodinil stock solution was used to compare the sensitivity of CPE and OL-AgNPs-MCPE to cyprodinil. Fig. 12 shows that OL-AgNPs-MCPE was 3 times more sensitive to cyprodinil than CPE (Fig. 13).

1.0×10^{-4} M acetylthiocholine chloride (ATCh) and acetylcholinesterase (AChE) were used for the electrochemical determination of thiocholine. Fig. 13 shows that the peak was not observed at CPE for thiocholine. OL-AgNPs-MCPE is more

sensitive to thiocholine than CPE. The obtained currents at nearly 850 mV were plotted against the thiocholine concentration and the linearity showed (Fig. 14).

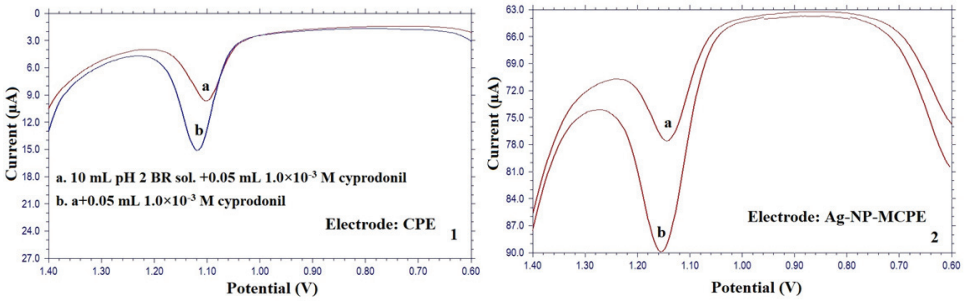

Fig. 13. Square wave voltammograms for cyprodinil on: 1) CPE and 2) OL-AgNPs-MCPE (pH 2.0 BR buffer solution; f = 15 Hz; ΔE = 25 mV).

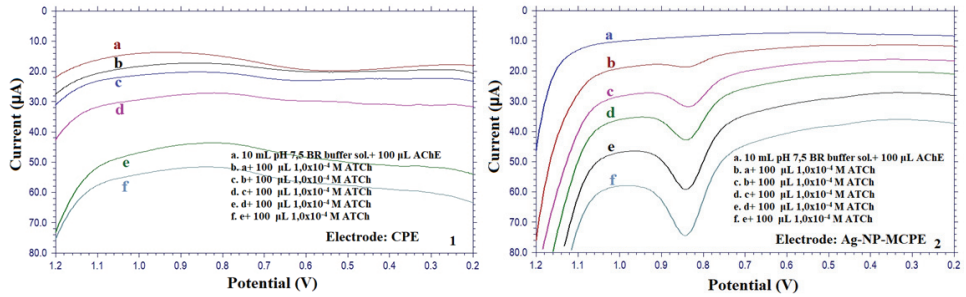

Fig. 14. Square wave voltammograms for thiocholine on 1) CPE and 2) OL-AgNPs-MCPE (pH 2.0 BR buffer solution; f = 15 Hz; ΔE = 25 m).

As a result, this study showed a successful approach to synthesizing silver nanoparticles using olive leaves extract, which could be used in electrochemical determination of H_2O_2, mepanipyrim, cyprodinil and thiocholine.

CONCLUSION

In this study, a new modified carbon paste electrode was prepared for the determination of the most commonly used pesticides (mepanipyrim and cyprodinil), H_2O_2 and thiocholine. For this purpose, eco-friendly silver nanoparticles were synthesized using waste olive leaves extract. In addition, the optimal parameters for synthesizing silver nanoparticles, using olive leaves extract, and characterization were studied. The application of silver nanoparticles in the preparation of modified carbon paste electrodes provides successful. Voltammetric determination of H_2O_2, mepanipyrim, cyprodinil and thiocholine. Consequently, the present study showed an innovative approach for synthesizing silver nanoparticles, using olive leaves extract, which can be used in various electrochemical determinations.

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ИЗВОД

ПОТЕНЦИЈАЛ ЕЛЕКТРОХЕМИЈСКИХ АНАЛИЗА ПЕСТИЦИДА ПОМОЋУ НАНОЧЕСТИЦА СРЕБРА СИНТЕТИСАНИХ ЗЕЛЕНИМ ПУТЕМ НА БАЗИ ОТПАДНОГ ЛИШЋА МАСЛИНЕ

EZGI ADAK[1,2], MERVE KESKIN[3] и HALIT ARSLAN[1]

[1]Department of Chemistry, Faculty of Science, Gazi University, 06500, Ankara, Turkiye, [2]Gazi University Graduate School of Natural and Applied Science, 06500, Ankara, Turkiye и [3]Vocational School of Health Services, Bilecik Seyh Edebali University, 11210, Bilecik, Turkiye

Пестициди су хемикалије које негативно утичу на људско здравље и животну средину. Несавесна употреба пестицида доводи до присуства њихових остатака у храни и пољопривредним производима, као и до загађења воде и земљишта. Брзо и једноставно одређивање остатака пестицида је важно за контролу животне средине. Из тог разлога, неопходно је развити алтернативне методе за одређивање остатака пестицида које би могле заменити традиционалне технике, као што је хроматографија. Електрохемијски сензори су аналитички уређаји нове генерације развијени за одређивање пестицида који омогућавају брзу и практичну анализу. Електроде од угљеничне пасте, које се користе у дизајну електрохемијских сензора, модификоване су коришћењем различитих материјала као што су наночестице сребра. Наночестице сребра, које се користе за модификацију, могу се синтетизовати хемијским методама, али су ове методе штетне по животну средину, јер се у поступку синтезе користе токсичне хемикалије. Из тог разлога, развијена је техника зелене синтезе као алтернатива хемијским техникама. У овој студији, отпадни листови маслине коришћени су у зеленој синтези сребрних наночестица као прекурсора електрона. Наночестице (OL-AgNP) су окарактерисане и припремљене су модификоване електроде од угљеничне пасте. Електроде су коришћене у одређивању тиохолина (производа хидролизе ацетил тиохолина ензимским реакцијама), H_2O_2 (производа многих реакција ензима оксиредуктазе) и широко коришћених пестицида ципродинила и мепанипирима. Резултати су показали да су модификоване електроде од угљеничне пасте биле осетљивије од електрода од угљеничне пасте за све аналите. Јасно је показано да се модификоване електроде од угљеничне пасте могу користити у одређивању пестицида.

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