Nanomaterial-based sensors for water remediation, healthcare and food monitoring applications

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A photonic crystal material for the online detection of nonpolar hydrocarbon vapors

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Abstract

A modern level of nanotechnology allows us to create conceptually new test systems for chemical analyses and to develop sensitive and compact sensors for various types of substances. However, at present, there are very few commercially available compact sensors for the determination of toxic and carcinogenic substances, such as organic solvents that are used in some construction materials. This article contains an overview of how 3D photonic crystals are used for the creation of a new test system for nonpolar organic solvents. The morphology and structural parameters of the photonic crystals, based upon a crystalline colloidal array with a sensing matrix of polydimethylsiloxane, have been determined by using scanning electron microscopy and by the results of specular reflectance spectroscopy based on the Bragg–Snell law. A new approach has been proposed for the application of this sensor in chemical analysis for the qualitative detection of saturated vapors of volatile organic compounds due to configuration changes of the photonic bandgap, recorded by diffuse reflectance spectroscopy. The exposure of the sensor to aromatic (benzene, toluene and p-xylene) and aliphatic (n-pentane, n-heptane, n-octane and n-decane) hydrocarbons has been analyzed. The reconstitution of spectral parameters of the sensor during the periodic detection of saturated vapors of toluene has been evaluated.

Introduction

Photonic crystals (PhCs) used for chemical sensors can be divided into three groups depending upon their structure, that is, one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D) [1-10]. 2D and 3D structures used as chemical sensors are studied in most projects. 2D structures consist of a monolayer of spherical particles placed on a substrate. 3D struct-
tures, which appear in the form of a crystalline colloidal array (CCA), are called opal structures (spherical particles close-packed in an ordered structure). If the structure has been placed in a matrix and the particles have been removed, then it is an inverse opal structure [11-13]. A photonic bandgap (PBG) appears in colloidal crystals due to the periodic modulation of the refractive index. At the bandgap, selective reflection of light is observed, which is connected to a low photon density of states within the materials [14]. Most of the configuration changes of the photonic bandgap in opal and inverse opal structures occur due to swelling or compression of the polymer matrix or gel.

To date, four main methods for the modification of photonic crystals are used for the creation of stimuli-responsive materials: (a) formation of a sensitive polymer matrix, (b) impregnation of the reagent, (c) immobilization of the reagent and (d) preparation of the sensor elements from molecularly imprinted polymers.

Organic solvents are usually detected by using polymer matrix sensors (Table 1) through matrix interaction [7-9,15,16]. The impregnation and immobilization methods are rather close; they are used for the determination of inorganic ions (Cu$^{2+}$, Pb$^{2+}$, Hg$^{2+}$, Ni$^{2+}$ and Cd$^{2+}$) [2,11,17-19] and organic molecules of simple and complex structure (glucose, organophosphates, urea, creatinine, ciprofloxacin and sarin) [5,6,20-27]. The development of a sensor device with molecularly imprinted polymers allows for the determination of organic compounds (nicotinamide, sulfonamides, bisphenol A and diethylstilbestrol) with a more complex structure [12,13,28,29].

The color shift (blueshift or redshift) or the color intensity of the sensor serves as an analytical signal for such sensors. The standard method for measuring an analytical signal is specular reflectance spectroscopy within the visible range; however, the interaction of a 2D PhC with the analyte is also analyzed by changing the diameter of the Debye diffraction ring [2,5,6,10].

Currently, very few works are devoted to the study of the mechanism that leads to the shift of the PBG in 2D and 3D photonic crystals. This is caused by the variety of flow processes in the structure, which are significantly influenced by the filling of the structure, the structural heterogeneity within a volume, the presence of foreign chemical substances and the size variation of the matrix and particles during the chemical analysis process. However, one cannot ignore sensors based on molecularly imprinted polymers for the selective detection of volatile organic compounds [30-32]. In most cases, the response of such sensors is a change in mass recorded using a quartz microbalance. A simpler design and research method made it possible to investigate in more detail the processes occurring during the absorption of solvents. The ability to control selectivity in molecularly imprinted sensors and the simple visual response in photonic crystal sensors make it promising and even mandatory to combine these two approaches.

In our previous study, the optimal parameters of polystyrene (PS) particles for sensor matrices for saturated vapors of volatile organic compounds have been determined [33]. In this work, we determined the parameters of the sensor structure and examined online the detection of high concentrations of aromatic and aliphatic hydrocarbon vapors in air. The detection was

| Table 1: Some photonic crystal structures for chemical sensing of organic solvents. |
| Geometry | Material | Response | Analyte | Limit of detection | Ref. |
| 3D PhC (CCA) | polystyrene | redshift (<50 nm) | methanol vapors | 5% ($V_{methane}/V_0$) | [7] |
| 3D PhC (PCCA)$^a$ | polystyrene-Ag/ polydimethylsiloxane | redshift (<50 nm) | chloroform, chlorobenzene, tetrahydrofuran, dichloromethane and dimethoxyethane liquids | 5 µL (5 nm) | [8] |
| 3D PhC (CCA) | polystyrene | redshift (<40 nm) | methanol, ethanol, isopropanol, 1-propanol and n-butanol vapors | 2% ($V_{ethane}/V$) | [9] |
| 3D PhC (PCCA) | polymethylmethacrylate/ methyl cellulose | redshift (<80 nm) | ethanol, n-propanol, isopropanol and n-butanol liquids and vapors | NA | [15] |
| 3D PhC (CCA) | polystyrene | redshift (<150 nm) | methanol and ethanol benzene, toluene, p-xylene, n-pentane, n-heptane, n-octane and n-decane vapors | NA | [16] |
| 3D PhC (PCCA) | polystyrene/ polydimethylsiloxane | redshift | 0.3 mg/m$^3$ (toluene) | this work |

$^a$Polymerized crystalline colloidal array.
performed by using 3D PhC-based sensors, which are a CCA of polystyrene submicrometer particles embedded in a polydimethylsiloxane (PDMS) layer. The matrix interaction was responsible for the main mechanism, which was monitored by configuration changes of the photonic bandgap using diffuse reflectance spectroscopy.

Results and Discussion
Determination of the morphology and the structural parameters of the sensor
A comparison between the specular reflectance and the diffuse reflectance spectra tested in the “specular component included (SCI)” and “specular component excluded (SCE)” modes has shown (Figure 1a) that the maximum of the diffuse reflectance spectra of the PhC sensor in the SCI mode coincides with the maximum of the specular reflection spectra resulting from flat (111) surfaces at an 8° angle. This applies regardless whether the sensor has a PDMS layer or not. However, the diffuse reflectance spectra of the sample without a polydimethylsiloxane layer, measured in the SCE mode, had a significant intensity decrease at the assumed maximum point of the reflection. For a sensor with a PDMS layer, the spectrum intensity of the specular reflectance component, regarding the diffuse reflectance spectrum, decreases. This is expressed in the smaller influence of the viewing angle on the color of the sensor and a simpler visual registration of the color due to a decrease of the iridescence effect, which is an important requirement for the testing system.

A reflection peak was approximated by a quadratic function ($y = ax^2 + bx + c$) to determine the PBG position. The fitted coefficients of the quadratic function of spectral maxima were used to determine the dependence of the photonic bandgap position on the exposure time and the incident angle by the analysis of the diffuse and specular reflectance spectra.

According to the literature data, the structure has a face-centered cubic lattice (FCC), therefore, it can be assumed that the structure is filled by 74% with PS and that the remaining volume is filled with air or polydimethylsiloxane [34,35]. Figure 2 shows the surface of a crystalline colloidal array obtained by using scanning electron microscopy.

The investigated samples have an ordered lattice structure similar to crystals; therefore, the Bragg equation has been applied for the analysis. Since the diameter of particles in the lattice is in the submicrometer region, diffraction occurs in the visible spectrum, and it becomes necessary to consider the refraction of light during propagation through materials with different refractive indices. In the case of the CCA with FCC lattice, the Bragg–Snell law can be written as follows:

$$m\lambda = 2d_{111}\sqrt{n_{\text{eff}}^2 - n_{\text{air}}^2 \sin^2 \theta},$$

where $m$ is the order of a diffraction maximum, $\lambda$ is the wavelength of the reflectance maximum, $d_{111}$ is the interplanar distance between crystallographic (111) planes, $n_{\text{eff}}$ is the effective refractive index of the medium.

![Figure 1: Optical characteristics of sensors based on 3D PhC (matrix thickness $\approx 90 \mu m$): (a) diffuse reflectance and specular (8°) reflectance spectra of the sensor before and after the PDMS layer formation (the difference in reflection intensity is caused by different apertures and the area of samples) and (b) dependence of $m^2\lambda^2$ on $\sin^2 \theta$ for the sensor with the PDMS layer (red circles) and without (blue circles), where $m$ is the order of the photonic bandgap, $\lambda$ is the wavelength of the photonic bandgap, $\theta$ is the incident angle. Black lines show the linear correspondence of the experimental data.](image)
Figure 2: (a) Photo of the sensor and (b) electron microscopy image of a CCA of polystyrene particles without a PDMS layer on a glass substrate (effective particle size = 201 nm). The data obtained with dynamic light scattering slightly overestimates the diameter in comparison with the data obtained from microphotographs. This may be related to the specificity of the dynamic light scattering (DLS) method (hydrodynamic diameter measurement).

The effective refractive index of the CCA, \( n_{\text{air}} \), is the refractive index of the medium (in our case air) from which light falls in and \( \theta \) is the incidence angle [8,9,36]. The incidence angle was varied from 8° to 65° in the experiments.

Knowing the volume fraction of particles in the CCA (\( f_p \)) and their refractive index (\( n_p \)), as well as the volume fraction of air or other substances (\( f_f \)) that fill the spaces between the particles and the corresponding refractive index (\( n_f \)), an effective refractive index of the CCA can be calculated by using the following formula:

\[
 n_{\text{eff}}^2 = \frac{f_p n_p^2}{1 - f_f n_f^2} + f_f n_f^2.
\]  

The interplanar distance between crystallographic planes (111) in a FCC lattice is related to the effective particle diameter \( D \) by the following equation:

\[
 d_{111} = \frac{\sqrt{2/3}}{D}.
\]  

From the linear dependence (Figure 1b) and Equations 1–3, an average diameter of spherical particles and the effective refractive index of the photonic crystal can be obtained [37,38]. The effective refractive index of the structure without PDMS is 1.525, with PDMS it is 1.599, and the average diameter of the particles is 197.5 nm and 201.5 nm, respectively. The obtained average diameters of the polystyrene particles are consistent with the SEM results, and the effective refractive indices are slightly overestimated in comparison with the theoretically calculated values (without PDMS: 1.477, with PDMS: 1.568) [39,40]. This may be due to the presence of surfactants in the interparticle space, defects of the crystal lattice, and a different ratio of volume fractions.

The use of diffuse reflectance spectrometry in further experiments is necessary to obtain integrated optical characteristics of the stimuli-responsive matrix as an analogue of visual recordings. This allows one to optimize the development of sensors with a visual recording of analysis results.

**Kinetics of interaction between the sensor and solvent vapors**

Of interest is the use of a 3D PhC-based sensor for online measurements of the concentration of nonpolar solvents and their vapors. Exposure to saturated solvent vapors allows the analyte to be delivered more evenly to the surface of the stimuli-responsive matrix than applying a liquid sample to the surface of the sensitive layer, which is an important feature in studying the mechanism of interaction. The following analytes were studied: benzene, toluene, \( p \)-xylene (the BTX aromatics), \( n \)-pentane, \( n \)-heptane, \( n \)-octane and \( n \)-decane, which have a high vapor pressure under normal conditions.

As a result of exposure to aromatic and aliphatic nonpolar solvents, a redshift in the PBG is observed using diffuse reflectance spectroscopy. The diffusion of vapors of organic solvents into the PDMS layer and the CCA leads to their swelling and, to a lesser extent, to a change in the effective refractive index of the structure (Figure 3). Since the experimental observation of the photonic bandgap shift was more than 100 nm, this would require a very large change in the effective refractive index. Consequently, the degree of swelling of polydimethylsiloxane...
predominantly affects the formation of a response when exposed to hydrocarbons. In this regard, it can be assumed that the sensor will be able to detect other hydrocarbons leading to a swelling of PDMS, such as diethyl ether, tetrahydrofuran and chlorobenzene [41]. Therefore, the main factor can be considered the affinity of solvents to PDMS, namely the polarity and rate of diffusion of the solvent through the matrix. In addition, the polydimethylsiloxane matrix protects the polystyrene CCA from the effects of high concentrations of hydrocarbons, thus, it acts as a “conductor” and “dispenser” of the analyte.

An analysis of the vapor effects of the analytes was performed through studying kinetic curves (Figure 4a). It was found that nonpolar aromatic compounds have some response delay, but a steeper rise of the S-curve, which is visually expressed as a more contrasting color change of the sensor matrix. The effect of vapors of nonpolar aliphatic organic solvents, in contrast, leads to an instantaneous photonic bandgap shift, but there is no sharp jump in the kinetic curve. The response time means the moment when the PBG shift rate is maximum; this parameter is well determined by the first derivative of the curves from Figure 4b. This feature allows one to distinguish qualitatively the analytes. This behavior can be explained by the rapid dissolution of the necks (“bridges”) between neighboring particles that occur during CCA assembly and hold this array, preventing it from moving apart due to the swelling of the polydimethylsiloxane layer, whereas when exposed to the test alkanes, this does not happen so quickly, and the shift of the lower layers is delayed, resulting in broadening of the photonic bandgap and a less pronounced color.

**Qualitative detection of nonpolar low-molecular-weight organic compounds**

It was found that the response rate increases exponentially among \(n\)-pentane, \(n\)-heptane, \(n\)-octane and \(n\)-decane. This is...
consistent with an exponential decrease in vapor pressure and a decrease in the rate of diffusion of the compounds into the polymer matrix (Figure 5). The effect of \( n \)-decane vapor does not lead to a significant change in the sensor color, but only shifts the PBG by just \( \approx 10 \) nm, nevertheless, the approach proposed here allows one to detect slight changes in the sensor color.

Aromatic and aliphatic hydrocarbons were screened for the color change time of the sensor. For the series benzene, toluene, \( p \)-xylene and \( n \)-pentane, \( n \)-heptane, \( n \)-octane and \( n \)-decane, an increase in the response time is observed that is close to exponential. This allows for the detection of the total toxic effect considering the different analytical response rates and the toxicity of the detected compounds.

Since the absorption of hydrocarbon vapors is responsible for the delivery of the analyte to the photonic crystal, a change in the sensor sensitivity is possible by varying the thickness of the sensitive PDMS layer. In the experiments, sensors with a polydimethylsiloxane layer thickness from 10 \( \mu \)m to 2 mm were investigated for detecting low concentrations of vapors and liquid hydrocarbons. As the limit of detection depends on the matrix thickness, the main obstacle to its reduction is the development of a technique for uniform application of polydimethylsiloxane. In some experiments, it was possible to detect toluene vapors with a concentration of ca. 0.3 mg/m\(^3\) using a sensor with a sensitive layer thickness about 20 \( \mu \)m.

**Effects of organic solvent mixtures on the sensor**

Of particular interest is the detection of analytes in complex objects. An example is the detection of toluene in the presence of xylenes. We discovered that the response rate of the composite sensor is affected by the \( p \)-xylene/toluene ratio in the analyzed mixture. The relationship shown in Figure 6, corre-

![Figure 6: The dependence of the sensor response rate on the content of toluene in \( p \)-xylene (matrix thickness about 100 \( \mu \)m).](image)

![Figure 5: Response rates of sensor matrices (red) and vapor pressure (blue): (a) response time for aromatic hydrocarbons (matrix thickness about 280 \( \mu \)m) and (b) response time for normal alkanes (matrix thickness about 90 \( \mu \)m).](image)
lates well ($R^2 = 0.995$) with a 3rd-degree polynomial curve:

$$t = -2.0 \times 10^{-5}C_{\text{PhMe}^3} + 4.8 \times 10^{-3}C_{\text{PhMe}^2} - 0.49C_{\text{PhMe}} + 23.$$

The rate of sensor response to the mixture increases sharply even with a low content of toluene. This factor indicates the possibility of detecting small concentrations of volatile organic compounds with a higher vapor pressure in complex objects. The experimental results also show the possibility of using a sensor to assess the total toxic effect of BTX vapors.

This approach shows that this sensor can be used for the qualitative analysis of complex matrices such as fossil fuel due to the different types of kinetic curves, for example, as shown for aliphatic and aromatic hydrocarbons, according to the criteria obtained after processing the kinetic curves using chemometric methods of analysis.

**Reversibility of optical characteristics of the sensor during cyclic exposure to toluene**

After the first cycle of exposure to both aromatic and aliphatic solvents, the initial color of the sensor changes, associated with the degradation of the CCA. It should be noted that benzene, toluene and $p$-xylene, unlike, for example, $n$-pentane or $n$-heptane, can lead to the irreversible destruction of the stimuli-responsive matrix due to dissolution or adhesion of PS particles. Therefore, an experiment was carried out with cyclic exposure to toluene vapor. The sensor matrix was exposed to saturated toluene vapors for 8 min. Then, the sensor was allowed to stand for a day to recover the photonic bandgap to its initial position, although 90% of recovery was reached already after 2 min, and the experiment was repeated.

From the results presented in Figure 7a, we can conclude that there is a wide spread in both the initial position of the PBG and

**Figure 7**: Reversibility of the response to toluene vapor: (a) position of the reflection maximum: green – before exposure to toluene vapor, red – at the response point, blue – 8 min after the exposure (data obtained with an “eye-one Pro” mini-spectrophotometer). (b, c) The starting position of the maximum reflection and initial photo images of the sensor before the next cycle of exposure to toluene vapor (data obtained with a “Ci7800” spectrophotometer).
the final one, but this is explained by the heterogeneity of the sensor degradation over the surface area and the small aperture of the mini-spectrophotometer (4.5 mm), which is smaller than the treated sensor area. Before each experiment, the diffuse reflectance spectra were recorded using a device with a larger aperture (10 mm) than the sample size (Figure 7b,c). Even though a partial degradation of the sensor occurs after each detection, it is already linear after the second detection ($R^2 = 0.988$) and can be taken into account accordingly.

**Conclusion**

An approach is proposed for the qualitative determination of aromatic and aliphatic hydrocarbons using stimuli-responsive materials based on 3D photonic crystals. The kinetic regular interactions of organic nonpolar solvents with a photonic crystal-based sensor, having a PDMS sensor matrix, were studied by diffuse reflectance spectroscopy. Vapors of a $p$-xylene/toluene mixture containing the components in different proportions were detected. The possibility of determining the concentration of compounds in a two-component mixture is confirmed.

It was found that after stopping the exposure, the position of the photonic bandgap is almost completely recovered. This fact allows one to perform chemical cycles or online environmental monitoring. However, when exposed to aromatic solvents, sensor degradation is observed, but its linear direction should be noted.

**Experimental Materials**

Submicrometer particles from linear polystyrene have been synthesized in the Institute of Fine Chemical Technologies RTU MIREA [42]. The sensor matrix has been developed from PDMS “Sylgard 184 silicone elastomer” (Dow Corning, USA). The following organic solvents have been used as analytes: benzene “pur.” and toluene “p.a.” (Reakhim, Russia); $p$-xylene “pur.”, $n$-pentane, “puriss.”, $n$-heptane “puriss. spec.” and $n$-decane “pur.” (EKOS-1, Russia); $n$-octane “pur.” (Acros Organics, Belgium/US).

**Instruments**

The average hydrodynamic radius of the PS particles has been determined by using the DLS method on a “Zetasizer Nano ZS” (Malvern Panalytical Ltd, UK) device. Microstructures of sensor matrices have been tested by using the SEM method on an “NVision 40” (Carl Zeiss, Inc., Germany) device and a specular reflectance spectrophotometer “Lambda 950” (PerkinElmer, Inc., USA) in the visible range of the electromagnetic spectrum. The diffuse reflectance spectra have been recorded on a spectrophotometer “Ci7800” (X-Rite, Inc., USA) in the visible range of the electromagnetic spectrum in SCI and SCE modes. The studies of kinetics have been carried out using a mini-spectrophotometer “eye-one Pro” (X-Rite, Inc., USA). The thickness of the stimuli-responsive layer (PDMS and CCA) has been measured by using a “Constant K5” thickness gauge (KONSTANTA LLC, Russia) with an ID2 induction converter. The SEM images have been obtained at the Centre of Shared Equipment of IGIC RAS.

**Sensors**

A composite sensor based on 3D PhC, which has a sandwich design, has been used for the experiments. The opal structure is formed on a glass or polymer (polycarbonate or polyleylene terephthalate) substrate. The structure has a close-packed crystal lattice, the nodal points of which contain spherical submicrometer particles of polystyrene with a hydrodynamic diameter of 239.5 nm (polydispersity index 0.101), determined by the DLS method. The CCA has been obtained by self-organization from a water–ethanol suspension [43]. The formed structure was covered with a hydrophobic polymer material layer, that is, polydimethylsiloxane of a specified thickness, which serves as a sensitive layer and mechanically protects the CCA. Sensors with a glass carrier have better optical characteristics but are inferior to polymer regarding material strength.

**Method**

Figure 8a shows the scheme of the experimental equipment. It consists of the spectrophotometer “eye-one Pro” and a peripheral device, which is made of black composite material based on epoxy resins for visible-light absorption and to elude backward reflection. The diffuse reflectance spectra have been recorded automatically by using the standard software “i1Share v1.4” (X-Rite, Incorporated, USA) and a scripting language program that allowed for receiving data on a preset periodic base. Temperature, pressure and humidity were monitored by a BMP280 sensor (Robert Bosch GmbH, Germany).

A sample of the PhC sensor (Figure 8b) was installed on a cuvette with an analyte solution and fixed with a clamp to avoid vapor leakage during the exposure. The sensitive side of the sample was faced into the cuvette with an analyte and the diffuse reflectance spectra were recorded through the optically transparent carrier. A negligible reflection of the polycarbonate substrate is in the blue spectrum region (below 420 nm) and does not overlap with the reflection of the photonic crystal. As a result of the experiment, we obtained spectra measured at a predetermined time interval (Figure 8c). The color of the sensor changed from green to red (Figure 8d).
Figure 8: Key points of the experiments: (a) scheme of the experimental equipment; (b) a photo image of the sensor; (c) redshift upon exposure of saturated hydrocarbon vapors on the sensor; (d) photo image of the sensor before and during the exposure.

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References
Piezoelectric nanogenerator for bio-mechanical strain measurement

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Abstract

Piezoelectric materials have attracted more attention than other materials in the field of textiles. Piezoelectric materials offer advantages as transducers, sensors, and energy-harvesting devices. Commonly, ceramics and quartz are used in such applications. However, polymeric piezoelectric materials have the advantage that they can be converted into any shape and size. In smart textiles, polyvinylidene fluoride (PVDF) and other piezoelectric polymers are used in the form of fibers, filaments, and composites. In this research, PVDF nanofibers were developed and integrated onto a knitted fabric to fabricate a piezoelectric device for human body angle monitoring. Scanning electron microscopy and X-ray diffraction analyses were used to study the morphology and to confirm the beta phase in fibers. The results reveal that the nanofibers made from solutions with high concentration were smooth and defect-free, compared to the fibers obtained from solutions with low concentration, and possess high crystallinity as well. Under high dynamic strain more output voltage is generated than under low dynamic strain. The maximum current density shown by the device is 172.5 nA/cm². The developed piezoelectric nanofiber sensor was then integrated into a knitted fabric through stitching to be used for angle measurement. With increasing bending angle, the output voltage increased. The promising results show that the textile-based piezoelectric sensor developed in this study has a great potential to be used as an angle measuring wearable device for the human body due to its high current density output and flexibility.
Introduction

Smart textiles are normally elevated to value-added textile products with improved properties and characteristics [1]. They exhibit properties of a textile with some added characteristics. Smart textiles are obtaining by combining conventional textile techniques, such as knitting or weaving, with different technologies of electronics [2]. The world is moving towards intelligent or smart textiles. In 2012, the size of the smart textile market was almost $289.5 million and surged to $1,500 million in 2020. The integration of electronically active fibers or yarns in textile substrates is the basis of smart textiles [3]. Textile-based sensors and electrodes are composed of conductive fibers, threads, or fabrics [4]. Their use for physiological and medical examination has been rising rapidly in the last couple of years. Textile-based sensors, being flexible, are easy to fit in a garment and create no barrier to the wearer. Nowadays, wearable sensors based on conductive threads and conductive polymers are capable of measuring vital signs of the human body [4,5]. Tognetti et al. [6] designed and developed a resistive strain sensors for movement analysis. They integrated an electrically conductive elastomer into a fabric, which was then able to detect the posture and the movement of the human body. Retrieved data from these strain sensors were compared with conventional motion tracking systems. The results show promising performance for body posture classification and reconstruction. Similarly, for measuring human body angles, piezoresistive sensors were developed and characterized under bending and stretching regarding the application as strain sensors [7]. Knitted piezoresistive fabrics were used to develop sensors that were a wearable type of a goniometer. These sensors were then tested under static and dynamic conditions. For another application, researchers designed and developed a purely textile-based capacitive pressure sensor to be integrated and embedded into the garments to monitor and measure human body pressure. These sensors were beneficial for pressure sore prevention, rehabilitation, and the detection of movement during activities. Further, these sensors were comfortable and bendable and were applied onto the upper portion of an arm to detect the deflection of the forearm during muscle bending [8]. Park et al. [9] developed a self-powered piezoelectric sensor for monitoring the pulse rate in real time. A pressure sensor was attached to the epidermis for monitoring pulse and assessing personal health status. Traditional sensors for pulse monitoring can detect biосignals of the human body but they have the limitation of power supply, which will restrict the operation of the wearable devices for medical purposes. Hence, piezoelectric sensors were used for monitoring bio-signals of the human body without limitation of power supply. Moreover, Lorussi and co-workers developed a smart textile garment by embedding a strain sensor into an ordinary garment. For the piezoelectric effect, the conductive blend was applied onto the fabric, which resulted in a change in resistance under strain. This phenomenon was used in gloves, car seats, and leotards for determining body posture, shape, and gesture. They primarily focused on studying a leg pad that was able to perceive the knee movement and posture [10]. Piezoelectric sensors have a wide range of applications including sidewalks or crosswalks that collect energy from vibrations, which can be store in batteries [11]. Moreover, piezoelectric sensors can be used at workplaces and gyms to collect energy from machine vibrations [12]. These sensors are embedded under the shoes so that the pressure exerted during walking or running can be converted into energy and can be used for different applications. Piezoelectric sensors can also be used under mats and floors so the pressure due to footsteps can be utilized as a source to generate energy [13]. Besides these, piezoelectric sensors can also be used for sensing human body motion and monitoring physical health parameters, such as electrocardiograms [14-19]. Polyanvinylidene fluoride (PVDF), having a semi-crystalline structure, is generally synthesized through polymerization of vinylidene difluoride [20]. It generally has four crystalline phases, namely α, β, ε, and γ. Among them, the beta phase possesses the highest dipole movements, while the other phases are usually non-polar as their structural packing is anti-parallel [21]. Usually, PVDF is non-reactive towards acids and bases. It was discovered in 1969 that PVDF can produce electrical signals. Thus, it can be used in various applications of energy harvesting, in various forms such as fibers, films, monofilaments, and powder. This material is trending in textile-based research where different researchers are working to manufacture smart textiles to generate energy [22,23]. Nanofibers have many technical applications such as in air and liquid filtration [24,25], tissue engineering [26,27], drug delivery [28], wound dressings [29], sound adsorption [30], cosmetics [31], and sensor devices [32-34]. In filtration processes, electrospun nanofibers can be employed for removing volatile organic compounds (VOCs) from the atmosphere. To protect people from bacteria, viruses, smog, and dust, nanofibers are utilized in medical face masks. These masks will not allow the particles to be inhaled because of the small pore size of the nanofibrous scaffold, while oxygen molecules are small enough to pass through these pores. Nanofibers are also used in other medical applications, for instance, for developing artificial organs and blood vessels, and in gene and drug delivery [35]. Monitoring joint angles through wearable systems enables human posture and gesture to be reconstructed as a support for physical rehabilitation both in clinics and at the patients’ home [36]. To date, wearable sensors used for monitoring body movements in the market are battery-based. The battery needs to be worn all the time. Also, it needs to be charged or replaced, which makes its application impractical. In this work, we present a proof of concept for using a nanofi-
brous-based piezoelectric sensor composed of PVDF, which is capable of monitoring body angles. This sensor will be able to replace the battery being used in commonly available products and is more breathable, lightweight, and flexible. The developed sensor has been characterized through advanced techniques. The current density has been calculated and compared with the current state of the art. To the best of our knowledge, the PVDF-based nanofibrous device developed in this study is superior to previously reported ones.

**Experimental**

**Materials**

Polyvinylidene fluoride (PVDF) obtained from Alfa Aesar was used as a piezoelectric material. Dimethylformamide (DMF) and acetone from Sigma-Aldrich were used as solvents without any further purification. A conductive tape was used to make electrodes. Knitted fabric was used for the integration of the nanofibrous mesh for human body angle measurement.

**Fabrication of the nanofibrous mesh and its characterization**

PVDF solutions with varying concentrations (12, 14, and 16 wt %) were prepared in an acetone/DMF mixture (1:2.3 by volume). PVDF was dissolved in the acetone/DMF mixture at 120 °C in a sealed container under stirring for 4 h followed by incubating the solutions at room temperature for 24 h before electrospinning. A conventional electrospinning process was used to create the piezoelectric electrospun nanofibers. The polymeric solution was pumped from a metallic syringe needle of 0.4 mm inner diameter at a flow rate of 3.5 mL/h. The fibers were collected on a stationary collector placed at a working distance of 15 cm. A constant voltage of 15 kV was used for all the experiments. The nanofibrous meshes were first dried at room temperature in the fume hood for 24 h followed by drying in a vacuum oven until constant weight to ensure the complete evaporation of the solvents. PVDF nanofibers were characterized through scanning electron microscopy (SEM) and X-ray diffraction (XRD) to determine morphology and crystalline structure, respectively.

**Sensor development, its embedding, and testing**

The prepared PVDF nanofibrous mesh was folded into a square shape (4 cm²) with 2 mm thickness for sensor development. Subsequently, conductive tape was attached to both sides of the film in a way that it covered the maximum area of the sheet (Figure 1).

The developed piezoelectric sensor was tested in knee angle measurements using a digital oscilloscope. The sensor was exposed to low and high dynamic strains with varying frequencies to examine their effect on the output voltage. Then the developed sensor was integrated and stitched on a knitted fabric to check the effect of the bending angle on the output voltage.
Staple spun polyester thread was used to stitch the sensor onto the knitted fabric by using a lockstitch machine. The sensor was worn on the knee and the bending angle of the knee was changed from 0° to 45°, 90°, and 120° to check the piezoelectric output with a digital oscilloscope.

Results and Discussion

SEM analysis

SEM was used to study the diameter and morphology of PVDF nanofibers developed through three different PVDF solutions (12, 14, and 16 wt %). The secondary electron images, taken at 10,000× and 40,000×, magnifications of prepared PVDF electrospun films from a 12 wt % polymer solution are shown in Figure 2A. The images show that the nanofibers have a more bead-on-string-like structure than the nanofibers obtained from 14 wt % solution (Figure 2B) due to incomplete solvent evaporation. The optimum polymer solution concentration is essential to obtain defect-free smooth fibers [14, 37]. The nanofibers obtained from 16 wt % solution were smooth and presented a bead-free morphology (Figure 2C). Therefore, these fibers were selected for developing the piezoelectric sensor. The average diameter of the fibers obtained from different solutions is provided in Figure 2D.

XRD analysis

PVDF exhibits four crystalline structures: α, β, γ, and δ [38]. Normally, all phases of PVDF show almost similar peaks with the exception of unique peaks that are used to identify the crystalline structure. X-ray diffractograms were used to analyze the crystalline structure [39] of prepared PVDF nanofibrous meshes obtained from different solutions to identify the beta phase in the fibers, which is primarily accountable for piezoelectric characteristics. PVDF showed its strongest peak near θ = 20°. The alpha phase has a peak around 18° [40], the γ phase has its intense peak at 19.2° [41], while the beta phase exhibits peaks between 20.04 and 22.03° (Figure 3).

It can be seen that the beta phase is dominant in the fibers obtained from the 16 wt % solution compared to the solutions of 12 and 14 wt %. The crystallinity was calculated from the XRD diffractograms and came out to be 52.3%, 54.6%, and 57.7% for the fibers made from 12, 14, and 16 wt % solutions, respectively. This phenomenon can be correlated with the high concentration of polymer solution. During evaporation of the solvent, polymer chains are more likely to form crystalline structures because they are closer together than in the solutions with lower concentration. Also, when the polymer
amount increases in the solution it increases generation capacity [37].

Digital oscilloscope analysis
The nanofibrous mesh made from the 16 wt % solution was selected for developing the sensor (Figure 4F). These nanofibers have a smooth and defect-free morphology with highly crystalline regions, which indicate the complete evaporation of the solvent and the presence of a large amount of polymer. To check the piezoelectric output of the developed sensor, a digital oscilloscope was used and the sensor was tapped with the fingers. Silicon gloves were used to avoid any possible static charges influence [42].

The sensor was placed on a smooth surface and the wires of the sensor were connected to the probes of a digital oscilloscope. Low dynamic strain was applied onto the sensor by pressing the sensor with a finger and then lifting the finger. The process was repeated continuously to obtain the effect of low strain on the piezoelectric output. Figure 4A illustrates the result of the digital oscilloscope showing output voltage when the sensor was under low strain. The graph showing the piezoelectric output of PVDF nanofibrous sensor under low dynamic strain is presented in Figure 4B. It shows that under low dynamic strain the energy generation is small. To check the impact of high strain, the previous process was repeated by pressing the sensor with a high dynamic strain. Figure 4C demonstrates the result of the digital oscilloscope showing output voltage when the sensor was under high dynamic strain. The result of the piezoelectric output of PVDF nanofibrous sensor under high dynamic strain is shown in Figure 4D. Under high dynamic strain, the generated energy is higher than under low dynamic strain, which shows the direct relation between pressure and piezoelectric output. To check the impact of the frequency on the piezoelectric output, the sensor wires were attached to the probes of the digital oscilloscope and a high-frequency dynamic strain was applied onto the sensor by pressing the sensor with a finger and lifting the finger at high speed. Figure 4E shows the result of the digital oscilloscope showing the output voltage of the sensor under dynamic strain at high frequency.

To show the potential application of the developed sensor, the relation of the bending angle with the output voltage was established. For this, the sensor was integrated into a knitted fabric through stitching and a prototype representing a knee medical pad was developed (Figure 5A). The medical pad was worn onto a knee, and the knee was moved at different angles to check the output. The knee was moved from 0° degrees to 120° (Figure 5B). The effect of the bending angle on the output voltage was measured. When the bending angle was 45°, the output voltage was less than the output voltages at 90° and 120° because, with increasing angle, the sensor was more strained. Therefore, the bending angle and the output voltage are directly related. Figure 5C illustrates the voltage change with the change of the bending angle of the knee. The current density of the
device has been calculated and compared with the current state of the art (Table 1). Clearly, the highest efficiency is shown by the device developed in the current study.

**Conclusion**

An experimental study on a textile-based piezoelectric sensor for human body angle monitoring has been performed. In this research, the polymeric material PVDF was used for the development of a piezoelectric nanofibrous sensor. SEM and XRD analyses were performed to determine morphology and crystalline phases of the developed nanofibers, respectively. The SEM analysis of nanofibers confirmed smooth, defect-free, and uniform fibers produced from a solution of high concentration (16 wt %). Additionally, the highest content of the beta phase was present in the nanofibrous mesh developed from the highly concentrated solution. Therefore, these fibers were selected for developing a piezoelectric sensor for subsequent studies. For this, the sensor was integrated into a knitted fabric through stitching to make a wearable textile-based piezoelectric sensor for human body angle monitoring. The piezoelectric output was measured by using a digital oscilloscope. The output voltage was high for high dynamic strain, which was also confirmed by...
changing the angle of the knee. The higher angle exerts more strain onto the sensor, which generates a high voltage in return. The piezoelectric output also increased when the frequency of the dynamic strain was increased. The textile-based piezoelectric sensor developed in this study has a great potential to be used as an angle measuring wearable device for the human body due to its high current density output and flexibility.

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**References**

1. Orzan, C. M.; Florescu, M. S.; Zara, I. A.; Orzan, O. A. *Ind. Text. (Bucharest, Rom.)* 2020, 71, 572–575. doi:10.35530/it.071.06.202018
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A non-enzymatic electrochemical hydrogen peroxide sensor based on copper oxide nanostructures

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Full Research Paper

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Abstract

This article describes the synthesis of nanostructured copper oxide on copper wires and its application for the detection of hydrogen peroxide. Copper oxide petal nanostructures were obtained by a one-step hydrothermal oxidation method. The resulting coating is uniform and dense and shows good adhesion to the wire surface. Structure, surface, and composition of the obtained samples were studied using field-emission scanning electron microscopy along with energy-dispersive spectroscopy and X-ray diffractometry. The resulting nanostructured samples were used for electrochemical determination of the H₂O₂ content in a 0.1 M NaOH buffer solution using cyclic voltammetry, differential pulse voltammetry, and i–t measurements. A good linear relationship between the peak current and the concentration of H₂O₂ in the range from 10 to 1800 μM was obtained. The sensitivity of the obtained CuO electrode is 439.19 µA·mM⁻¹. The calculated limit of detection is 1.34 μM, assuming a signal-to-noise ratio of 3. The investigation of the system for sensitivity to interference showed that the most common interfering substances, that is, ascorbic acid, uric acid, dopamine, NaCl, glucose, and acetaminophen, do not affect the electrochemical response. The real milk sample test showed a high recovery rate (more than 95%). According to the obtained results, this sensor is suitable for practical use for the qualitative detection of H₂O₂ in real samples, as well as for the quantitative determination of its concentration.

Introduction

Hydrogen peroxide, a strong oxidant and an essential intermediate product in many biomedical reactions, has recently attracted widespread research interest. In high concentrations it can cause serious harm to human health and the environment, despite the fact that, in living organisms, H₂O₂ is a by-product of metabolism for a wide range of biological and chemical processes, occurring under the influence of external stimuli and intracellular processes [1,2]. Disruption of the natural regulation
process and increasing concentration of H$_2$O$_2$ in the blood can cause severe diseases such as Alzheimer’s and Parkinson’s [3], premature aging of cells [4], death of nerve cells [3,5,6], loss of brain mass [7], and cancer [8-11]. For this reason, targeted monitoring of the concentration of H$_2$O$_2$ in body fluids can be used in the diagnosis of these diseases [12-15]. Rapid and accurate determination and control of H$_2$O$_2$ concentration is an important task in many other areas, including pharmaceuticals [16-18], environmental protection [19], and industrial areas (especially food production) [20-25].

Measurement techniques including fluorescence [26,27], luminescence [28], spectrometry [29,30], and electrochemistry [31-33] are widely used for H$_2$O$_2$ determination. Currently, the electrochemical method is most widely used due to its simplicity, selectivity, and low detection limit. Modified (with enzymes) and unmodified electrodes are used as working electrodes. In the case of modified electrodes, the surface is functionalized by redox-active enzymes (the most popular being horseradish peroxidase) [34-36], and detection is carried out through physicochemical processes of interaction between H$_2$O$_2$ and the enzyme. This type of sensor has high catalytic activity, sensitivity, and selectivity. However, enzyme sensors have a significant disadvantage, namely enzyme instability. Due to the nature of enzymes, they can be easily damaged thermally and chemically during production, transportation, and use of electrodes. In addition, enzymes are quite expensive, which significantly increases the production cost and total price of this type of sensor. Recently, research has focused on the development of non-enzymatic electrochemical sensors for the detection of H$_2$O$_2$ [37-39]. In this type of sensor, H$_2$O$_2$ interacts with the electrode material directly. Certain catalytic processes occurring between H$_2$O$_2$ and the electrode material provide an unambiguous electrochemical response and, as a consequence, the selectivity of the sensor. This type of sensor is characterized by good reproducibility of measurement, low production cost, fast response, high sensitivity and selectivity, and chemical and mechanical stability in aggressive environments [40-46]. Nanostructured materials are widely used as the working surface of the electrode [47-49]. The most common are transition metal nanoparticles [33,37,50-54], carbon nanotubes [8], metal oxides [55-64], graphene [32,33], and ordered mesoporous carbon [38,65,66]. Compared to bulk materials, nanostructures have higher catalytic activity and a significantly increased surface area-to-volume ratio, which makes it possible to significantly increase both sensitivity of the sensor and rate of detection of H$_2$O$_2$. Among the nanostructured materials used, the most promising candidate is copper oxide (CuO) [56,67-71]. It has selectivity for the determination of H$_2$O$_2$, high catalytic activity, and a variety of morphologies (e.g., nanoneedles, nanoplates, and nanorods). Various techniques have been used in the preparation of nanostructured epitaxial CuO coatings, such as thermal oxidation of copper electrodes in an oxygen atmosphere [72,73], hydrothermal chemical oxidation of copper surfaces [56], and hydrothermal synthesis using various precursors containing copper ions [74,75]. Copper oxide nanostructures can also be obtained as a powder and then applied to electrodes by dip- or drop-coating techniques, using a porous substrate or binder polymers [69,76,77]. However, despite the widespread use and simplicity of this method of electrode preparation, it has a number of significant disadvantages. First, there is the problem of homogenization of the nanostructured suspension in solution. Second, nanostructures are distributed randomly during the process of deposition, which can affect the electrochemical activity of the electrode and reduce the repeatability of the experiment. Third, the obtained coatings are characterized by their low adhesion and poor mechanical stability, and can, thus, be easily damaged during production, storage, and measurement. These disadvantages can be avoided by using an in situ growth process of CuO nanostructures directly on a copper substrate, in the presence of certain surfactants or additives. This method makes it possible to obtain nanostructures with a large active surface area, which ensures efficient electron charge transfer between CuO nanostructures and the copper substrate due to the formation of high-density, single-crystal nanopetals. Nanostructures are produced in one step, and can be directly used as sensor electrodes without additional treatments such as surface modification or enzyme immobilization. This article describes the process of obtaining wire electrodes with nanostructured CuO coatings by a one-step chemical hydrothermal oxidation method and their application in electrochemical measurements for the detection of H$_2$O$_2$. The article proves the higher efficiency of nanostructured electrodes compared to electrodes with less developed surface. The article shows the influence of the time of hydrothermal synthesis on the morphology of nanostructures and, as a result, the change in the sensitivity of the sensor. The most important electrochemical measurements were carried out to determine H$_2$O$_2$ concentration in aqueous solutions using the obtained sensor. It is shown that the obtained non-enzyme sensor has high sensitivity and selectivity toward H$_2$O$_2$. Experiments were also carried out to detect H$_2$O$_2$ in real milk and mouthwash samples.

Materials and Methods

Materials

Ammonium persulfate ((NH$_4$)$_2$S$_2$O$_8$, CAS number: 7727-54-0), sodium hydroxide (NaOH, CAS number: 1310-73-2), and hydrogen peroxide solution (H$_2$O$_2$, 30%, CAS number: 7722-84-1) were purchased from Merck. Ascorbic acid (C$_6$H$_8$O$_6$, CAS number: 50-81-7), uric acid (C$_4$H$_5$N$_4$O$_3$, CAS number: 69-93-2), dopamine hydrochloride


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an X-ray diffractometer (RIGAKU Smart Lab, Cu Kα) integrated into the FESEM system. The crystalline structure of the samples was defined using energy-dispersive spectroscopy (EDS, Inca Synergy) integrated into the FESEM system.

The morphology of the surface of the nanostructured CuO samples was studied via field-emission scanning electron microscopy (FESEM, Tescan MAIA 3). The chemical composition analysis was performed via energy-dispersive spectroscopy (EDS, Inca Synergy) integrated into the FESEM system.

The crystalline structure of the samples was defined using an X-ray diffractometer (RIGAKU Smart Lab, Cu Kα (λ = 1.543 Å) using parallel beam scanning geometry and an additional Ge(220) × 2 bounce monochromator.

CuO layer synthesis on copper wires
A smooth film coating of copper oxide was obtained by annealing the copper wire in an oxygen atmosphere. Before annealing, the copper wire was washed several times with water and ethanol to clean the surface of possible contamination. The wire was then fixed in a metal holder and placed in a Linn High Therm (Germany) furnace, where it was gradually heated to 500 °C and held at this temperature for 30 min. Then, the oven was turned off and left to cool naturally. The result was a wire with a uniform black coating.

Nanostructured samples were obtained by a one-step chemical hydrothermal oxidation. For this, copper wire was rinsed with water and ethanol in order to clean the surface of possible contamination. To prepare the working solution, 10 mL of a 10 M NaOH solution and 26 mL of H₂O were combined. The wire samples were immersed in the resulting solution and then poured into a heat-resistant glass beaker with a lid. The beaker was placed in an oven preheated to 90 °C for 3 h, and then left to cool naturally. The obtained samples, covered with a nanostructured oxide layer, were repeatedly washed with distilled water in order to get rid of residual reagents, and then dried in an oven at 90 °C for 3 h in order to remove moisture.

To compare the dependence of the sensitivity of nanostructured samples on their morphology, samples were obtained after 1 and 6 h of synthesis time.

The morphology of the surface of the nanostructured CuO samples was studied via field-emission scanning electron microscopy (FESEM, Tescan MAIA 3). The chemical composition analysis was performed via energy-dispersive spectroscopy (EDS, Inca Synergy) integrated into the FESEM system.

The obtained wire samples were cut into 2 cm long pieces, and at one end were stripped to pure copper over 5 mm length to provide electrical contact with the equipment. The measurements were carried out using an electrochemical station (Zanher, Germany), supplemented by a custom-made electrochemical cell (for more details about its structure, see our publication [71]). During the measurement, a three-electrode cell was used, using oxide-coated copper wire as a working electrode, 0.4 mm diameter Ag/AgCl wire as a reference electrode, and a 6 × 6 mm PCB electrode with ENIG surface finish as a counter electrode.

Electrochemical measurements
Cyclic voltammetry (CV) was carried out in the range from −0.8 to 0.1 V vs Ag/AgCl, with U_start = 0 V vs Ag/AgCl and a scan rate of 100 mV/s. As buffer solution, 0.1 M NaOH (pH 12.7) was used. For the determination of H₂O₂, 0.1, 0.25, 0.5, 0.65, 0.85, 1, and 5 mM concentrations were used. Measurements were carried out five times for each of the indicated concentrations, and the curves in the following sections show the averaged data from all measurements. To determine the optimal scanning parameters that provide the maximum sensitivity of the sensor, the dependence of the electrochemical response on the pH of the buffer solution and on the scanning speed was studied.

Impedance spectroscopy was carried out in the frequency range from 1 Hz to 100 kHz at an applied signal voltage of about 0.3 V.

Differential pulse voltammetry
Before the measurement, the samples were maintained for 30 s at U = −0.8 V vs Ag/AgCl. The measurements were carried out using the following parameters: voltage range from −0.8 V to 0.1 V vs Ag/AgCl, pulse amplitude = 50 mV, pulse step = 3 mV, pulse width = 200 ms, and pulse frequency = 2 Hz. As buffer solution, 0.1 M NaOH was used. For the determination of H₂O₂, 0.033, 0.066, 0.1, 0.17, 0.25, 0.37, and 0.5 mM concentrations were used. The measurements were carried out five times for each of the indicated concentrations, and the curves in the following sections show the averaged data from all measurements.

To determine the scanning parameters that provide the maximum sensitivity of the sensor, the dependence of the differential pulse voltammetry (DPV) response on the pH of the buffer solution and on the pulse frequency was studied.

Current response study
For the current response study (i–t measurement), a constant voltage U = −0.7 V vs Ag/AgCl was applied to the cell.
and the current was measured. 0.1 M NaOH was used as buffer solution. The measurement was started at 0 µM concentration, and after 600 s (time required for stabilization) the first 10 µM portion of H₂O₂ was added. Subsequent portions were added every 30 s with the following steps: 10 µM for the concentration range of 0–100 µM, 20 µM for the concentration range of 120–300 µM, 50 µM for the concentration range of 350–800 µM, and 100 µM for the concentration range of 900–1800 µM. The measurement was carried out with constant stirring using a magnetic stirrer.

Interference study

A constant voltage $U = -0.7$ V vs Ag/AgCl was applied to the cell and the current was measured. As buffer solution 0.1 M NaOH was used. The experiment was started at 0 µM concentration of H₂O₂, then every 60 s either H₂O₂ or an interfering substance at a concentration of 100 µM was added to the solution, in the following order: H₂O₂, ascorbic acid, uric acid, dopamine, NaCl, glucose, and acetaminophen. Then, the whole cycle was repeated two times. The measurement was carried out with constant stirring using a magnetic stirrer.

Real sample study

To demonstrate the possibility of practical application of the obtained nanostructured electrodes for the analysis of real samples, samples of ultrahigh-temperature processed (UHT) milk were investigated. H₂O₂ is present in milk samples either as a result of enzymatic activity or as an antibacterial agent [20-22]. For the experiment, we used 3.2% fat milk and Listerine antiseptic mouthwash from a local supermarket. To reduce the sample matrix effect, the samples were diluted in a 1:2 ratio with 0.1 M NaOH buffer solution. The resulting solution was maintained at pH 12.7. The amperometric response method was used for the analysis with $U = -0.7$ V vs Ag/AgCl.

Results and Discussion

CuO structure

The morphology of CuO is shown in Figure 1. The SEM image (Figure 1a,b) shows the surface morphology of a thermally obtained copper oxide film. The resulting film is a homogeneous, polycrystalline oxide layer consisting of grains of arbitrary shape. In practice, this layer exhibits poor adhesion to the surface and can be easily damaged mechanically during post-processing.

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**Figure 1**: SEM images of copper oxide samples. (a, b) General view and morphology of a CuO film obtained by thermal oxidation on a copper wire; (c, d) general view of a copper wire with CuO layer obtained by chemical hydrothermal oxidation; (e) 3D flower-like nanostructured formations on the surface of the main CuO layer (f).
Figure 1c–f shows the morphology of the copper oxide layer obtained by chemical hydrothermal oxidation. The resulting coating is characterized by a high degree of uniformity, good adhesion to the copper surface and stability during post-processing. The resulting coating consists of a dense uniform layer of CuO petals several nanometres thick (Figure 1f). The surface of the main layer is covered with chaotically distributed, micrometre-sized 3D flower-like formations assembled from individual petals (Figure 1d,e).

EDS microanalysis showed that the samples consist of Cu (58.96 atom %) and O (41.04 atom %), which confirms the high chemical purity of the samples obtained and the absence of foreign impurities.

Figure 2 shows the XRD analysis results. The diffractogram shows only peaks corresponding to CuO and pure Cu (substrate peaks). Extraneous phases and inclusions were not detected. A low amorphous background indicates a high degree of crystallinity of the obtained samples. The X-ray diffraction pattern shows a large number of crystallographic planes corresponding to the CuO (tenorite) lattice; however, the dominant orientation corresponds to the direction perpendicular to the (002) and (111) planes.

The spherical shape of the obtained flower-like nanostructures indicates that their nucleation centre is not located in the plane of the substrate. The formation of spherical structures can be explained as follows: the presence of a large number of OH\(^{-}\) ions makes it possible to generate a large number of nucleation centres in solution in a short time. The particles begin to agglomerate in order to minimize the total surface energy, forming spherical seeds, which, according to the mechanism of dissolution–secondary precipitation [78,80], overgrow with CuO petals, thereby forming 3D structures in solution. Then, under the influence of gravity, these structures gradually descend to the substrate, where they are captured by the surface and immobilized.
Electrochemical measurements

Figure 3 shows the CV results for CuO in the solution containing 0.1 M NaOH and H₂O₂ at various concentrations. The curve shows a pair of oxidation peaks corresponding to Cu⁰/Cu⁺ and Cu⁺/Cu²⁺ transitions, as well as a pair of reduction peaks corresponding to Cu²⁺/Cu⁺ and Cu⁺/CuO transitions [68,81]. Figure 3a shows that the addition of H₂O₂ to the buffer solution affects the peak current values. The value of the maximum peak current decreases with increasing H₂O₂ concentration. Figure 3b compares the CV results for a pure Cu wire, a CuO film obtained via thermal oxidation (TO), and a nanostructured CuO film obtained by hydrothermal synthesis (HS). Measurements were carried out in 0.1 M NaOH solution containing 5 mM H₂O₂. Figure 3c shows the comparison of CV curves obtained at different pH values of buffer solution containing 5 mM H₂O₂. Figure 3d compares the CV curves obtained at different scan speeds. Measurements were carried out in 0.1 M NaOH solution containing 5 mM H₂O₂. Figure 3e shows the electrode stability study over multiple CV cycles (n = 10). Measurements were carried out in 0.1 M NaOH solution containing 5 mM H₂O₂. Figure 3f presents the EIS analysis (frequency range from 1 Hz to 100 kHz at an applied signal voltage of about 0.3 V). Measurements were carried out in 0.1 M NaOH solution containing 0–200 μM H₂O₂.
The mechanism of electron transfer in the modified electrode can be explained as follows: In this catalytic process, during the reduction of $\text{H}_2\text{O}_2$ on the CuO surface, $\text{Cu}^{2+}$ is electrochemically reduced to $\text{Cu}^+$ and $\text{H}_2\text{O}_2$ to $\text{O}_2$. Then, $\text{Cu}^+$ on the electrode surface is electrooxidized back to $\text{Cu}^{2+}$, and the catalytic cycle is repeated [55, 81, 82].

The active surface area of an electrode can be calculated using the Randles–Sevcik equation [83-85], which at 25 °C is:

$$I_p = \left(2.69 \times 10^5\right) n^{3/2} A \cdot C^* \cdot D^{1/2} \cdot v^{1/2},$$

(4)

where $I_p$ represents the redox peak current (A), $n$ is the number of electrons transferred in the redox reaction, $D$ is the diffusion coefficient in solution ($D = 6.8 \times 10^{-5} \text{ cm}^2\text{s}^{-1}$), $C^*$ is the concentration (mol·cm$^{-3}$); $v$ is the scan rate (100 mV·s$^{-1}$), and $A$ denotes the effective surface area of the electrode (cm$^2$). The electrochemically active surface area was calculated to be 6.5 cm$^2$, that is, five times larger than the geometrical surface area of a bare electrode, which indicates the presence of a well-developed nanostructured surface.

Figure 4 displays the dependence of the sensor sensitivity on the morphology of CuO nanostructures obtained after different periods of synthesis time. It is shown that as a result of 1 h of growth, nanopetals are formed with a greater thickness and a significantly lower height than in the case of 3 h of growth. This change in aspect ratio leads to a decrease in the active surface area and, as a result, to a decrease in sensitivity (reduction of the current peak in the CV curves). An increase in the duration of hydrothermal synthesis to 6 h also leads to a change in the morphology of the nanostructures. The SEM picture shows that the nanoleaves grow together, forming dense spherical formations that are difficult for the solution to penetrate, which also leads to a decrease in the surface area and a deterioration in sensitivity (decrease of current peak value). Hence, it can be concluded that the chosen synthesis time of 3 h is optimal and provides maximum sensitivity.

Figure 5 shows the DPV results for the nanostructured CuO electrode. The measurements were carried out in 0.1 M NaOH buffer solution containing $\text{H}_2\text{O}_2$ at a concentration of 0–500 µM. The lowest considered concentration (33 µM) provides a noticeable electrochemical response, which indicates that the nanostructured CuO electrode has sufficiently high sensitivity. Figure 5b shows the dependence of the peak current values on the concentration of $\text{H}_2\text{O}_2$. The resulting dependence is linear over the entire concentration range.

In Figure 6a and Figure 7a show typical curves of the amperometric response for nanostructured CuO electrodes. After $\text{H}_2\text{O}_2$ injection, a fast, stable, and sensitive amperometric response was observed. The sharp jump in the current when $\text{H}_2\text{O}_2$ is added can be explained by a local increase in the $\text{H}_2\text{O}_2$ concent-
Figure 4: SEM images of CuO nanostructures obtained via hydrothermal oxidation method after (a) 1 h, (b) 3 h, and (c) 6 h. (d) CV curves of the CuO samples after 1, 3, and 6 h of synthesis time. Measurements were carried out in 0.1 M NaOH solution containing 1 mM H$_2$O$_2$.

A linear relationship was obtained in the range from 10 to 1800 µM ($R = 0.99874$). The sensitivity of the obtained CuO electrode is 439.19 µA·mM$^{-1}$. The calculated limit of detection (LOD) is 1.34 µM, assuming signal-to-noise ratio of 3. The results indicate that the nanostructured CuO electrode can be used for accurate and precise detection of H$_2$O$_2$. The obtained results are comparable to several published studies where CuO nanostructures were used for electrode modification for H$_2$O$_2$ detection (Table 1).

For the successful practical application as a sensor material, a high selectivity of the obtained coating is of importance. Therefore, the selectivity of the petal-like CuO electrode was evaluated using four different interfering substances, namely ascorbic acid, uric acid, dopamine, and NaCl. These substances are most commonly encountered in clinical and pharmaceutical applications together with H$_2$O$_2$. They are also oxidizing agents that can react with CuO during electrochemical tests, leading to a false increase in the current signal. The amperometric response
Figure 5: (a) DPV results for the nanostructured CuO electrode in 0.1 M NaOH buffer solution containing 33–500 μmol H₂O₂. (b) Dependence of the amperometric response on the concentration of added peroxide (SD = 3.5%, n = 5). (c) Comparison of DPV curves obtained at different pH values of buffer solution containing 500 μM H₂O₂. (d) Comparison of DPV curves obtained at different pulse frequencies. Measurements were carried out in 0.1 M NaOH solution containing 500 μM H₂O₂.

Figure 6: (a) Amperometric response of the nanostructured CuO electrode in 0.1 M NaOH with stepwise addition of H₂O₂ at concentrations from 10 to 1800 μM and (b) the corresponding calibration curve (SD = 3.5%, n = 5).

after sequential injection of 0.1 mM H₂O₂ and 0.1 mM interfering substance is shown in Figure 7a. There is an insignificant reaction of the sensor to the above substances, the current intensity of which is commensurate with the noise level. Thus, it can be concluded that the CuO petal-like electrode shows good selectivity for the detection of H₂O₂.
Figure 7: (a) Amperometric response of the nanostructured CuO electrode in 0.1 M NaOH with stepwise addition of H$_2$O$_2$ at concentrations from 100 to 300 μM and the most common interfering substances: (1) ascorbic acid, (2) uric acid, (3) dopamine, (4) NaCl, (5) glucose, and (6) acetaminophen. (b) Stability study and the dependence of the change in the electrochemical signal on the storage time of the samples.

Table 1: Analytical performance of the CuO sensor in this study compared with other reported H$_2$O$_2$ sensors.

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Morphology of nanostructured CuO</th>
<th>Linear range (μM)</th>
<th>Sensitivity (μA/mM)</th>
<th>LOD (μM)</th>
<th>Reference</th>
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</thead>
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<tr>
<td>Cu$_2$O/GCE</td>
<td>nanocubes</td>
<td>0.3–7.80</td>
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<td>CuO/Cu wire</td>
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<td>10–1800</td>
<td>439.19</td>
<td>1.34</td>
<td>this work</td>
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Table 2 shows the result of an amperometric study of real milk and mouthwash samples. As the possible amount of H$_2$O$_2$ can be below the detection limit, the samples were spiked with different amounts of H$_2$O$_2$ above the detection threshold and a standard sample recovery test was performed. It can be seen that the electrode has a high recovery rate (over 95% for all cases) and a low relative standard deviation for three samples of each spiked concentration not exceeding 5.5%. The results indi-

<table>
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<tr>
<th></th>
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<th>Milk</th>
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<tr>
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<td>RSD (%)</td>
<td>(n = 3)</td>
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<td>Found (μM)</td>
<td>Recovery (%)</td>
<td>RSD (%)</td>
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</table>
cate that this sensor can be successfully used to detect hydrogen peroxide in real samples.

To assess the long-term stability of the sensor, the obtained samples were stored under ambient conditions for one and four weeks. Measurements were taken every second day. The stabilities of each sample were assessed by the degree of reduction of the current peak value in the CV curve. For samples stored under environmental conditions (20 °C, 40% relative humidity) for one week, the signal level remained at 95% of the initial value. For samples stored under environmental conditions for a month, the signal level remained at 90% of the initial value. The influence of the environment and degree of sample degradation can be significantly reduced by ensuring that samples are stored in a vacuum desiccator. After a week of desiccator storage, the samples had not lost their original electrochemical properties at all, and after a month of storage they retained 95% of their initial values (Figure 7b). After a month of storage, no significant morphological changes were observed, which proves the stability of the samples. These results show that the nanostructured CuO coating has long-term stability and resistance to environmental influences, which is another advantage compared to enzyme sensors.

**Conclusion**

This article describes the preparation of a nanostructured coating of CuO and its application as a working electrode for the electrochemical determination of H$_2$O$_2$. The resulting coating is distinguished by high homogeneity and adhesion to the copper wire, which ensures high mechanical and chemical resistance of the sample. The nanostructured CuO coating develops a petal-shaped surface, which possesses significant peroxidase-like electrocatalytic activity, and makes it possible to detect H$_2$O$_2$ with a high degree of sensitivity compared to samples with less developed surface. It has been shown that the optimal time for hydrothermal synthesis is 3 h, since this period of time allows one to obtain a morphology with maximum electrochemical response towards H$_2$O$_2$.

The resulting electrode displays a linear current response in a concentration range from 10 to 1800 µM. The sensitivity of the resulting electrode was 439.19 µA·mM$^{-1}$ and the calculated limit of detection (LOD) was 1.34 µM. The electrochemically active surface area was calculated to be 6.5 cm$^2$. Sensitivity testing showed a lack of electrochemical response to the most common interfering substances, showing the high selectivity of this electrode. This study also showed high long-term stability of the resulting coating stored under ambient conditions (the signal level remained at 95% of the initial value after one week and at 90% after a month). Storage in a vacuum desiccator helps to improve the stability of samples (the signal level remained at 100% of the initial value after one week and at 95% after a month). Real milk sample and mouthwash analysis demonstrated a high recovery rate (over 95%), which makes this sensor suitable for qualitative and quantitative detection of H$_2$O$_2$.

Further research will be aimed at studying this sensor in healthcare to analyse changes in the concentration of H$_2$O$_2$ in biological fluids. Also, a promising option to study more complex analytes and to significantly increase the sensitivity is the use of this nanostructured CuO sensor as part of a multisensor system based on several types of metal oxides (e.g., Co$_3$O$_4$, TiO$_2$, NiO, and Fe$_2$O$_3$).

**Funding**

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**References**


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A nonenzymatic reduced graphene oxide-based nanosensor for parathion

Sarani Sen¹,², Anurag Roy³, Ambarish Sanyal¹ and Parukuttyamma Sujatha Devi⁴

Abstract
Organophosphate-based pesticides (e.g., parathion (PT)) have toxic effects on human health through their residues. Therefore, cost-effective and rapid detection strategies need to be developed to ensure the consuming food is free of any organophosphate-residue. This work proposed the fabrication of a robust, nonenzymatic electrochemical-sensing electrode modified with electrochemically reduced graphene oxide (ERGO) to detect PT residues in environmental samples (e.g., soil, water) as well as in vegetables and cereals. The ERGO sensor shows a significantly affected electrocatalytic reduction peak at −0.58 V (vs Ag/AgCl) for rapid quantification of PT due to the amplified electroactive surface area of the modified electrode. At optimized experimental conditions, square-wave voltammetric analysis exhibits higher sensitivity (50.5 μA·μM⁻¹·cm⁻²), excellent selectivity, excellent stability (≈180 days), good reproducibility, and repeatability for interference-free detection of PT residues in actual samples. This electrochemical nanosensor is suitable for point-of-care detection of PT in a wide dynamic range of 3 × 10⁻¹¹–11 × 10⁻⁶ M with a lower detection limit of 10.9 pM. The performance of the nanosensor was validated by adding PT to natural samples and comparing the data via absorption spectroscopy. PT detection results encourage the design of easy-to-use nanosensor-based analytical tools for rapidly monitoring other environmental samples.

Introduction
Crop production is constantly increasing to fulfil the demands of the growing population. The protection of crops against insects is a big challenge for our society. Pesticides have indiscriminately been used in all sectors of agriculture. Among the various pesticides available for the aforementioned purpose, organophosphates (OPs) are commonly employed in agricul-
ture, households, gardens, and veterinary practices. This prac-
tice also jeopardizes food safety in all stages of the food supply
chain, even after pesticide use. Due to its high nondegradability,
pesticides can stay more often on the surface of fruits and vege-
tables; sometimes, it can also penetrate into the peel of vegeta-
bles and fruits [1]. Organophosphorus insecticides react with
biomolecules either via deoxyribonucleic acid (DNA) alkyl-
ation or acetylcholinesterase (AChE) phosphorylation, involved
in the initiation of the carcinogenic process and acute cholin-
ergic toxicity, respectively [2]. Parathion (PT) is a highly toxic
OP-based insecticide, potentially harmful to human health, and
it may even cause death upon ingestion, inhalation, or dermal
penetration [3,4]. Due to its extreme toxicity, it is necessary that
easy-to-use, cost-effective diagnostic kits for routine screening
of pesticides in fruits and vegetables are developed.

The high-throughput analytical methods such as chromato-
graphic (gas, liquid) and spectroscopic (mass, absorption, fluo-
rescence) techniques are time-consuming, laborious, costly,
require specific and sophisticated instruments and trained
personnel, and most often are not portable to enable on-site
detection [5,6]. Electrochemical nanosensors are one of the
preferred methodologies due of their fast and straightforward
responsive nature, high sensitivity, and selectivity leading to
real-time detection [7]. A combination of a receptor, an analyte,
and a transducer is made up to obtain an electrochemical sensor,
in which the surface of the electrode induces redox characteris-
tics via selective binding with the analyte under a voltage for a
different analyte which results in a quick qualitative signal. This
approach promotes real-time label-free methods, providing
more consistent and reproducible results.

Most of the electrochemical nanobiosensors for the detection of
OPs (e.g., methyl parathion, ethyl parathion, fenitrothion, chlor-
pyrifos, paraoxon, ethion, and acephate) are based on the inhibi-
tion of acetylcholinesterase (AChE) activity (an indirect
method) [8-10]. Some organic molecules and metal cations also
act as an inhibitor of AChE. Thus, interference-free detection of
OP in agricultural samples using enzyme-based nanosensors is
challenging. The stability of biananosensors also extensively
depends on the viability of the corresponding biomolecule
during the matrix immobilization course [9]. The main draw-
backs of using biananosensors for the selective detection of OP
in actual samples are i) the high cost of the enzyme, ii) low
stability of biomolecules at room temperature, and iii) difficul-
ties in using interference-free selective detection of a specific
OP. However, nonenzymatic electrochemical nanosensors could
easily be employed as rapid, cost-effective, easy-to-handle,
selective, sensitive, and point-of-care (POC) analytical tools for
monitoring environmental pollutants [2,11]. They can also
detect residual OPs based on their electrocatalytic activity and
affinity toward nanomaterials, such as nanoparticles, carbon
nanomaterials, and metal oxides [11]. In a few reports, hybrid
carbon nanomaterials such as ferrocene-thiophene modified by
carbon nanotubes, zinc(II) phthalocyanine-boron dipyrro-
methene attached single-walled carbon nanotubes were used for
the direct detection of pesticides [12-15]. So far, only limited
electrochemical nanosensors modified by nanomaterials have
been reported to detect PT [16,17]. However, their sensitivity
and detection limit for quantifying trace amounts of PT in envi-
ronmental samples are improved. The inherent electrochemical
behavior of nitroaromatic OPs (e.g., paraoxon, parathion, and
fenitrothion) exhibit well-defined redox activities at the elec-
trode surface, potentially leading to the fabrication of nonenzy-
matic electrochemical nanosensors for detecting specific OPs
on the electroactive surface [2,11,17-19]. For example, electro-
chemical sensing platforms modified with zirconia-embedded
PEDOT membrane, graphene nanoribbons doped with silver
nanoparticles, rGO doped with ZrO$_2$, and CuO–TiO$_2$ hybrid
nanocomposites were proposed to detect methyl parathion [19-
22]. Rajaji et al. (2019) modified glassy carbon electrodes with
graphene oxide encapsulated 3D porous chalcopyrite (CuFeS$_2$
) nanocomposites to detect methyl paraoxon in vegetables [23].
Recently, Jangid et al. (2021) also described the electrocatalytic
activity of fenitrothion on glassy carbon electrodes modified
with nitrogen and sulfur co-doped activated carbon-coated
multiwalled carbon nanotubes [24]. Nevertheless, the fabrica-
tion process of the sensing platform was not cost-effective,
stable, and sensitive in order to develop a robust electrochemi-
cal nanosensor for on-site monitoring of organophosphates in
agricultural samples. To date, no reliable sensing system is
available for the rapid quantification of parathion residues in
environmental samples. Thus, the primary goal of this report
was to showcase the fabrication of a more effective, economi-
cal, electroactive surface in a simplified way to selectively
detect PT residues in real samples. Thus, a robust sensing
matrix can be used for designing a nonenzymatic POC device
with a low detection limit and long-term stability at room tem-
perature.

Graphene oxide (GO), consisting of a monolayer of sp$^2$-
hybridized carbon atom network, has already been used in elec-
trocatalysis, nanoelectronics, biananosensors, and sustainable
energy storage systems due to its larger active surface area, en-
hanced electron transport facility, excellent mechanical, ther-
nal, and electrical stability [11,25-27]. The electronic structure
and surface physicochemistry of graphene are beneficial for
electron transfer. Several graphene-based nanocomposites based
on complex synthesis processes are reported as excellent
sensing matrices for detecting various analytes [11,21-23,28].
The “green synthesis” of graphene via electrochemical reduc-
tion is the most economical strategy for the mass production of
graphene compared to chemical or thermal reduction of GO [27,29]. Since no hazardous chemicals (e.g., hydrazine) as reductants or rapid heat treatment at high temperatures are required for the synthesis of electrochemically reduced GO (ERGO), controlled synthesis of ERGO films could be possible via optimization of electrochemical parameters. These parameters are the range of the applied voltage, numbers of cycles, the scan rate of cyclic voltammetry, or reduction time at a fixed potential in chronoamperometry [30-32].

However, the desired size and thickness of the film can be increased by controlling the amount of precursor GO deposited onto the electrode surface [31,32]. Optimizing the process parameters is a robust scientific approach to achieving the highest sensing performance of an electroactive analyte.

In this work, we proposed a simple, robust, and reliable ERGO-modified nonenzymatic electrochemical nanosensor as a good alternative for a POC-based easy diagnostic platform to monitor the level of PT residues in environmental samples, such as water, soil, crops, and vegetables. A straightforward and economic fabrication process with high sensitivity, selectivity, and stability with the lowest detection limit is the foremost advantage emerging from this study for the rapid on-site monitoring of PT. For this purpose, the electrochemical reduction of GO was tuned using various electrolytic buffers with different pH values, supporting the variation of the physicochemical characteristics of ERGO discussed in this work. Besides, this study highlights the scope of an interference-free nonenzymatic approach through electrochemical nanosensing which can also be used in other biosensing applications.

Experimental Chemicals

The chemicals used in this work are summarized in Supporting Information File 1, Table S1. Sodium dihydrogen phosphate was used to prepare phosphate buffer saline (pH 4.6, 7.4, 9). Acetate buffer (20 mM, pH 4.5), and Britton–Robinson (BR) buffer (40 mM, pH 4) consisting of phosphoric acid, boric acid, and acetic acid were also prepared.

Synthesis of graphene oxide

Graphene oxide was synthesized from graphite powder using a modified Hummer’s method [30,31]. In detail, 100 mg of sodium nitrate (Merck) was added to 250 mg of graphite powder (Alfa Aesar) and further acidified with ≈5 mL of concentrated sulfuric acid (Merck) at a temperature range of 0–5 °C followed by vigorous stirring. In the next step, 600 mg of KMnO₄ (Merck) was sequentially added to the aforementioned solution, during which the solution temperature was increased to 35 °C. After 30 min of the addition of KMnO₄, a brownish-grey paste was obtained. Deionized water (100 mL) was added to the paste under constant stirring at 90 °C for 30 min, followed by drop-wise addition of 30% H₂O₂ (Merck, India). Finally, a dark brown solution was filtered and thoroughly washed with 100 mL of distilled water until a neutral pH value was achieved. The black product obtained after filtration was dispersed in water and sonicated for 1 h to get a well-dispersed suspension. Finally, the suspension was centrifuged twice at 3000 rpm for 15 min. The product (GO) was collected and dried at room temperature for further studies.

Fabrication of electrochemically reduced graphene oxide modified electrodes

Before surface modification of GO, a bare glassy carbon electrode (GCE, φ = 3 mm) was polished in 1.0, 0.3, and 0.05 micron alumina slurry (CHI Instruments) on micro cloth pads sequentially to a mirror-like finish with fine wet emery paper (grain size 4000), and rinsed with ultrapure water. Then the electrode was separately dipped into concentrated NaOH, nitric acid, and methanol for 120 s, followed by sonication in alcohol for 2 min, and finally dried in air. The as-prepared GO colloidal suspension (2 mg·mL⁻¹) was deposited onto the surface of the pretreated GCE and dried at room temperature. The GO/GCE was submerged in 50 mM PBS, pH 4.6, for the electrochemical reduction of GO by a potentiostat technique at a potential of −0.9 V for 900 s using an Ag/AgCl reference electrode. The buffer and pH values of the electrolytes were optimized to fabricate electrochemically reduced GO (ERGO) modified GCE designated as ERGO/GCE.

Cyclic voltammogram (CV) measurements to assess the electrochemical behavior of parathion were performed from +0.5 to −1.0 V versus Ag/AgCl, with a scan rate of 100 mV·s⁻¹. Square-wave voltammetry (SWV) analysis was performed from −0.3 to +0.9 V versus Ag/AgCl, with pulse amplitude of 100 mV, frequency of 25 Hz, and modulation time of 10 s in 50 mM PBS. The nanosensor was cycled 25 times for signal stabilization before PT detection.

Preparation of environmental and food samples for residual parathion analysis

The practical application of the proposed electrochemical nanosensor was studied by sensing PT in the groundwater, soil, tomato, and rice samples with different concentrations of PT. The groundwater and soil were collected from local agricultural land in Kolkata, India. As parathion is highly soluble in alcoholic compounds, we have used ethanol to extract residual PT from the collected food and environmental samples. The soil sample (1 g) was stirred for 1 h in 50% ethanol to disperse all organic and inorganic soil molecules in the liquid phase. Tomato as a sample vegetable was purchased from the local
market in Kolkata, India, and washed with running water before preparing the sample. The tomato samples (30 g) were smashed with 30 mL of 50% ethanol, and the juice was collected for further filtration. Boiled rice (20 g) was also smashed with 20 mL of 50% ethanol. All the samples were stored at 4 °C after filtration (pore size = 0.45 micron) to remove all the solid impurities. Actual samples were spiked with different concentrations of PT during electrochemical analysis. Each concentration of PT was tested five times, and the average value was represented with standard deviation. The results also validate the standard spectrophotometric analysis.

Quantification of parathion using spectrophotometry
The ultraviolet-visible (UV–vis) absorption spectroscopic study was performed to validate the results of the proposed nanosensor. A stock solution (1 mM) of PT was prepared in 99.9% ethanol (Empura, Merck). A 5 mL volume of working solutions of 1 to 35 µM was prepared in ethanol for monitoring the UV spectra in the range of 200–400 nm with a scan rate of 2 nm/s. The absorbance change of PT due to π–π* transition was noted at 273 nm using ethanol as blank, and a calibration curve was plotted to compare the results obtained from the proposed nanosensor.

Material characterization
Voltammetric studies were carried out using an IVIUMStat electrochemical analyzer (Model: A09050, Iviumstat Technologies, USA), which was connected by a three-electrode system, including a modified and/or unmodified GCE as the working electrode, a saturated Ag/AgCl as the reference electrode (RE), and a platinum wire as the counter electrode (CE). The electrochemical impedance spectroscopy (EIS) study of the modified electrodes was carried out in 5 mM of [Fe(CN)₆]³⁻ and [Fe(CN)₆]⁴⁻ with 0.1 M KCl within the frequency range from 1 MHz to 0.01 Hz, amplitude of 10 mV, at a fixed potential of 0.28 V.

The UV–visible absorbance spectra were obtained on a UV–vis–NIR spectrophotometer (SHIMADZU UV-3600). The Raman spectra of the samples were recorded in the 4000–400 cm⁻¹ region with a resolution of 1 cm⁻¹ using a Renishaw via a Reflex micro-Raman spectrometer with an argon ion (514.6 nm) laser. The X-ray photoemission spectroscopy (XPS) data were obtained from a PHI 5000 Versa probe II scanning XPS microprobe (ULVAC-PHI, U.S.) with monochromatic Al Kα (hv = 1486.6 eV) radiation, and a beam size of 100 µm. The Fourier transform infrared (FTIR) absorption spectra of GO and ERGO were collected in the 4000–400 cm⁻¹ region on a Perkin Elmer spectrometer as KBr (Sigma-Aldrich, Germany) pellets. The crystalline phase of GO and RGO was characterized by X-ray diffraction (XRD) using a X’pert pro MPD XRD (PAN analytical B.V., the Netherlands) with Cu Kα radiation (λ = 1.5406 Å).

Scanning electron microscopy (SEM) of the modified electrode was conducted on a JEOLEVO® 18 special edition (model: ZEI S EVO-MA 10) at an acceleration voltage of 15 kV. The morphological characteristics of the electrodeposited ERGO were obtained by field-emission scanning electron microscopy (FESEM, model: LEO 430i, Carl Zeiss) and high-resolution transmission electron microscopy (model: Tecnai G2 30ST, FEI) operating at 300 kV.

Results and Discussion
Optimization and characterization of electrochemically reduced graphene oxide formation
Figure 1A shows the UV spectra of GO and its change following the electrochemical reduction of GO. It is observed that the absorption peak of GO at 223 nm due to the π–π* transition of the C=C bond disappeared in ERGO. The amount of residual oxygenated functional groups in ERGO films is likely to vary depending on the experimental conditions, such as applied potential, reduction times, and the electrolyte used [25]. The process parameters for electrochemical reduction were optimized to develop better functioning electrodes. Raman spectroscopy has been frequently used as a reliable technique to optimize the electrochemical parameters for the synthesis of ERGO in terms of the intensity ratio of D- (disordered band) to G-band (graphitic band) (I_D/I_G). It measures the change in size of the sp² ring clusters in a network of sp³- and sp²-bonded carbon [33]. Previous reports have indicated the possibility of converting GO to ERGO at different electrochemical parameters, but its effect on the residual oxygenate of the electrolyte was optimized to increase the deoxygenation of the GO sheet during ERGO formation. Figure 1B depicts three significant Raman peaks of GO at 1350 cm⁻¹ for the D band (associated with defects in the sp² lattice), 1596 cm⁻¹ for the G band (due to vibrations of the hexagonal lattice), and 2700 cm⁻¹ for the 2D band (related to numbers of layers in the graphene sheet). Table 1 shows the values of I_D/I_G at different electrolytic buffers during one-step electroreduction of GO at a constant potential of −0.9 V. The intensity of I_D/I_G predominantly increased for ERGO compared to the that of the as-prepared GO, which suggests a decrease in size of the sp² domain due to extensive deoxygenation of the graphene sheets after electrochemical reduction. The comparative values of I_D/I_G (Table 1) also indicate that a higher defect in the sp² domain was observed at acidic pH values of the electrolytic buffer during electrochemical reduction of GO. The highest value of
$I_D/I_G$ was found to be 1.454 for the conversion of ERGO using PBS (pH 4.5), which suggests the formation of higher defects between the graphene layers during electrochemical reduction [26,34]. Thus, 50 mM PBS, pH 4.5, has been chosen for an efficient conversion of GO to ERGO.

Figure 1C shows the characteristic FTIR spectra of GO and ERGO (in PBS, pH 4.5) to identify the change of functional groups due to electrolytic reduction of GO. The predominant characteristic absorption peaks of GO include a broad peak at 3426 cm$^{-1}$ corresponding to the O–H stretching vibration origi-
nating from carboxyl groups. Besides, an intense peak at 1641 cm\(^{-1}\) was assigned to the C=O stretching of carboxyl and/or carbonyl groups, a sharp peak at 1387 cm\(^{-1}\) corresponding to a –OH bend, and a strong peak at 1068 cm\(^{-1}\) ascribed to an alkoxy and/or epoxy C–O stretching vibration. The significant reduction of the FTIR signal intensity of ERGO for –OH, –C=O, and –C–O suggests the successful formation of ERGO due to the electrochemical deoxygenation of GO, which corroborates the Raman analysis.

Figure 1D depicts a characteristic XRD peak of GO at \(2\theta = 9.98\) (interplanar spacing = 0.843 nm) corresponding to 001 reflections. Two characteristic peaks of ERGO at \(2\theta = 21.15\) (interplanar spacing = 0.413 nm) and \(2\theta = 29.65\) (d-spacing = 0.343 nm) for the reflection of (020) and (200), respectively, confirm the successful formation of ERGO from GO.

Figure 2 represents the deconvoluted C 1s and O 1s XPS spectra of GO (Figure 2A and Figure 2B) and modified ERGO (Figure 2C and Figure 2D) electrodes. An asymmetric peak centered on \(≈284.8\) eV appeared due to the graphitic nature of GO and ERGO (Figure 2A and Figure 2B). Four different carbon types are observed from the deconvolution of the peaks shown in Figure 2A. They show an increase in binding energies evidencing the presence of C–OH, C–C, C–O–C, and C=O bonds in GO. The O 1s spectra of synthesized GO can be deconvoluted into three peaks, corresponding to contributions from carbonyl and carboxyl-type oxygen (531.4 eV), C–OH type (532.5 eV), and hydroxyl (533.6 eV). The intensity of the peaks is significantly reduced in RGO samples (Figure 2C and Figure 2D) compared to pristine GO, indicating considerable deoxygenation. The C 1s spectra of RGO (Figure 2C) can also be deconvoluted into four peaks at 284.7, 285.96, 292.8, and 295.7 eV. However, the relatively intense doublet appeared at 292.8 ± 0.1 eV and beyond 295 eV every time we performed the scan. Peaks in the range of 290 eV in these types of materials are mainly due to aromatic \(\pi-\pi^*\) transitions. However, considering the intensity of the peak and our repeated measurements, we believe that the presence of a well-defined deconvoluted doublet peak beyond 290 eV corresponds...
to K 2p$_{3/2}$ and K 2p$_{1/2}$, which may have resulted from the contribution coming from the potassium salt present in the buffer during electrochemical conversion. The deconvoluted analysis of the peaks and the relative atomic percentages of GO and RGO are summarized in Supporting Information File 1, Table S2.

Figure 3A–D depicts TEM micrographs of as-prepared GO and synthesized ERGO at different pH values, indicating that the intensity of the electrons is attenuated by the platelets of graphene sheets with varying transparencies due to thickness variation [26,31]. Dark areas of the micrograph suggest thick stacking layers of GO and/or RGO with intercalated oxygen-containing functional groups. A few layers of graphene sheet in ERGO (in PBS, pH 4.5) have areas with higher transparency due to the exfoliation of stacking layers of GO. This suggests an increased surface area due to delamination of graphene layers (thickness of about one to a few layers) by electrochemical reduction. The high-resolution TEM of ERGO shows a d-spacing of 0.413 nm (Figure 3E), indicating a reduced graphite nature of GO. This confirms that the oxygen functional groups were removed from the graphene layers by electrochemical reduction of GO, decreasing the interspacing distance between graphene layers which facilitates electron transport. Thus, the conductivity of ERGO was enhanced compared to that of GO. The SEM micrograph of ERGO (Figure 3G) also shows graphene sheet exfoliated layers compared to GO (Figure 3F). The FESEM image also depicts the flaked nanostructure of RGO (Figure 3H).

Electrochemical characterization of the modified electrode

The electronic properties of graphene materials depend on the number of layers and the distance between the layers, which can be changed by a variation of the synthesis protocol to achieve a higher electroactive surface area and electrical conductivity. Figure 4A displays a higher oxidation/reduction peak current of Fe$^{2+/3+}$ redox couple for the synthesized ERGO in PBS pH 4.5. It forms the highest electroactive surface area compared to other electrolytic buffers and pH values to prepare ERGO/GCE. To confirm the increase in the electroactive surface area of ERGO/GCE in comparison to bare GCE, CV was performed at different scan rates (10–300 mV/s) in 1.0 mM K$_3$Fe(CN)$_6$ as a redox probe (Supporting Information File 1, Figure S1). The electroactive surface areas were calculated according to Randles–Sevcik equation (Equation 1) [28,32]:

$$ I_p = \left(2.69 \times 10^5\right)^{3/2} n^{3/2} A_c D_r^{1/2} v^{1/2} C_0, $$  

where $I_p$ is the peak current (A), $v$ is the scan rate (V s$^{-1}$), $n$ is the number of electrons transferred ($n = 1$), $A_c$ is the electrode active area (cm$^2$), $D_r$ is the diffusion coefficient ($7.6 \times 10^{-6}$ cm$^2$·s$^{-1}$), and $C_0$ is the concentration of K$_3$Fe(CN)$_6$ (mol·cm$^{-3}$). From the slope of the plot of $I_p$ vs $v^{1/2}$, the effective surface area for bare GCE and ERGO/GCE was calculated to be 0.0707 and 0.121 cm$^2$, respectively, which indicates that the effective electroactive surface area of ERGO has been improved by $\approx$71.14% due to exfoliation of graphene sheets.

![Figure 3](image-url) Figure 3: (A) TEM images of as-synthesized GO, ERGO synthesized in different electrolytes: (B) PBS pH 4.5, (C) pH 7, and (D) pH 9.6. (E) HRTEM image of ERGO in PBS pH 4.5. (F) SEM micrographs of as-synthesized GO, (G) ERGO in PBS pH 4.5 and (H) FESEM of ERGO in PBS pH 4.5 at different magnifications.
Electrochemical impedance spectroscopy was performed to investigate the electron transfer capability of ERGO (Figure 4B). Supporting Information File 1, Table S3 depicts the values of charge-transfer resistance ($R_{ct}$), capacitance ($C_{dl}$), and Warburg impedance ($W$) of bare GCE, GO/GCE, and ERGO/GCE. The Nyquist plot of the bare GCE electrode depicts a semicircle with $R_{ct}$ of 4.692 Ω. A nearly straight line for ERGO with a negligible $R_{ct}$ (1.618 Ω) value suggests opened porous microstructures of ERGO, which makes the graphene sheets more accessible to the electrolyte. It also facilitates electron transfer and diffusion of ions during the electrochemical process [28,34].

Electrochemical behavior of parathion at modified nanosensors
Figure 5A depicts the CVs (first cycle) of bare GCE, GO/GCE, and ERGO/GCE in PBS (0.05 M, pH 7) in 10 μM PT. The CV of PT on bare GCE (inset of Figure 5A), shows a reduction
peak at −0.65 V and a little anodic peak due to autocatalysis of PT. A robust cathodic peak at −0.56 V and an anodic peak at +0.015 V were mainly observed on GO/GCE due to the absorption of PT through π stacking interaction between aromatic moieties of GO and the benzene ring of PT. In comparison, the highest cathodic/anodic peak was obtained at −0.58 and −0.05 V, respectively, for the electro-reduction/oxidation of PT on ERGO/GCE. The oxidation/reduction potentials of PT on ERGO/GCE were shifted to less positive values, effectively inhibiting the surface fouling caused by the reaction products, making ERGO-modified GCE more suitable for determining PT. The electrocatalytic ability of PT (10 µM) on the modified ERGO/GCE was investigated in PBS (pH 7) (Figure 5B) in the potential range from +0.2 to −1.0 V with a scan rate of 100 mV·s⁻¹ and compared with the control group (bare GCE and GO/GCE). It is in good agreement with the literature reports that a sharp cathodic peak (E_{pc1}) at −0.58 V was observed in the first cycle due to the reduction of the nitro group of PT (NO₂–PT) to form its hydroxylamine derivatives (NHOH–PT) involving a four electron-transfer process as shown in Figure 5C [16-18,35]. An anodic peak appeared at −0.05 V in the backward segment of the first cycle, which is related to the oxidation of NHOH–PT to a nitroso group (NO–PT). This reversible two-electron-transfer process further generated a reduction peak (E_{pc2}) at −0.11 V during the second potential scan of CV (Figure 5C). Nitroaromatic OPs such as parathion, methyl parathion, ethyl parathion, and fenitrothion, paraoxon exhibit this kind of electrocatalytic behavior, which is consistent with previous reports [21,23,24]. In this study, we chose the irreversible reduction peak of PT (NO₂–PT to NHOH–PT) of the first cycle due to its suitability for important measurements in nanosensor applications.

The amount of exfoliated GO dispersed on bare GCE is vital in optimizing the sensing matrix. Figure 6A depicts the CVs using a variation of deposited GO on bare GCE to prepare ERGO/GCE to measure the reduction and oxidation peak current for 10 µM PT in PBS, pH 7. Figure 6B shows that the highest reduction peak for 10 µM PT was obtained using 8 µL of GO to prepare modified ERGO/GCE. As the autocatalytic response for the electrochemical oxidation/reduction process is an absorption process, the accumulation time is another vital parameter to
achieve the highest response for monitoring the amount of parathion residue in samples [35,36]. It has been shown in Figure 6 that as the immersion time of the modified electrode in a PT solution increased, the accumulation of PT on the electrode surface also enhanced. It was found that the highest peak current for 10 μM PT was obtained after immersion for 240 s in the PT solution. A further increase in the accumulation time was unaffected as the active area of the electrode surface was saturated (Figure 6C).

Effect of scan rate and pH values on the electrolyte
The effect of scan rate on the reduction of PT at ERGO/GCE was investigated by applying different scan rates from 10 to 250 mV·s⁻¹ (Figure 7A). The linear peak current increase with the scan rate suggests a surface-confined diffusion-controlled electrocatalytic process [21]. The slope of log Ip as a function of log ν is 0.611 (>0.5), which confirms an adsorption-based reduction of PT on the modified electrode surface (Figure 7B).

The reduction peak potential was shifted towards a more negative potential by increasing the scan rate. A linear equation of Ep as a function of log ν was represented as

\[ E_p = E^{\circ} + \frac{2.303RT}{nF} \log \left( \frac{RTk^*}{F} \right) + \frac{2.303RT}{nF} \log \nu \]  

where α is the transfer coefficient; n is the number of electron transfers; and R, T, F represent constants (R = 8.314 J·K⁻¹, T = 298 K, F = 96480 C·mol⁻¹). The standard redox potential (E°) was found to be −0.523 V from the linear plot of Ep as a function of ν (E_p = −0.908ν − 0.523), at a scan rate 0 V·s⁻¹. The standard heterogeneous rate constant (k°) for electrocatalysis of PT was 38.81 s⁻¹. The value of αn was calculated to be 0.672, and the n value was found to be 0.954 (i.e., one-electron transfer process [37]).
The protonation reaction influences the electrochemical reaction. Figure 7C shows the effect of pH on the electroreduction of PT (40 μM) by varying the pH values of PBS from 4.6 to 9. The irreversible reduction potential of PT was shifted towards a more negative potential as the pH values of the electrolyte varied from 4.5 to 9 (Figure 7D). The slope of the reduction peak ($E_{pc}$) and oxidation peak ($E_{pa}$) potential of PT as a function of pH is near −59 mV, which suggests that the same number of $e^{-}$ and $H^{+}$ is involved in the reaction [38,39].

Optimization of square-wave voltammetry parameters

Square-wave voltammetry analysis is more accurate compared to an electrochemical method such as cyclic voltammetry and differential pulse voltammetry. It can minimize background current to obtain an intense, sharp, and well-defined peak of the targeted analyte at a particular potential. To obtain the maximum peak current, the parameters of SWV were optimized using 10 μM PT in PBS (pH 7). The variation of reduction peak current with accumulation potential (A), starting potential of scan (B), frequency (C), and pulse amplitude (D) are shown Figure 8A–D.

Analytical performance and selectivity of the proposed nanosensor

Figure 9A represents SWV curves obtained from the ERGO modified electrode for sequential additions of PT into phosphate buffer (pH 7). A sharp increase in the reduction peak current was observed for each addition after dipping the electrode into a particular solution for 240 s at an applied potential of −0.1 V (i.e., deposition potential). The peak was shifted to a negative potential as the concentration of PT enhanced, indicating a diffusion-controlled process [40]. The concentration-dependent linear plot depicts good linearity (Figure 9B, Figure 9C) with a calibration equation of $I_p$ (μA) = 3.5735 [PT] + 12.018 ($R^2 = 0.9871$) for the range of 0.1–11 μM, $I_p$ (μA) = 0.2916 [PT] + 3.7526 ($R^2 = 0.9936$) for 3–15 nM, $I_p$ (μA) = 19.176 [PT] + 4.2723 ($R^2 = 0.9367$) for 0.03–0.15 nM. The corresponding sensitivity was found to be 50.5 μA·μM⁻¹·cm⁻² with a wide linear range for quantification.
of PT. The limit of detection (LOD = [(3 * standard deviation of blank)/slope of the lowest range of linear curve (i.e., 0.03–0.15 nM)]) and limit of quantification (LOQ = [(10 * SD of blank)/slope]) were calculated as 10.9 pM and 36.5 pM, respectively, from the lower calibration equation [39,41].

The selectivity of the proposed ERGO/GCE modified nanosensor (Figure 9D) was investigated in the presence of other possible substances in water and soil samples. Square-wave voltammetry measurements were performed in PBS (50 mM, pH 7.0) containing 10 μM PT along with some inorganic ions (e.g., Na⁺, K⁺, Fe²⁺, Fe³⁺, Cd²⁺, Ni²⁺, Mg²⁺, Mn²⁺, NH₄⁺, Cl⁻, NO₃⁻, CO₂⁻, SO₄²⁻, CH₃COO⁻), nitroaromatic compounds (e.g., 4-nitrophenol, 2,4-dinitrophenol), and other pesticides, such as acephate, chlorpyrifos, dicofol, lindane. As shown in Figure 9D, the modified electrode showed almost the same peak current when PT coexists with other substances. This indicates that the added substances have no significant effect on PT sensing in environmental samples. Other interfering OP (acephate, chlorpyrifos) and organochloride (dicofol, lindane) pesticides also did not significantly affect the response current of PT reduction as they have different redox potential and adsorption potential on the modified electrode surface.

Reproducibility, repeatability, and stability are essential parameters for practical applications of electrochemical nanosensors. Inter-assay measurements of 10 μM PT using five independent ERGO/GCE were performed, and a 3.4% relative standard deviation (RSD) was obtained for five replicate scans, indicating good reproducibility of the proposed nanosensor. Similarly, a single modified electrode exhibits good repeatability with an RSD of 1.81% for five repeated measurements performed in PBS (50 mM, pH 7.0) containing 10 μM PT.

The analytical performance of the ERGO/GCE, such as detection limit and linear range, are compared with previously re-
ported modified electrodes for the detection of PT (Table 2) [16-18,36,42-44]. The proposed electrode showed better stability, sensitivity, and the lowest detection limit in comparison to previous reports [16-18,36,42-44]. As ERGO showed thermal and mechanical stability, ERGO/GCE could be a suitable electrode material for rapid screening of PT in actual samples.

To determine the storage stability, the electrocatalytic response of 10 μM PT was monitored in seven-day intervals for the first two months, and it retained about 96.17 ± 0.2% of its initial response. It was shown a consistent response to PT sensing during two months of storage. After that, the response was measured in intervals of 10 days, and 90.53 ± 0.3% of the initial response was retained after six months. This indicates good stability of the modified electrode at room temperature (Supporting Information File 1, Figure S2A). The feasibility of the proposed robust sensing platform was demonstrated by quantifying environmental samples such as groundwater and a soil sample from an agricultural land. Food (e.g., boiled rice) and vegetable (e.g., tomato collected from local market) samples were also analysed. As the concentration of PT in the collected samples was negligible, a specific amount of PT was spiked from the standard PT solution (1 mM). Supporting Information File 1, Figure S2B depicts the SWV response of groundwater spiked with 1.5, 2.5, and 5 μM PT, and detailed experimental results are shown in Table 3. The amount of spiked [PT] was monitored by the SWV response, and the results were validated using standard UV results. The UV spectra with increasing PT concentration (1–35 μM) are shown in Supporting Information File 1, Figure S3A. The concentration of PT in real samples was further calculated from the standard calibration curve obtained from UV spectra at 273 nm (Supporting Information File 1, Figure S3B). The quantitative spiked recoveries of PT ranged from 97.0–102.4%, with an RSD of 0.998–1.62%. In addition, the proposed method also depicts satisfactory relative error (1.53–3.96%) with standard absorption results for the quantification of PT in environmental samples.

**Conclusion**

A newly developed inexpensive and environmentally friendly technique, using an interference-free nonenzymatic approach was developed to fabricate a nonenzymatic electrochemical nanosensor based on ERGO for rapid detection of PT. The electrochemical parameters were optimized to achieve the highest performance of the ERGO-modified electrode, and the structure was characterized by Raman, XRD, XPS, TEM, FESEM, and EIS techniques. Square-wave voltammetry was performed to achieve excellent nanosensor performance, such as higher sensitivity, low detection limit (10.9 pM), linear response range (3 × 10^{−11}–1 × 10^{−6} M), and fast response time. The proposed ERGO/GCE nanosensor exhibits excellent electrocatalytic activity, long-term storage stability, reproducibility, repeatability, low-cost fabrication, and strong anti-interference ability to quantify PT residues in real samples. The low RSD value (0.998–1.62%) and relative error (1.9–3.9%) obtained from UV data confirmed the accuracy of this method, showing that the electrochemical nanosensor has good reliability for PT detection in real samples. It can be concluded that the feasible nonenzymatic electrochem-

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**Table 2:** Experimental sample table for a comparative analytical performance of the proposed nanosensor with the reported nonenzymatic nanosensor.

<table>
<thead>
<tr>
<th>Modified electrode</th>
<th>Method</th>
<th>Molecule</th>
<th>Linear range (μM)</th>
<th>LOD (nM)</th>
<th>pH</th>
<th>Samples</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>NanoTiO₂-SAM/GCE</td>
<td>DPV</td>
<td>PT</td>
<td>0.05–10</td>
<td>10</td>
<td>PBS 5</td>
<td>cucumber, cabbage</td>
<td>[17]</td>
</tr>
<tr>
<td>NanoAg/Naf ion/GCE</td>
<td>DPV</td>
<td>PT</td>
<td>0.103–0.62</td>
<td>80</td>
<td>BR buffer, pH 2.56</td>
<td>water</td>
<td>[16]</td>
</tr>
<tr>
<td>ZrO₂/MAS/Au</td>
<td>SWV</td>
<td>PT</td>
<td>0.017–3.4</td>
<td>2.8</td>
<td>pH 6, 0.1 M KCl</td>
<td>vegetables, water</td>
<td>[43]</td>
</tr>
<tr>
<td>SPAN(sulfonated Pani)/GCE</td>
<td>DPV</td>
<td>PT</td>
<td>0.01–10</td>
<td>1.5</td>
<td>BR buffer 2.5</td>
<td>urine sample</td>
<td>[42]</td>
</tr>
<tr>
<td>ordered mesoporous carbon/GCE</td>
<td>DPV</td>
<td>PT</td>
<td>0.015–0.5</td>
<td>3.4</td>
<td>PBS 6</td>
<td>–</td>
<td>[44]</td>
</tr>
<tr>
<td>NiO-SPE</td>
<td>DPV</td>
<td>PT</td>
<td>0.1–30</td>
<td>24</td>
<td>0.05 M BR buffer, pH 6.0</td>
<td>urine, tomato</td>
<td>[18]</td>
</tr>
<tr>
<td>Al-doped mesoporous cellular foam</td>
<td>SWV</td>
<td>PT</td>
<td>0.01–1 mg/L</td>
<td>17.16</td>
<td>0.1 M KCl, pH 6.0</td>
<td>cabbage</td>
<td>[36]</td>
</tr>
<tr>
<td>ERGO/GCE</td>
<td>SWV</td>
<td>PT</td>
<td>3 × 10^{−5}–11</td>
<td>10.9 × 10^{−3}</td>
<td>PBS, pH 7</td>
<td>groundwater, soil, tomato, rice</td>
<td>present work</td>
</tr>
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</table>
Table 3: Experimental sample table for recovery studies of spiked PT in actual samples.

<table>
<thead>
<tr>
<th>Real samples</th>
<th>Added (μM)</th>
<th>Detected (μM)</th>
<th>Detected by UV–vis</th>
<th>Recovery (%)</th>
<th>Relative error (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ground water</td>
<td>1.5</td>
<td>1.46</td>
<td>1.52</td>
<td>97.33</td>
<td>3.947</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>2.48</td>
<td>2.52</td>
<td>99.20</td>
<td>1.587</td>
<td>1.369</td>
</tr>
<tr>
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<td>5</td>
<td>5.12</td>
<td>5.2</td>
<td>102.4</td>
<td>1.538</td>
<td>1.369</td>
</tr>
<tr>
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<td>0.97</td>
<td>1.01</td>
<td>97.00</td>
<td>3.961</td>
<td>1.620</td>
</tr>
<tr>
<td></td>
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<td>2.95</td>
<td>3.07</td>
<td>98.33</td>
<td>3.909</td>
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<td></td>
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<td>4.96</td>
<td>5.04</td>
<td>99.20</td>
<td>3.909</td>
<td>1.240</td>
</tr>
<tr>
<td>tomato</td>
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<td>0.99</td>
<td>96.00</td>
<td>3.030</td>
<td>1.120</td>
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<tr>
<td></td>
<td>3</td>
<td>2.9</td>
<td>3.04</td>
<td>96.67</td>
<td>4.605</td>
<td>0.992</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>7.07</td>
<td>7.18</td>
<td>101.00</td>
<td>1.532</td>
<td>1.119</td>
</tr>
<tr>
<td>rice</td>
<td>0.5</td>
<td>0.51</td>
<td>0.58</td>
<td>102.00</td>
<td>1.260</td>
<td>0.991</td>
</tr>
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<td>5</td>
<td>5.12</td>
<td>5.16</td>
<td>102.40</td>
<td>0.775</td>
<td>0.991</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10.11</td>
<td>10.25</td>
<td>101.10</td>
<td>1.366</td>
<td>1.230</td>
</tr>
</tbody>
</table>

cal nanosensor could be a good alternative for on-site monitoring of PT usage in agricultural fields. The robust and straightforward electrochemical-sensing platform could also be a promising path for selective and sensitive analysis of other pesticides and environmental pollutants based on electrocatalytic activity.

Supporting Information
Table S1: Chemical sample table indicating corresponding CAS, supplier and other details. Table S2: Experimental sample table for composition analysis using binding energies of GO and RGO by XPS. Table S3: Experimental sample table for the modified glassy carbon electrode electrochemical characteristics. Figure S1: CV of ERGO/GCE at different scan rates (10–300 mV/s) in 1.0 mM K₃Fe(CN)₆ solution with 1 M KCl. Figure S2: (A) Storage stability of the proposed sensing matrix (ERGO/GCE), (B) SWV of PT (1.5, 2.5, 5 μM) added in groundwater. Figure S3: (A) Absorption spectra of parathion and (B) corresponding calibration plot.

Supporting Information File 1
Additional figures and tables. [https://www.beilstein-journals.org/bjnano/content/supplementary/2190-4286-13-65-S1.pdf]

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References

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