A Novel Responsive Sensor for *Penicillium italicum* Fruit Fungus Based on Mesoporous CaMn$_4$O$_8$-G-SiO$_2$ Nanocomposite

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Abstract
In this study, we present the development of a novel CaMn$_4$O$_8$–G–SiO$_2$ (CaMnGS) sensor to detect the presence of *Penicillium italicum* mold. CaMnGS as ternary type nanocomposite was synthesized using a self-assembly technique. CaMnGS sample demonstrated outstanding stability, high selectivity, and notable characteristics for *Penicillium italicum* fungus detection. For *Penicillium italicum* fungus sensing, the CaMnGS displayed a large linear range of (50–100) μL, and a low detection limit of 0.50 μL. Significantly, the CaMnGS sensor was capable of swiftly detecting the *Penicillium italicum* fungus in wastewater. The CaMnGS has been proven to improve the selectivity for *Penicillium italicum* and had tremendous potential for *Penicillium italicum* fungal sensing. The mesoporous CaMnGS sensor may also detect the presence or absence of the fungus *Penicillium italicum*. This method might be used to identify the fungus *Penicillium italicum*. This is also the first attempt to discuss the fabrication of an electrochemical sensor employing a mesoporous CaMnGS nanoparticle composite as a platform for the selective detection of *Penicillium italicum*.
Graphical Abstract

*Penicillium italicum* sensing

**Keywords** Sensor · Graphene · *Penicillium italicum* · Electrochemical

1 Introduction

*Penicillium italicum* is one of the most troublesome molds, and accounts for nearly 25% of citrus output globally; during storage and transportation, its infection is widespread [1]. *Penicillium italicum*, sometimes known as blue mold, is the costliest postharvest issue for citrus crops. It is a wound pathogen that exclusively infects damaged citrus trees. Fruit-to-fruit contact may transmit the illness. *Penicillium italicum* may develop particularly at or below 10 °C, therefore cold storage does not preclude its presence or infection. This is what causes it to be infectious, even when fruit are stored in standard, protected conditions [2]. Numerous research efforts have been conducted to eradicate and inhibit the development of *Penicillium italicum* in citrus species [3], but no studies on the identification of *Penicillium italicum*

species other than phylogenetic approaches [4] have been published. Traditional culture techniques are time-demanding, whilst alternative technologies, like ELISA, PCR, confocal and fluorescence microscopy, and SPR, are not widely available, and may be labor-intensive and expensive [5].

A Mn-based sensor is made up of micron-sized arrays arranged in a certain order on a tiny patch. Mn-based sensing systems are superior to other sensor devices, because of their efficiency, simplicity, and noninvasive nature. Mn-based biosensors can collect and analyze electrical signals from biofluids, including interstitial fluid and blood. The Mn-based sensor may either be an active component of biosensor, or collect samples for signal processing [6]. For two decades, manganese oxide-based molecules have intrigued scientists. There is also a charge and spin–orbit ordering in these molecules. Solid fuel cells, oxygen sensors, and
magnetoresistance switching [7] all use manganese oxide compounds based on CaMnO₃ as electrode. Nanomaterials made from graphene are being studied for many new applications. Their vast surface area, electrical and thermal conductivity, mechanical strength, and optical transmittance make them stand out. Easy production, excellent stability in multiple solvents, and surface functionalization make graphene a desirable material for electrochemical sensors [8–14].

The development of mesoporous materials has grown exponentially in recent decades. Mesoporous silica particles have very interesting applications in sensing. To preserve or modify the optical or binding properties of the pores of the material, sensors are covalently bound to the surface of the material that is acting as a solid support. The sensing compound is loaded into the pores without covalent interaction. The selectivity is achieved by interacting the analyte with the layer decorating the material adds to the amplifying impact of high transducer loading into the pores [15, 20].

Mycotoxins are often detected in centralized labs using procedures that include HPLC, fluorescence, SPR, chemiluminescence, ELISA, quartz crystal microbalance, and solid-phase extraction. These techniques are sensitive and selective, but necessitate heavy equipment, complicated sample preparation and clean-up processes, expert operators, and overall high expenses. Thus, disposable biosensors with point-of-care capabilities are a viable tool for mycotoxin detection. Recently, nanomaterials have been employed to construct biosensors with better electrocatalytic capabilities. The most widely used nanomaterial for biosensors is graphene. Graphene’s unique qualities include huge surface area (2630 m²/g), excellent electrical and thermal conductivity, good biocompatibility, cheap cost, heterogeneous electron transfer (HET), and high stability (125 Gpa). Graphene-based nanomaterials also offer superior macroscopic conductivity and electrocatalytic activity [21].

To achieve the all of properties, we designed a graphene-based electrode with unique characteristics and an increased redox electrochemical signal that resulted in sensitivity and selectivity for *Penicillium italicum* fungi. In this work, we used a CaMn₄O₈–G–SiO₂ (CaMnGS) modified electrode to detect *Penicillium italicum* fungi as a biomarker of infections.

## 2 Experimental

### 2.1 Materials and Instruments

Calcium chloride dihydrate (CaCl₂·2H₂O), Manganese (II) chloride tetrahydrate (MnCl₂·4H₂O), Ethanol, natural graphite powder, hydrochloric acid (HCl, 36 wt.%), sulphuric acid (H₂SO₄, 97%), and potassium permanganate (KMnO₄) were purchased from Sigma–Aldrich. Pluronic F-127 was purchased from Duksan Pure Chemicals Co. Ltd., Korea. Tetraethyl orthosilicate (SiC₆H₂₀O₄) and ethylene glycol (99.5%) were purchased from Samchun Pure Chemical Co., Ltd., Korea. *Penicillium italicum* fungi were collected from orange fungus. All solutions were prepared using distilled water. Electrochemical measurements were made using a PG201 Potentiostat/Galvanostat (Volta lab™, Radiometer, Denmark). A platinum electrode was employed as a counter electrode. Ag/AgCl was used as the reference electrode, and nickel foam was used as the working electrode.

### 2.2 Synthesis of Mesoporous CaMn₄O₈ (CaMn)

CaMn₄O₈ nanoparticles were synthesized using a self-assembly technique. Calcium chloride dihydrate (CaCl₂·2H₂O) and Manganese (II) chloride tetrahydrate (MnCl₂·4H₂O) were dissolved in ethyl alcohol (250 mL) under continuous stirring at room temperature (RT) separately, and after 15 min. 250 mL DI water was added. A pink suspension was then formed. The molar ratios were set at 1:1:1 for precursor reagents. A surfactant ethanol solution (7 g of block copolymer Pluronic F127 dissolved in 300 mL of ethanol) was then added. After stirring at 50 °C for 5 h and evaporating for 24 h, a sol–gel solution was obtained, which was then calcined at 700 °C (1 °C/min) for 3 h.

### 2.3 Synthesis of Mesoporous CaMn₄O₈–G (CaMnG)

After 0.1 g of graphite oxide (GO) was added into 550 mL of water, the reaction mixture was irradiated with a high-intensity ultrasonic probe at RT in air for 70 min. The sonicated graphene oxide solution was added to the solution of CaMn₄O₈ under continuous stirring. Then, 60 mL of 1 M sodium hydroxide solution was dropwise added into the sonicated precursor mixture for optimum pH. The resulting mixture was stirred for 3 h at 120 °C to effectively combine graphene with CaMn₄O₈, and then calcinated at 700 °C for 3 h.

### 2.4 Synthesis of Mesoporous CaMn₄O₈–G–SiO₂ (CaMnGS)

The CaMn₄O₈–G solution was drop-by-drop added to a beaker containing 0.15 g of mesoporous silica powder, which was synthesized using our previously reported method [21] and stirred at 120 °C for 24 h. Next, the mixture was ultra-sonicated for 30 min. The powder was then filtered, washed with ethanol, and dried at 70 °C overnight. After that, it was calcined at 700 °C at a heating rate of 10 °C/min, and then held at 700 °C for 5 h.
2.5 Fabrication of Mesoporous CaMn$_4$O$_8$–G–SiO$_2$ (CaMnGS) Electrode

CaMn$_4$O$_8$ (CaMn), CaMn$_4$O$_8$–G (CaMnG), and CaMn$_4$O$_8$–G–SiO$_2$ (CaMnGS) sensors were all working electrodes and were made of copper foil. Electrochemical paste was deposited on 15 mm×40 mm copper foil. The copper foil was then doped with CaMnGS. To form a paste, ethyl cellulose was homogenized with CaMn, CaMnG, and CaMnGS, respectively. It was next sintered for 1 h at 100 °C. Pressure was then applied to obtain a homogenous surface of the electrode. All samples had the same thickness, size, and coating.

2.6 Characterization of Materials

The phase structure and purity of the as-synthesized products were examined by X-ray diffraction (XRD; Rigaku, Japan) with Cu–K$_\alpha$ radiation (λ = 1.5406 Å) at 40 kV and 30 mA over the 2θ range (20–70)°. Morphologies of the obtained samples were studied by scanning electron microscopy (SEM). EDS analysis was performed by SEM (JSM-76710F, JEOL, Japan), transmission electron microscopy (TEM) (JEM-4010, JEOL, Japan), and high-resolution TEM (HRTEM) (JSM-76710F, JEOL, Japan), operated at an accelerating voltage of 300 kV. X-ray photoelectron spectroscopy (XPS), Diffuse Reflectance Spectroscopy (DRS), and Raman spectroscopy (RAMAN) analyses were performed utilizing WI Tec. alpha 300 series. Porous characterization of CMGS structure was performed with a full analysis of N$_2$ adsorption–desorption tests (BEL-SORP-max, BEL Japan Inc.) (PG201 Potentiostat/Galvanostat, Volta lab™, Radiometer, Denmark).

2.7 Electrochemical Measurements

Three electrodes were used in this experiment, namely a platinum counter electrode, an Ag/AgCl reference electrode, and an aqueous buffer solution as the electrolyte. The detection limit was calculated using Eq. (1) shown below [22, 23]:

\[
\text{LOD} = 3.3(S_v/S)
\]  \hspace{1cm} (1)

where, $S_v$ is the standard deviation of the response of the curve, and $S$ the slope of the calibration curve. CV measurements were made from (−0.3 to +0.2) V versus Ag/AgCl at 10 mV s$^{-1}$.

3 Results and Discussion

3.1 Characterization of the CaMnGS Sample

Figure 1 a shows the XRD patterns of CaMn, CaMnG, and CaMnGS, respectively. A crystal structure for CaMn$_4$O$_8$ has now been established. Orthorhombic perovskite structure is seen in CaMn$_4$O$_8$. The (004), (011), (200), (112), (020), (013), (116), and (020) planes in the XRD pattern of mesoporous CaMnGS are connected to various XRD peaks in the XRD pattern. The orthorhombic structure was clearly visible in every pattern in the produced material, with no traces of impurity phases [24].

Figure 1 (b) shows the EDS analysis of CaMnGS that was used to identify its elemental composition. EDS identified a high concentration of C, O, Ca, Mn, and Si elements, but no additional contaminants. The CaMnGS sample quantitative investigation discovered significant amounts of C (3.47%), O (48.44%), Ca (25.50%), Si (5.9%), and Mn (23.53%), in addition to other elements.

SEM, TEM, and HRTEM images of CaMn, CaMnG, and CaMnGS samples were collected for electron microscopy studies to examine sample morphology, as shown in Fig. 2. According to the results of TEM and HRTEM images, the self-assembly synthesis yields agglomerated CaMn nanoparticles [25], with an average diameter as indicated in Fig. 2a. When combined with graphene and mesoporous SiO$_2$, as shown in Figs. 2b and c, SEM images of CaMnG and CaMnGS reveal the material’s well-distributed nature. As shown SEM images, the particle size of CaMnG increased when graphene was added to CaMn, and the particle size of CaMnGS increased further when mesoporous SiO$_2$ was added to CaMnG. CaMnG and CaMnGS are both confirmed by HRTEM images. In Fig. 2f, a HRTEM image representing mesopore is presented. The pore size shows a size of about 18 nm.

Figure 3 shows the (a) N$_2$ adsorption–desorption isotherms, (b) surface area plot, (c) t-plots, and (d) pore distribution. Mesoporous materials are known to exhibit the H2 Type IV isotherm and a Type H2 hysteresis loop in the (0.6–0.9) $P/P_0$ range. In the adsorbent, mesopores were discovered to have a pore size distribution of 13.97 nm, confirming the existence of mesopores. The type of adsorption isothermal curve is an important factor that indicates the shape of pores. The formation of an isothermal curve hysteresis loop is largely over all range, which indicates the formation of mesopores uniformly. Uniform formation of mesopores will increase the effect of sensor sensitivity and reactivity with an increase in adsorption amounts.

XPS analysis was used to determine the surface chemical compositions of the mesoporous CaMnGS. Figure 4 shows the Ca, Mn, C, O, and Si results. The CaMnGS nanocomposite’s chemical states and surface composition were confirmed using an XPS examination, which was carried out in the lab.

Figure S1a of the Supplementary information shows the XPS analysis of Ca2p that was used to detect the chemical state of Ca in CaMnGS. To distinguish one peak from the
other, the Ca2p spectra has two peaks with binding energies of (348.12 and 351.62) eV for Ca2p3/2 and Ca2p1/2 respectively. According to the binding energies of calcium in the CaMnGS [26], the Ca2p peaks in the XPS spectrum are consistent. There are two peaks at (641.85 and 654.02) eV for Mn 2p3/2 and Mn 2p1/2, which are characteristic of Mn3+-based materials [27] in Fig. S1b of the SI. This shows that the CaMnGS surface prefers Mn substitution. Figure S1c of the SI shows the C1s spectra exhibit peaks at (284.08 and 289.08) eV that are thought to represent C–C bonding and O–C=O. Figure S1d of the SI, which depicts O1s spectra, shows C–O bonding at 532.02 eV [20]. The presence of SiO2 on the CaMnGS surface is seen in Fig. S1e of the SI at 102.03 eV [22].

Figure 5(a) shows the EIS findings. Due to its high electrical conductivity, the CaMnGS has a charge–transfer resistance of 25 Ω cm−2. The initial charge–transfer resistance (between 3 ~ 150 Z' regions) of CaMnGS was found to be lower than that of CaMn and CaMnG. This suggests that graphene addition significantly increases the electrical conductivity, and therefore, the electrochemical performance in a CaMnGS
sample. As illustrated in Fig. 5b, Raman spectra may be used to study the graphene oxide D and G bands for structural information. A ratio of ID/IG of CaMn was not shown, which indicates that peaks were not shown because it did not contain graphene. In the case of CaMnG, a relatively large amount of graphene is contained, and the ratio of ID/
IG shows a relatively high peak intensity of 0.92. In addition, the ratio of ID/IG of CaMnGS was close to 1.0, which was relatively small. This shows a small peak intensity due to the reason that the amount of graphene is less than the amount of CaMnG.

Both of these bands are associated with the out-of-plane breathing mode of the Sp² atom induced by defects and E₂g phonons near the Brillouin zone center. The G band is created by the stretching of carbon sp² atom bonds, whereas the D band is caused by structural disorder [27]. The ID/IG ratio (derived from the intensity of the D and G bands) may be used to describe the graphitic structural disorder in carbon materials. Because of the various functional groups generated during the oxidation of graphite powder, GO has a very disordered structure, as seen in Fig. 5b. The D band is located at 1352 cm⁻¹, while the G band is located at 1596 cm⁻¹.

3.2 Electrochemical Behavior of CMGS

Figure 6 depicts the linear CV curve for the electrochemical behavior of the (a) CaMnGS, (b) CaMnG, and (c) CaMn in the absence of the Penicillium italicum fungus at 10 mVs⁻¹. The maximum peak current shows 6.6 x 10⁻¹ mA cm⁻² for CaMnGS electrode, while CaMn and CaMnG are found at (2.0 x 10⁻³ and 3.0 x 10⁻⁴) mA cm⁻², respectively. According to CaMn, CaMnG, and CaMnGS, the peak current improved in CaMnGS due to the CaMnGS electrode electroconductivity, which was discovered to have further increased by adding graphene and SiO₂ to the working electrode surface. It has been shown that graphene and SiO₂...
work synergistically to enhance electron transport between the redox probe and electrode surface. The electrochemical performance of the CaMnGS working electrode has been significantly improved because of the addition of graphene and SiO2 to the porous structure. In part, this is due to the large surface area of the composite material, and the functional group located there [28].

Exposure to *Penicillium italicum* fungus reduced the current for CaMn and CaMnG to $1.0 \times 10^{-4}$ and $5.0 \times 10^{-5}$ mA cm$^{-2}$, respectively, as shown in Fig. 7(a), but Fig. 7(b) shows that the peak current for CaMnGS electrode decreased to $2.0 \times 10^{-5}$ mA cm$^{-2}$.

This is due to the CaMnGS electrode SiO2 surface modification, which increases the rate at which electrons move from the electrolytic solution to the electrode. There are chemical and biological components on the CaMnGS surface that drastically restrict the pace at which electrons may be transferred. The study confirmed that CaMnGS electrodes for biosensing must be modified with conductive nanomaterials on the surface to collect the tiniest of biological activity signals. A decrease in current density and an increase in insulating quality are achieved by *Penicillium italicum*.
covalently adhering to the electrode surface [28]. For further study, it was decided to build a biosensor to measure the quantitative amount of *Penicillium italicum* to simultaneously identify the fungus, which is discussed in more detail in the next section.

Chronoamperometry was also used to measure *Penicillium italicum* fungus, as seen in Fig. 8.

The CaMnGS electrode was utilized to facilitate a suitable reaction, which was then delivered to the electrochemical detection cell for chronoamperometry, together with the various concentrations of *Penicillium italicum*. As the *Penicillium italicum* concentration rises, the observed current diminishes. Covalent connections formed between the *Penicillium italicum* fungus and the biosensor inhibit the movement of electrons from the solution to electrode surface. In terms of linear detection range, low detection limit, and analysis time, the proposed biosensor possesses all of these features, and more [29]. The best biosensor response was discovered at pH 7.0, which is a critical factor in biosensor response. Lower reaction was seen at higher pH values. Biosensor sensitivity was only about half of what it was when the pH level was higher in the acidic range. In the next part, we study pH dependence to determine the suitable pH value.

Because the pH of the electrolyte might affect the peak potential and peak current of the modified electrode, measuring the proton-to-electron ratio involved in the electrode reaction is more advantageous than determining other parameters. Because a change in pH results in a change in the current density of the electrode, the two are related. As a result, the pH range is also critical for biosensors. Figure 9

![Fig. 7](image1.png)  
**Fig. 7** a Linear curve of CaMn and CaMnG and b CaMnGS electrode with 40 μL *Penicillium italicum* fungus solution in PBS at scan rate of 10 mV s⁻¹ (pH 7.0)

![Fig. 8](image2.png)  
**Fig. 8** Chronoamperometry of CaMnGS electrode with different conc. of *Penicillium italicum* solution in 0.1 M PBS (pH 7.4). All experiments run at room temperature (20 °C)

![Fig. 9](image3.png)  
**Fig. 9** The pH dependence of CaMnGS electrode
shows the results of a pH scan performed at a rate of 10 mVs\(^{-1}\) throughout the pH range (4 to 9).

There is a tiny shift in current density in the pH range (4 -- 9), which we can see. During a study of CaMnGS peak currents, it was discovered that pH 7.5 was the most appropriate for biological chemical detection applications.

Additional comparators for evaluating the CaMnGS sensor selectivity included kitchen fungus, bread fungus, and biofilm. An electrode was immersed with a variety of strains of the same concentration, as seen in Fig. 10, to estimate the current density. *Penicillium italicum* exhibited a lower value when the other two species were injected dropwise, although the value of *Penicillium italicum* was $5 \times 10^{-5}$ mA cm\(^{-2}\).

The sensor was able to correctly identify *Penicillium italicum*, as shown by the results. This phenomenon happened due to the hydrogen bonding of *Penicillium italicum* with mesoporous CaMnGS sensor. *Penicillium italicum* binds with hydrogen bonding and collapses the electron transfer by blocking the active site. Then any other biological material can’t bind. Detection of the selectivity was carried out after the appropriate pH and thermal states had been established.

Figure 11 depicts the temperature-dependent sensitivity of CaMnGS electrodes to *Penicillium italicum*.

For the CaMnGS sensor, the sensor sensitivity was evaluated to ensure that the sensor sensitivity of *Penicillium italicum* was affected by temperature changes. To demonstrate its capacity to detect temperature, the CaMnGS electrode performed well in temperature ranges of 20 to 70 °C. When the temperature rises over 65 °C, the *Penicillium italicum* is eliminated, as shown by our sensor, and the CaMnGS sensor may transfer electrons and enhance the conductivity of the sensor, confirming this. In parallel, the temperature dependency test provides the thermal stability determination and confirmation of the absence or presence of *Penicillium italicum*. While at 37 °C, the current density is $3.0 \times 10^{-5}$ mA cm\(^{-2}\), due to the presence of *Penicillium italicum* in the sensor, which prevents electron transfer, when the temperature is raised to 65 °C, the *Penicillium italicum* is destroyed, resulting in an increase in the current density to $1.0 \times 10^{-4}$ mA cm\(^{-2}\). As a result, the ideal sensor temperature to detect *Penicillium italicum* well is (30 to 50) °C [30].

Electrochemical properties of rare metal also offer superior macroscopic conductivity and electrocatalytic activity [31].
The sensor effect measured at a temperature of 20 °C showed similar results at a temperature condition of 25 °C. In addition, the temperature conditions at 37 °C showed similar results to the sensor effect measured at 40 °C. Therefore, in this study, sensor effects were presented under three typical temperature conditions.

4 Conclusions

For *Penicillium italicum* detection and sensing, CaMnGS mesopores were produced. When compared to *Penicillium italicum*, the CaMnGS produced mesoporous CaMnGS demonstrated high sensitivity and specificity. CaMnGS has been proven to improve the selectivity for *Penicillium italicum*. Because of its ability to both qualitatively and quantitatively identify *Penicillium italicum*, it is a viable solution for low-resource regions. *Penicillium italicum* may be detected in food samples using affordable point-of-use and high-throughput quantitative methods devised by the researchers. *Penicillium italicum* contamination in fruit may be detected using simple procedures in the field, in storage, and in transit situations. Consequently, the high sensitivity, cheap fabrication costs, and real-time operation of the suggested detection method are very promising, opening the way for a cost-effective and real-time sensor. *Penicillium italicum* infections may be detected and treated early, preventing the development of multidrug-resistant *Penicillium italicum*.

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Declarations

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