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<th>A novel non-enzymatic lindane sensor based on CuO-MnO\textsubscript{2} hierarchical nano-microstructures for enhanced sensitivity</th>
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In this work, we present new results about the non-enzymatic detection of lindane by using CuO/MnO$_2$ hierarchical nanostructure. The linear range for the determination of lindane is up to 700 μM with a limit of detection of 4.8 nM which is much lower than for other non-enzymatic lindane sensors.

In 1825, Michael Faraday reported to the Royal Society of London the synthesis and isolation of hexachlorocyclohexane (HCCH), otherwise known as benzene hexachloride. The reaction generates stereoisomers of HCCCH, among these isomers, only the Lindane (γ-HCCH) exhibits insecticidal property. Lindane has been used worldwide as a general, broad-spectrum insecticide for a variety of purposes, including fumigation of stored grain products, pest control on domestic animals, mosquito control, to kill soil-dwelling and plant-eating insects. Many countries have been using γ-HCCH as a topical treatment for head lice and scabies. Lindane is not bio-degradable; therefore, its accumulation has been detected in human blood, breast milk, and adipose tissue around the world. As a consequence of its toxic, carcinogenic, bioaccumulative and suspected endocrine disrupting properties, HCCH was listed in 2009 as persistent organic pollutants (POPs) by the Stockholm Convention. For this reason, research and development into new techniques for the degradation of lindane by various methods of dechlorination has attracted a great deal of interest over recent years. Presently, analysis of organohalide contamination often relies on the sophisticated analytical techniques such as gas chromatography, liquid chromatography or gas chromatography–massspectrometry (GC–MS). The overall analytical process is complex, expensive, and relies on skillful manpower. Therefore, a simple method for sensitive and selective detection of lindane is highly desirable. Electrochemical biosensors are promising alternatives to conventional analytical tools since they offer a rapid, specific, and sensitive protocol for the detection of environmental pollutants, including pesticides. A major limitation for the development of γ-HCCH biosensor is the availability of bioreceptor probe, which can specifically detect the target analyte. Lindane is insoluble in water and has a very high reduction potential, the direct reduction of lindane using the aqueous medium is a challenging task. Polarographic study in ethanol/water; γ-HCCH gave only one reduction wave and it has been concluded that the reduction takes place through a six-electron transfer process to form benzene. It has been known that certain metals, notably nickel, manganese and cobalt, can catalyze, the reduction of organohalides at much more moderate potentials. The overall reaction is R + H$^+$ + 2e$^-$ → RH + X. The source of electrons for these reactions may be either chemical or electrochemical. Metal and transition metal oxide nanoparticles have emerged as a viable alternative in fabricating highly efficient non-enzymatic electrochemical sensors. In particular, MnO$_2$ and CuO nanomaterials are considered as one of the most appealing inorganic materials and have drawn attention in bioanalytical chemistry, especially in electrochemical sensing. Researchers have recognized that metal oxides composites always show excellent electrochemical performances over the single metal oxide alone. Therefore, a facile route for large-scale production of CuO/MnO$_2$ composites is still highly demanded.

In this communication, we report a facile method for the synthesis of CuO/MnO$_2$ hierarchical nanostructure. Further, we demonstrated the applicability of CuO/MnO$_2$ hierarchical nanostructure as an active electrocatalyst towards reduction of lindane. The CuO/MnO$_2$ sensors exhibited low detection limit, and wide linear range toward the detection of lindane, which can be ascribed to the novel structure of CuO/MnO$_2$ composites.

Materials prepared in this study were subjected to XRD investigation (Fig. 1a). XRD pattern of the CuO can be indexed to monoclinic CuO (JCPDS 410254) and that of MnO$_2$, can be indexed to monoclinic K-birnessite (JCPDS no. 80-1098) (Fig. 1a). The diffraction peaks from both CuO and K-birnessite MnO$_2$ are clearly observed in the XRD pattern of CuO/MnO$_2$. In the XRD pattern of the CuO/MnO$_2$ composites, several diffraction peaks from the CuO have disappeared due to the thick coating of the MnO$_2$ (Fig. 1a). FESEM and TEM images are presented in Fig. 1b-d. Clearly, CuO are made up of a great number of leaves like structures (Fig. 1b). The FESEM image of MnO$_2$ is shown in Fig. 1c, which clearly illustrates the nanostructured hierarchical flower-like morphology. FESEM image of CuO/MnO$_2$ revealed that CuO had been homogeneously wrapped with a layer of dense MnO$_2$ nanoflakes (Fig. 1d). From TEM image (Fig. 1d, inset), clearly the MnO$_2$ nanoflakes are interconnected and quite uniformly grown over the surface of the CuO, forming an attractive porous heterostructure. HRTEM images of the CuO/MnO$_2$ (Fig. S1, ESI†), indicate that porous MnO$_2$ layer are composed of numerous tiny nanoflakes.
The textural properties of metal oxides were investigated by N2 adsorption/desorption measurements (Fig. S2, ESI†). CuO/MnO2 exhibits type IV isotherm with H3 hysteresis loop. The increase in slope at 0.45 corresponds to capillary condensation, typical of mesoporous materials, while the further increase at higher relative pressures indicates inter-particle porosity with slit-shaped pores.18c BJH pore size distribution of CuO/MnO2 shows a pore size distribution centered at ~22.7 nm (Fig. S2, inset). The surface areas of 31.3, 55, and 77.6 m2/g and pore volumes of 0.112, 0.15, and 0.157 cm3/g were obtained for CuO, MnO2, and CuO/MnO2, respectively. To investigate surface property of the composite, FT-IR spectrum analysis was conducted (Fig. S3, ESI†). Samples show characteristic absorption bands in the range of 400 to 800 cm−1, which can be assigned to Mn-O stretching vibrations (Fig. S3, ESI†).19 The FT-IR spectra of CuO/MnO2 exhibited very weak signals corresponding to the vibrational modes of CuO due to the high loading of MnO2.19 Diffused reflectance UV–visible investigation was used to determine the electronic structure and optical properties of CuO/MnO2 (Fig. S4, ESI†). The band gap value for the CuO was found to be 1.6 eV.19 The observed optical band gap for CuO/MnO2 was found to be 1.91 eV. Furthermore, the increase in the optical band gap in CuO/MnO2 materials can be explained on the basis of quantum size effect.11 The study suggests that MnO2 acted as a chemical modifier and caused a structural change or electronic effect.

CuO/MnO2 modified electrode was constructed to obtain the optimized analysis parameter for lindane reduction. Fig. S5 (ESI†) shows cyclic voltammetric responses at the CuO/MnO2 modified electrode before and after the addition of lindane (200 µM). In the absence of lindane, no peak was observed. When lindane was added, distinct peak at -1.5 V (vs Ag/AgCl) appeared, which is completely irreversible, implying that the reaction with lindane at CuO/MnO2 modified electrode is rapid (Fig. S5, ESI†).8c The effect of potential scan rate on the reduction peak currents was investigated in the range from 20 to 300 mV s−1 (Fig. S6, ESI†). Reduction peak currents are linearly proportional to the square root of the scan rate, which shows that lindane undergoes a diffusion controlled reduction; the non-zero intercept can be explained as an adsorption process after diffusion to the electrode surface (Fig. S6, ESI†). The results of the voltammetric analyses are collected in Table S1. A survey of the data reported in Table S1 showed that the transfer coefficient (α) value less than 0.5. It is well established that dissociative electron transfer (ET) which is concerted has a very small value of α typically around 0.3.12 Moreover, there is no reverse peak obtained for a wide range of scan rates, which shows that the reduction process is irreversible.12 The removal of a chlorine atom by the reduction of polychlorinated benzenes always leads to a product that is more difficult to reduce.13 In contrast, cleavage of carbon–chlorine bonds in lindane take place very fast and readily form fully reduced benzene.13 Differential pulse voltammetric (DPV) response at CuO/MnO2 modified electrode for different concentrations of lindane is shown in Fig. 2. The current obtained is proportional to the concentration of lindane in the reaction vessel (Fig. 2, inset).

A linear response was obtained between the peak current and lindane concentration (Fig. 2) with linear regression equation of, I(µA) = -4.18-0.022C (µmol L−1) (R² = 0.9998) in the concentration range of 1 µM–700 µM with the sensitivity of 0.12 µA µM−1 cm−2 and a lower detection limit of 4.8 nM. DPV method was used to investigate the comparative electrocatalytic activity of various metal oxides modified electrodes investigated in this study with CuO/MnO2 modified electrode. MnO2 modified electrode showed sensitivity of 0.10 µA µM−1 cm−2 in the concentration range of 40 µM–250 µM (Fig. S7, ESI†). The intercept of the calibration curve was not found to be zero, suggesting undesired non-Faradaic processes (Fig. S7 inset, ESI†).15 However, when the similar study was performed at CuO and bare glass carbon electrode in the presence of lindane, it did not show an increment in the reduction peak, suggesting no electrocatalytic activity for lindane reduction. The limit of detection was calculated by using IUPAC (International Union of Pure and Applied Chemistry) definitions, using standard approach of alternative (SA).13

\[
\text{LOD}_{\text{SA}} = 3S/q \quad (1)
\]

where S is the standard deviation of the blank signal, and q is the slope of the calibration curve. Based on these observations, one can easily conclude that among the materials investigated in this study, CuO/MnO2 modified electrode exhibited the highest activity towards lindane reduction. Fig. S8 (ESI†) shows a comparison of the DPVs obtained at CuO/MnO2 and MnO2 modified electrodes in the presence of 200 µM lindane. DPV measurements show that CuO/MnO2 modified electrode exhibits higher current response compared to MnO2 modified electrode. The current responses for 200 µM lindane at CuO/MnO2 and MnO2 modified electrodes are

![Fig. 1](Image 51x558 to 304x735)

**Fig. 1** (a) XRD patterns of materials synthesized in this study. SEM images of CuO (b), MnO2 (c), CuO/MnO2 (d); the inset show the corresponding TEM image of CuO/MnO2.

![Fig. 2](Image 330x475 to 545x613)

**Fig. 2** DPVs of lindane at varying concentrations at the CuO/MnO2 modified electrode using 0.05 M TBAB solution in 60:40 methanol-water (20 mL). Inset shows the calibration plot. DPV parameters were selected as: peak height = 50 mV; peak width = 200 ms; peak period = 400 ms; increment = 20 mV; pre and post-pulse width = 3 ms.
The interference study was carried out using continuous amperometry. The possibility of the interference was evaluated against several inorganic ions and organic compounds (1 mM each) such as cyclohexane (CH), chlorobenzene (CB), and benzene (B) (Fig. 3). The results indicate that CuO/MnO2-based sensor is specific to lindane. No interference was observed for 20 fold excess of triclosan, 1,3,5-trichlorobenzene (1,3,5-TCB), 4-chlorobenzaldehyde (4-CBA). It may further be noted that the response current decreased by 13.8% when 250 times higher concentration of 1,3,5-TCB was added. It should be noted that neither ethanol (1 mM), nor phenol (1 mM) elicited any response, these compounds being picked on the grounds of water solubility and structural features. The ionic makeup of other anions likely to be present such as sulfate (1 mM) or nitrate (1 mM), and 100 fold of NH4+, K+, Na+, Ca2+, Mn2+, Fe2+ and Co2+ has no effect on the peak currents corresponding to the lindane reduction signal. The reproducibility of CuO/MnO2 was evaluated by parallel DPV investigations of five numbers of identically fabricated CuO/MnO2 electrodes. The sensor electrodes show relative standard deviations (RSD) of only ~ 2.4%, which is significantly lower. This essentially suggests very high reproducibility of the CuO/MnO2-modified electrode and the process of electrode fabrication, which may be crucial for the most precise determination of electrode fabrication, which may be crucial for the most precise reproducibility of CuO/MnO2.

In summary, we have presented a simple and well performing novel non-enzymatic sensor for lindane. CuO/MnO2 nano-microstructure allows a detection concentration of lindane as low as 4.8 nM. This proposed electrochemical sensing methodology is thus expected to open opportunities for the detection of lindane in the ground water and other water bodies.

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Notes and references

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