



Nickel Oxide and Graphene Oxide Incorporated Molecular Imprinted Coatings for Electrochemical Recognition of Acetylcholine

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Herein, we synthesized molecular imprinted polymer (MIP) coatings containing nickel oxide (NiO) nanoparticles and graphene oxide (GO) for enhanced electrochemical recognition of acetylcholine. The preparation of NiO nanoparticles is carried out by polyol's method whereas GO was made by modified Hummer's scheme. Four different sensor coatings i.e., pristine-MIP, NiO-MIP, GO-MIP, and NiO-GO-MIP were prepared and integrated with screen-printed interdigital capacitors (IDCs), respectively for capacitive sensing of acetylcholine. Among all these sensor devices, NiO-GO-MIP layer coated IDC exhibited the highest capacitance shifts (ΔC) for acetylcholine. Additionally, the NiO-GO-MIP coated IDCs showed at least three times higher sensor response for acetylcholine as compared to dopamine and xanthine suggesting high selectivity of the receptor layer. It was noticed that the sensor response of two weeks old NiO-GO-MIP coated IDCs was similar to that of freshly coated IDCs which indicates that developed sensor coating is stable and robust that can be used for extended times without losing recognition performance.

Keywords: *Molecularly imprinted polymer, Interdigital capacitor, Nickel oxide, Graphene oxide, Acetylcholine.*

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1. Introduction

Acetylcholine [1] is an important neurotransmitter that plays a vital role in regulating the brain signaling system. It is the most abundantly found neurotransmitter that sends signals to central and peripheral nervous systems to carry out various biological functions including learning, memory [2], muscle contraction, sleep, and others. It is reported that an imbalance of acetylcholine concentration is associated with various physiological and neurological disorders [3]. Its dysfunctional concentrations in the body are correlated with many neuropsychiatric disorders including Alzheimer's [4] and Parkinson's [5] diseases. Thus, to diagnose nervous disorders, it is imperative to monitor acetylcholine concentration in patients using a fast, reliable, and inexpensive approach.

Currently, there are several methods reported in the literature [6] for highly sensitive and selective determination of acetylcholine, which include gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC) [7] coupled with microdialysis, chemiluminescence [8], radio-immunoassays, and others. Despite their high sensitivity for analyte detection, sample pretreatment before analysis, preparation of special reagents, and other tedious steps make these methods lengthy and laborious. Moreover, highly trained/skilled personnel are required to run these sophisticated instruments. Recently, amperometric and potentiometric sensor devices having enzyme-modified interfaces are also reported in the literature for acetylcholine sensing. Nevertheless, the development of smart miniaturized electronic sensors having non-

enzymatic recognition layers could be an interesting approach for detecting acetylcholine concentrations. In this perspective, molecular imprinting is a well-established approach to synthesizing biomimetic materials having tailored recognition sites. Molecularly imprinted polymers (MIPs) [9] have been widely reported for several chemical sensing and separation applications [10] for a variety of analytes. Their quick and uncomplicated preparation, high affinity for targets, low cost, high stability, and ability to integrate with various transducers make them highly competitive for tailoring chemical sensor recognition surfaces. It is reported that combining MIPs with organic/inorganic nanomaterials could further improve their sensing performance.

In the current work, a highly sensitive sensor coating comprises of MIPs having nickel oxide (NiO) nanoparticles along with graphene oxide (GO) is developed for acetylcholine sensing. The tailored recognition layers are combined with screen-printed interdigitated capacitors (IDCs) for the chemical sensing of acetylcholine. The developed sensor system was tested for its sensitivity, selectivity, and long-term stability.

2. Experimental

Nickel oxide (NiO) nanoparticles [11] were synthesized following polyol's method where nickel nitrate was used as a precursor and polyvinyl pyrrolidone as a capping agent. The pH of this mixture was maintained at 11 using sodium hydroxide under continuous magnetic stirring. The solution was then calcined at 350°C and the final product grey to black colored NiO nanoparticles were obtained which were washed thoroughly and dried for further use. Graphene oxide (GO) was prepared by oxidation of graphite powder following modified Hummer's method [12]. Molecularly imprinted polymer (MIP) gel was synthesized by taking acetylcholine as a template molecule along with methacrylic acid (monomer), ethylene glycol dimethacrylate (cross-linker), and azobisisobutyronitrile (initiator) following free radical polymerization in dimethyl formamide. To investigate the impact on sensitivity/selectivity due to the addition of NiO and GO individually or collectively to the MIP matrix, four different sensor coatings were prepared i.e., pristine-MIP, NiO-MIP, GO-MIP, and NiO-GO-MIP. The corresponding non-imprinted polymer (NIP) layers were prepared in an exactly similar manner except for the addition of a template for comparing non-specific layer-analyte interactions. The coatings were integrated by spin coating with screen-printed interdigital capacitors (IDCs) [13], separately for capacitive sensing of acetylcholine. The shift in capacitance ΔC i.e., sensor response was measured by

connecting the coated IDCs with the LCR meter as a function of analyte concentration.

3. Results and Discussion

The as-prepared NiO nanoparticles and GO are characterized by UV and FTIR spectroscopic methods. The obtained UV scans and FTIR spectra of NiO and GO are found in agreement with reported literature data indicating their successful synthesis. The synthesized MIP/NIP gels were characterized by FTIR which showed that both types of polymer possess similar polymer backbone structures furthermore, the incorporation of NiO/GO or both did not result in any observable change in both types of polymeric composite gels.

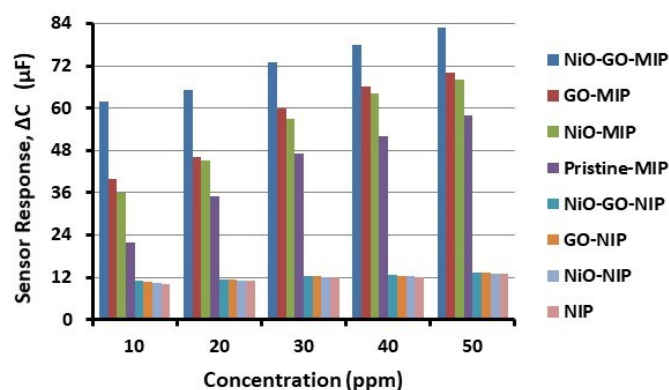


Figure 1 : Sensor response as capacitance shifts (ΔC) of NiO-GO-MIP, GO-MIP, NiO-MIP, and pristine-MIP coated IDCs, respectively for various acetylcholine concentrations. The sensor response of corresponding non-imprinted layers i.e., NiO-GO-NIP, GO-NIP, NiO-NIP, and NIP is also shown. All the layers were tested in the concentration range of 10-50 ppm.

Four sensor coatings i.e. pristine-MIP, NiO-MIP, GO-MIP, and NiO-GO-MIP were developed and integrated with separate IDCs. These devices were exposed separately to standard acetylcholine solutions in the concentration range of 10-50 ppm and subsequent capacitance shift ΔC was monitored. From figure 1 it can be observed that at 10 ppm acetylcholine concentration, the sensor response of the MIP fabricated device is 22 μF while the NIP layer showed a response of 10 μF only. The sensor response of pristine MIP is twice as large as NIP coating which suggests that the MIP layer contains adapted affinity interaction sites for enhanced molecular recognition of analyte while the corresponding NIP layer lack such functionality. To study the effect of NiO and GO in addition to MIP sensitivity, it was observed that incorporation of NiO and GO to MIP gels significantly improved sensor shifts than pristine MIPs. For instance, the addition of NiO to MIP increases the sensor response from 22 μF to 36 μF as shown by NiO-

MIP coating. And similarly, the incorporation of GO to MIP increases sensor response from 22 μF to 40 μF . In the next round, the combined impact of NiO nanoparticles and GO addition to the MIP layer was studied. It was observed that NiO-GO-MIP coated IDC exhibited the highest sensor response of 62 μF for 10 ppm acetylcholine. These results suggested that the combined addition of NiO nanoparticles and GO to MIPs have synergistically enhanced sensor signals. This could be due to the electrochemical characteristics of NiO [11,14] and GO [15]. Moreover, all the corresponding control layers i.e., NiO-NIP, GO-NIP, and NiO-GO-NIP exhibited similar responses as of pristine NIP layer. A similar trend could be observed for other higher concentrations of acetylcholine.

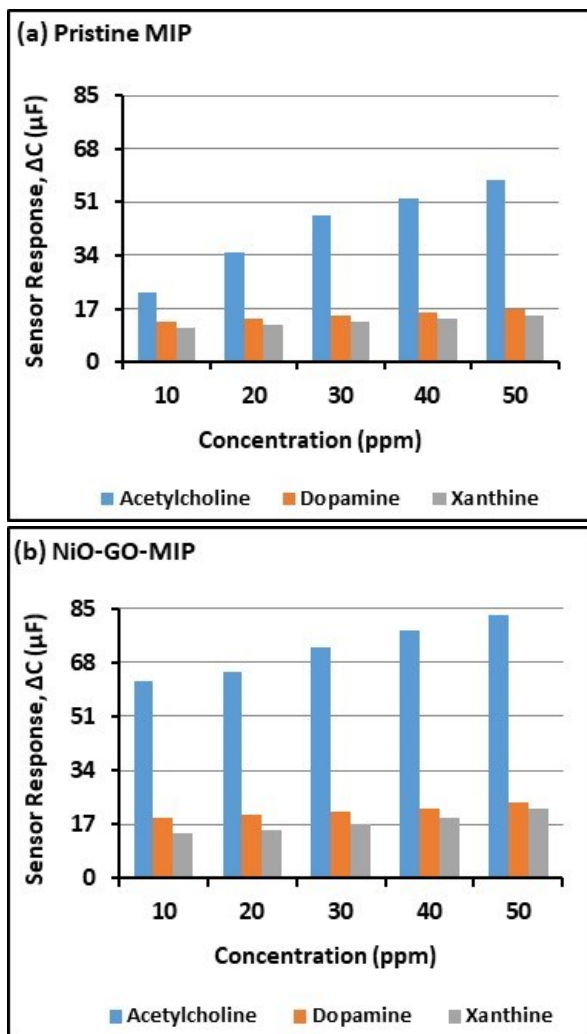


Figure 2. Selectivity pattern by (a) pristine MIP coated IDC and (b) NiO-GO-MIP for acetylcholine, dopamine, and xanthine in the concentration range of 10-50 ppm.

In the next round, the selectivity of NiO-GO-MIP coating was tested and compared with the pristine MIP layer. For this purpose, the sensor shifts for standard solutions of acetylcholine were recorded and compared with other

neurotransmitter drugs such as dopamine and xanthine at corresponding concentrations. Figure 2a shows the sensor shifts by pristine MIP coated device for acetylcholine, dopamine, and xanthine in the concentration range 10-50 ppm. While figure 2b showed the sensor signals of NiO-GO-MIP coated IDC for the same drugs. In figure 2a, it can be noticed that at 10 ppm, the pristine MIP layer showed a sensor-shift of 22 μF for acetylcholine while for dopamine and xanthine the signals are 13 μF and 11 μF , respectively. This shows that pristine MIP offers about two times higher shifts for acetylcholine as compared to other neurotransmitter drugs. In figure 2b, the response by NiO-GO-MIP coated IDC for acetylcholine is 62 μF while dopamine and xanthine exhibited sensor shifts of 19 μF and 14 μF , respectively. This shows that NiO-GO-MIP coating offers at least three times higher sensor shifts for acetylcholine than dopamine and xanthine. A similar trend for other tested concentrations could be observed. From these graphs, it can be demonstrated that the addition of NiO-GO to MIP coatings leads to better recognition of acetylcholine compared to dopamine and xanthine thus, improving the selectivity.

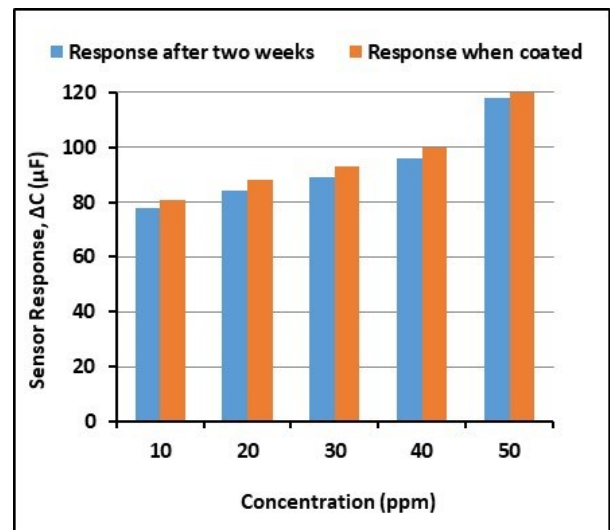


Figure 3. Sensor response of NiO-GO-MIP coated IDC for acetylcholine sensing. The response of the device was compared when it was freshly coated and after two weeks of storage.

To evaluate the stability of NiO-GO-MIP coatings, an already tested IDC was stored for two weeks in a refrigerator and then again subjected to sensor measurements for acetylcholine sensing under the same conditions. Figure 3 compares the sensor-shift (ΔC) data of a two-week-old device with the sensor data when the device was freshly coated and used for measurement. It can be observed that after two weeks of storage, the sensor signal for acetylcholine is almost the same as that of freshly coated IDC. For instance, at 10 ppm, the response of the

freshly coated device was 81 μF and after two weeks of storage, it was 78 μF . Overall at all concentrations, the two-week-old device retains more than 95% of recognition for acetylcholine sensing. This exhibited that NiO-GO-MIP coatings are robust and stable, and retain their recognition properties after storage thus, can be reused with high reproducibility.

4. Conclusions

The current study provides a facile and straightforward protocol for developing highly sensitive sensor coatings. The combined addition of NiO nanoparticles and GO to MIP coatings yield improved sensor signals for acetylcholine sensing than their respective individual additions to MIP coatings. Nevertheless, the incorporation of NiO and GO into MIP is a suitable way to enhance the sensitivity and selectivity for chemical sensing applications. Moreover, the sensor coatings are robust that can be reused after two weeks to achieve similar sensing performance compared to freshly coated devices.

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