



Novel One Step in Situ and Real Time Bioelectrochemical Enzyme-Sensor Based on Methanobactin Silver Nanoparticles for Rapid and Highly Sensitive Detection of Copper in Water Samples

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ABSTRACT

In this work, silver nanoparticles (AgNPs) were synthesized by green fabrication protocol accompanied with Methanobactin and modified onto the surface of screen printed gold electrode by deposition for successfully preparing the nanobiosensor. The established nanobiosensor was successfully applied to the detection of copper in real samples, and the detection limit of the established detection system using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) was as low as 1 nM/ml within 10 second of response time and the selectivity of the sensor is demonstrated by anti-interference experiment when compared with the detection limit which determined with 13.2 nM/ml and the response time within 30 second confirmed by fluorescence measurements, current electrochemical sensors based (Methanobactin + Ag) nanoparticles (AgNPs) results paving the way for the accurate and very fast monitoring of copper in drinkable water and food safety.

1. Introduction

Copper (Cu²⁺) is often added to food or water as additive, in the same time, the overdose of copper will pose a threat to human health, animals and plants, It is essential for people to ingest small amounts of copper every day in food and water. Ingesting too much copper can lead to illness and/or disease. Ingesting a high amount of copper, specially, in drinking water, can cause vomiting, nausea, abdominal pain, and/or diarrhea. Ingesting higher than recommended amounts of copper every day over time, such as in water or in copper supplements, can lead to severe illness, such as kidney and liver damage [1], so it is important to establish a rapid and one step in situ detection method for copper in related food and water. However, Cu²⁺ is one of the essential heavy metal that naturally occurs in rock formations. Human and animal bodies require a certain amount of copper to maintain wellness, and it is utilized to create numerous stuffs, including components for pipe systems of water supply. Copper may enter the drinking water as it flows through the home drainage system which is includes copper pipes in their modules. Consuming or ingesting copper

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typically doesn't result in sickness for the majority of healthy individuals and the common efficiency of copper intake process for human bodies [2]. Nonetheless, it's feasible that consuming or consuming excessive amounts of copper can lead to migraines, nausea, diarrhea, abdomen spasms, vomiting, liver damage, and kidney illness, also elevated copper concentrations could cause damage of red blood cells and might cause decrease the ability of red blood cells to transport oxygen. Elevated levels may impact male fertility of copper. Individuals affected by Wilson's disease and certain infants (children under twelve months old) are more reactive to copper [3]. Methanobactin is a tiny siderophore-like compound suggested to assist in copper capture and management by methanotrophs, which are methane-oxidizing bacteria that have an important role in the global carbon cycle. Copper is essential for methanotrophs metabolism, controlling the expression of two types of methane monooxygenase (MMO) systems, soluble MMO (sMMO) and particulate MMO (pMMO), along with other metabolic enzymes [4]. Furthermore, copper serves as a pMMO cofactor and promotes the development of intracytoplasmic membranes that contain pMMO. While Methanobactin might not only operate in copper absorption, it could also have a more direct involvement in pMMO loading and functionality. Initially known as a copper-binding compound (CBC), Methanobactin was identified a decade ago as a molecule produced by *Methylosinus trichosporium* OB3b that has a high affinity for Cu binding. The Methanobactin sequence from *M. trichosporium* OB3b is N-2-isopropylester-(4-thionyl-5-hydroxyimidazolate)-Gly1-Ser2-Cys3-Tyr4-pyrrolidine-(4-hydroxy-5-thionylimidazolate)-Ser5-Cys6-Met7, and its crystal structure depicts a single copper ion bonded to an N 2S 2 donor configuration [5-7]. Based on its structural resemblance to iron-siderophores, Methanobactin may exhibit antibacterial effects. The practical uses for Methanobactin may encompass its application in the semiconductor sector to extract copper from wastewater [8]. Although its potential significance is wide-ranging, crucial details regarding Methanobactin, like the oxidation state of the bound copper ion, are absent [9]. These small peptide which called methanobactin (Mb) secreted by methanotrophs can capture Cu(II), it can also combine with pMMO to form pMMO complexes in the soluble and participate in electron transfer of process catalyzed by pMMO. In addition, the Mb-Cu complex has peroxidase (POD) and superoxide dismutase (SOD) activity, which makes Mb or Mb-Cu complex be used as the superoxide anion scavenger to protect pMMO and maintain a particular redox state in the presence of oxygen. Mb contains sulfhydryl groups (or disulfide), which can adsorb the stability of elemental ions [10].

2. Methodology

All the Methanobactin molecules and silver nanoparticles which used in this study were prepared in previous work

2.1 Equipment and Apparatus

2.1.1 Cyclic Voltammogram measurements

All CV electrochemical measurements were carried out on a DropSTAT4000P potentiostat instrument (from DropSens) controlled by Autolab software using DropSens screen printed gold electrodes (SPGEs). These electrodes have a conventional three electrode configuration with gold

working electrode (4-mm diameter disk) and counter electrode (16 mm×1.5 mm curved line), and the potentials were ± 0.2 and ± 0.5 , at a scan rate of 100mV/s recorded against Ag/AgCl (16 mm×1.5 mm straight line) pseudo-reference electrode. Typical cyclic voltammograms results are shown in Figure (1).

2.1.2 Electrochemical Impedance Spectroscopy measurement

Impedance spectra were measured using an impedance analyzer (2000A) and gold interdigitated electrodes (from DropSens), 5 μm dimension for bands/gaps are available with reference number (G-IDEAU5) and the overlapping length is 6.76 mm. The AC voltage amplitude was 5 mV with the frequency varied from 100 mHz to 100 kHz; no DC bias was applied. Similarly to the CVs, the impedance spectra measurements were carried out on electrodes coated with Methanobactin+AgNPs suspension which were treated with solutions containing different concentrations of CuCl_2 . Typical results are shown as Nyquist plots in Figure (2&3).

3. Results

3.1 Cyclic voltammetry (CV) investigations

Generally, the CV graphs in Figure (1) are almost featureless for all concentration in the selected voltage range from -0.5V to +0.5V which was chosen deliberately in order to avoid electrochemical reactions on the electrodes. A slight increase in the anode current at -0.2V indicates the beginning of hydrogen reduction. The values of cathode current at -0.5V appear to decrease with the increase in mixed solution of Methanobactin and AgNPs with CuCl_2 concentration with the largest current shown for high concentration and the lowest for uncoated suspension electrode. This indicates that the companion adsorbed onto the surface of the screen printed gold electrodes and act as an conducting layer which increased the current. These data are very important since they establish a correlation between the values of anode and cathode current.

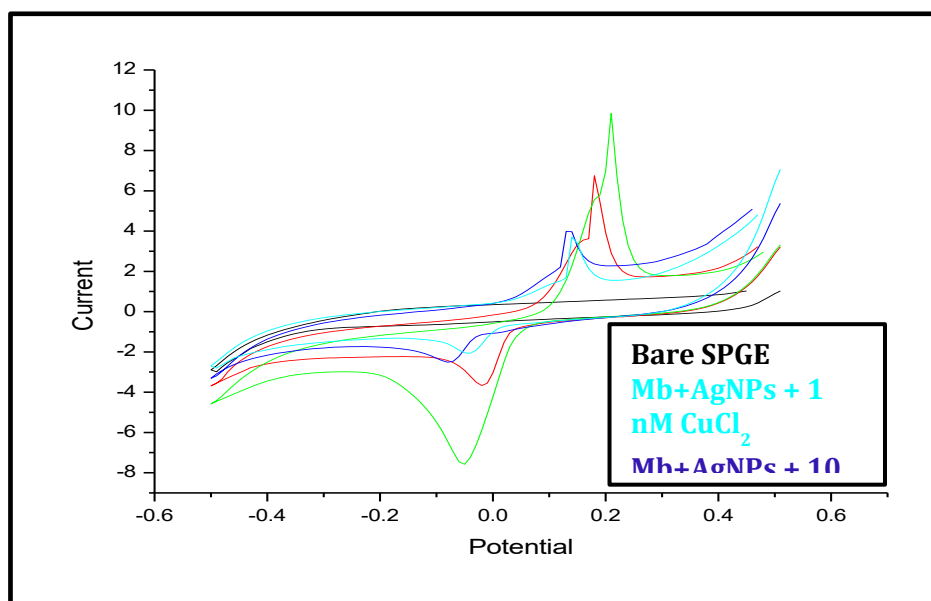


Figure 1: Cyclic voltammogram of Methanobactin+ AgNPs solutions treated with different concentration of CuCl_2 .

A series of CV measurements were carried out on liquid samples of mixed suspensions in SPGE which was mixed in 1:1 ratio with different concentrations of CuCl_2 and kept for 2 hours in (22-25) $^{\circ}\text{C}$ prior to CV measurements. The results of these measurements in Figure (1) show substantial increase in cathode current (I_c) for Methanobactin+ AgNPs solutions upon increasing the concentration of CuCl_2 . In addition, oxidation and reduction peaks appeared on Methanobactin+ AgNPs solutions which are definitely related to electrochemical reactions associated with the presence of CuCl_2 and octane. The anodic peak increases with the increase in CuCl_2 concentration. However, such electrochemical reactions do not appear with bare sample. It is known that CuCl_2 and Methanobactin+ AgNPs solutions act as cofactors for current increasing [11].

3.2 Electrochemical impedance spectroscopy (EIS)

The analysis of impedance on the altered electrodes was conducted meticulously. analyzed through the Nyquist plot to explore the electron transfer at with and without Methanobactin AgNPs compansission on SPGE. EIS of bare SPGE and Methanobactin+AgNPs modified SPGE is illustrated in Figures. 2&3. Methanobactin+AgNPs coated SPGE displays a semicircle in low frequency range followed by a linear section seen in the upper frequency range as seen in Figure. 2. Nevertheless, the semicircle of Methanobactin+AgNPs coated SPGE in the presence of Cu^{2+} ion solution serving as electrolyte observed to diminish and the size of semicircle notably vanish at greater Cu^{2+} concentration resulting from decreased stability and electronic conductivity see Figure 3. This suggested that this Methanobactin+AgNPs coated SPGE also displays greater ionic conductivity with charge transfer occurring quicker than that of the uncoated (Bare) GC electrode as shown in Figure 2.

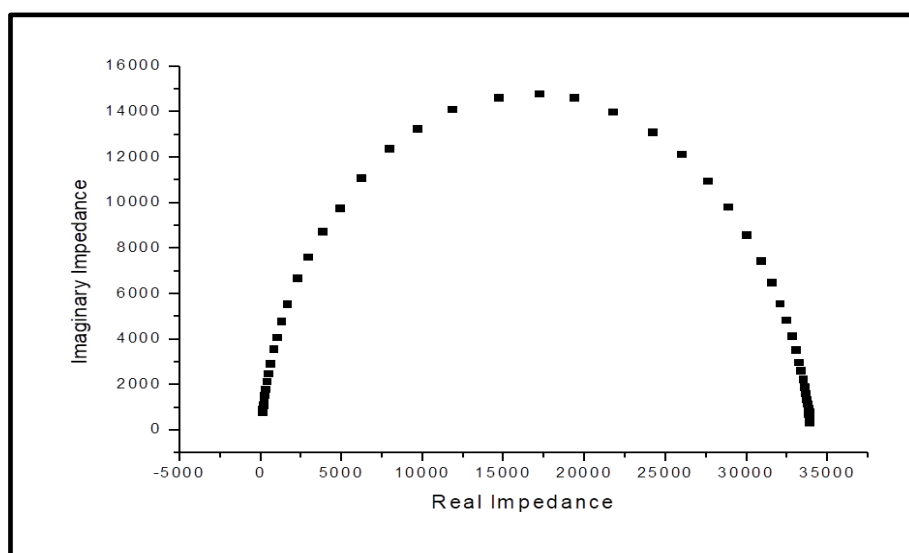


Figure 2: The Nyquist plots (-Z_{im} vs Z_{re}) for bare interdigitated electrodes.

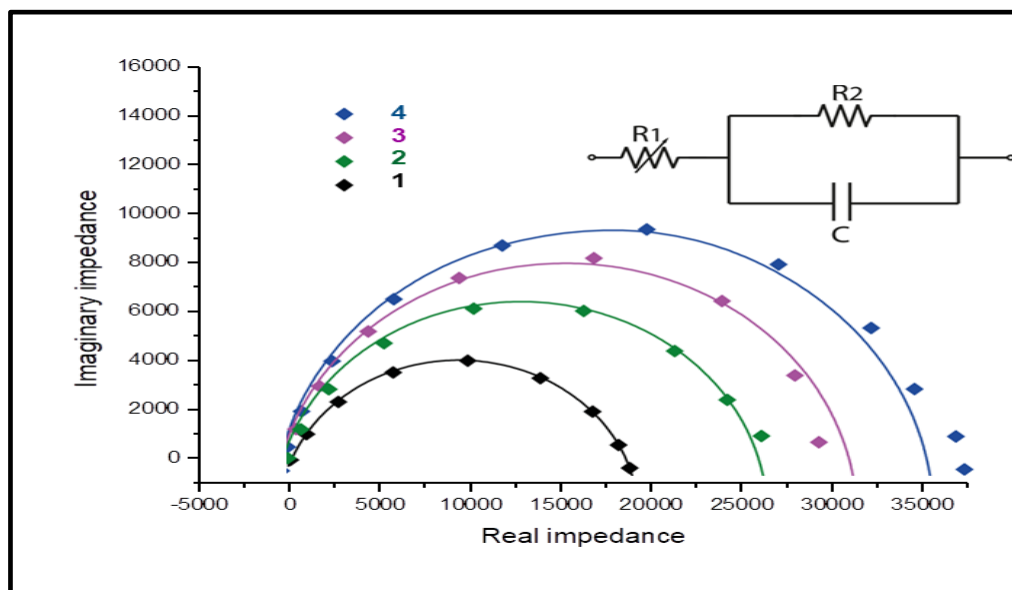


Figure 3: The Nyquist plots ($-Z_{im}$ vs Z_{re}) for interdigitated electrodes with Methanobactin+AgNPs suspension treated with Cu^{2+} ions in different concentrations (A); equivalent circuit (B).

The analysis of impedance spectra using an equivalent circuit model (shown as inset in Figure 2) was carried out according to the simplified circuit model impedance when the diffusion impedance Z_{diff} is neglected [12]. Study of the conductivity effect of CuCl_2 on optical and electrochemical properties of Methanobactin+AgNPs in suspension from proved the principle of CuCl_2 detection, from a practical point of view, the use of Methanobactin+AgNPs on the surface electrodes is much more promising for such sensor development [13-16]. A decrease in impedance was observed with increasing Cu^{2+} concentration, as evidenced by the decreasing height and diameter of the semi-circular Nyquist traces, which correspond to decreased capacitance and resistance of the sensor surface respectively [17-20]. R_s (solution resistance), R_{dl} (double layer resistance) are presented in Table 1. Good stability of the sensor was observed as the solution resistance was nearly constant at all stages of biosensor construction and testing; while R_{dl} increase due to treat the Methanobactin+AgNPs with Cu^{2+} ions.

Table 1: Values for the EIS parameters obtained from fitting the Nyquist plots shown in Figure 3 according to the equivalent circuit model.

Analyte suspension	R_s (Ω)	R_{dl} (Ω)
Bare	50.529	143.75
1 nM Mixed	48.726	125.82
10 nM Mixed	46.038	105.14
100 nM Mixed	45.414	83.666

Conclusions

In this research, we developed a sensitive electrochemical sensor based on Methanobactin+AgNPs nanostructure for detecting copper (II) ions in drink water bottles which are available in different sources of local market by utilizing a Methanobactin+AgNPs framework modified screen printed gold electrode (SPGE). The Methanobactin+AgNPs nanostructure coated SPGE was created by drop-casting a Methanobactin+AgNPs suspension with drink water sample onto SPGE surface. In the improved conditions, the Methanobactin+AgNPs framework modified screen printed gold electrode (SPGE) demonstrated remarkable effectiveness in identifying Cu^{2+} in water-based Methanobactin+AgNPs solutions.

Authors' contributions

HF, participated in the design of the study. The samples collection and interpretation were done by WM and HF. The manuscript preparation was conducted by all participated in the data editing. The authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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