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Proceeding Paper

Cancer Biomarker Methylmalonic Acid Detection by Molecularly Imprinted Polyaniline Paper Sensor †

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Abstract: Methylmalonic acid (MMA) plays a vital role in metabolism and energy production. It has been studied and reported as a sensitive early indicator for mild or serious Vitamin B12 deficiency. The normal range in health people is from 0.00 to 0.40 μM. Thus, most of MMA detection research was focused on Vitamin B12 deficiency with a small detection range. Recently, MMA has been reported to promote tumor progression due to age-induced accumulation. It was found that MMA concentration can reach as high as 80 μM in elderly people. MMA can be of great value as a promising biomarker for cancer diagnostics, as well as a therapeutic target for cancer treatment. Clinical determination of MMA concentration is by the method of gas chromatography mass spectroscopy (GCMS) or liquid chromatography mass spectroscopy (LCMS). However, these methods require extensive sample pre-treatment and large sample volume. They are also expensive and time-consuming. Hence, we proposed an attractive and effective strategy to detect MMA with a broad linear range by a low-cost molecularly imprinted polyaniline paper sensor. The polyaniline paper strip was fabricated by a one-step solution process using MMA as the template by molecular imprinting technology. The concentration of MMA was determined by the resistance change of the paper sensor. A calibration curve as a function of MMA concentration in aqueous solution was acquired with a correlation coefficient of 0.962. We demonstrated detection of the added MMA in plasma with a wide concentration range of 0 to 100 μM with a limit of detection (LOD) of 0.197 μM. This low-cost disposable paper sensor shows great potential in point-of-care MMA detection for cancer prognostics and diagnostics, especially in underserved communities.

Keywords: methylmalonic acid detection; cancer biomarker; molecular imprinting; polyaniline; paper sensor

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1. Introduction

Methylmalonic acid (MMA) plays a vital role in metabolism and energy production. It has been studied and reported that the concentration of MMA increases in blood and urine if adequate amount of vitamin B12 is not available in the body [1,2]. Therefore, MMA is viewed as a specific functional metabolic marker, which is a sensitive early indicator for mild or serious vitamin B12 deficiency. The normal range in health people is from 0.00 to 0.40 μM [3]. Hence, most research in the field of MMA detection were focused on Vitamin B12 deficiency with a small dynamic range. Recently, MMA has been reported to promote tumor progression due to age-induced accumulation [4]. It was found that MMA concentration can reach as high as 80 μM in elderly people. MMA can be of great value as a promising biomarker for cancer diagnostics, as well as a therapeutic target for

cancer treatment. Thus, it is quite urgent to investigate detection of MMA with a wide detection range, especially at high concentration conditions.

Many methods have been developed to detect MMA. Clinical determination of MMA concentration is by the method of gas chromatography mass spectroscopy (GCMS) or liquid chromatography mass spectroscopy (LCMS) [5,6]. However, these methods require extensive sample pre-treatment and large sample volume. They are also expensive and time-consuming. Electrochemical sensing has been reported as one of the reliable methods for determination of biomolecules [7,8]. However, this approach is limited by several issues. One of the major issues during sensing the biomolecules is that they tend to undergo oxidation/reduction at the closed potentials of the conventional electrodes. What's more, the electrodes have fouling effect which negatively affects the selectivity and reproducibility. These kinds of shortcomings can be improved by modifying the electrodes that play a vital role in the effective detection of biomolecules [9].

Molecular imprinting (MIP) is a promising process that works by the co-polymerization of functional monomers and cross-linking agents in the presence of a template molecule or its derivative [10,11]. The MIP approach has shown great advantages including simple preparation, potential reusability, and long-term stability [12]. MIP-based methods have been demonstrated as promising approaches to various chemical/bio sensing applications [13–17]. PdAu-polypyrrole tailored carbon fiber paper electrode has been reported to detect MMA, but it is limited by a narrow dynamic range, only from 4.01 pM-52.5 nM [18]. Molecularly imprinted polymer modified with graphene oxide and gold nanoparticles was also reported to detect MMA with a small linear range (<4 μM) [19]. However, these sensors have narrow detection ranges. What's more, they used gold-modified electrodes which will drastically increase the cost for each sensor.

Hence, we proposed an attractive and effective strategy to detect MMA with a broad linear range by a low-cost MIP polyaniline (PANI) paper sensor. The concentration of MMA was determined by the resistance change of the paper sensor. A calibration curve as a function of MMA concentration in aqueous solution was acquired with a correlation coefficient of 0.962. We also demonstrated detection of the added MMA in plasma with a wide concentration range of 0 to 100 μM. This low-cost disposable paper sensor shows great potential in point-of-care MMA detection for cancer prognostics and diagnostics.

2. Methods

2.1. Materials

MMA powder (99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Aniline (ACS reagent) and ammonium persulfate (APS, ACS reagent) were also purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid (HCl, 36–38% w/v) and acetic acid (ACS reagent) were purchased from Macron (Center Valley, PA, USA). Polyester paper substrates were obtained from Xerox (Norwalk, CT, USA). Silver conductive ink was purchased from Creative Materials (Ayer, MA, USA). Human plasma derived from whole blood donations was purchased from BioChemed Services (Winchester, VA, USA).

2.2. Synthesis of MMA Imprinted PANI on Paper Substrates

The synthesis process using MMA as the template was modified based on the fabrication process of molecularly imprinted polyaniline (MIP-PANI) paper sensor reported previously [20,21]. As control, the non-molecularly imprinted (NIP) PANI paper strips were synthesized following the same protocol without the addition of MMA template into the monomer solution.

2.3. Fabrication of PANI Paper Sensor

The as-prepared PANI paper strip was first attached to a piece of stencil with the size of 1 cm × 3 cm using a double-sided tape. Copper tape were cut into pieces with the size of 1 cm × 0.635 cm and then placed on both sides of the PANI paper strip on the stencil as

the electrodes, leaving a 1 mm gap between the PANI paper strip and each copper electrode. The PANI paper strip and the copper electrodes were physically connected by the conductive silver ink. The whole device was then kept at RT for at least 12 h to allow the silver ink to dry and ensure good conductivity between the paper strip and the electrodes. The NIP-PANI paper sensors were fabricated following the same procedures above as control.

2.4. Measurement of MMA Concentration

Aqueous solutions with various MMA concentrations were prepared with DI water. The stock solution was prepared by dissolving 200 μM MMA in human plasma solution. Sample solutions with a series of concentrations of 0, 20, 40, 60, 80 and 100 μM were prepared by diluting the MMA stock solution by human plasma. 10 μL of these sample solutions were dispensed on the surface of the MMA-MIP-PANI paper sensors. The direct current resistance (R) of the paper sensor was measured by a multimeter (8846A, Fluke, Everett, WA, USA) before and 30 min after sample dispensing. The resistance change of the NIP-PANI paper sensor was also recorded for each MMA concentration as control.

The MMA concentration is determined by the resistivity (ρ) change of the paper sensor before and after exposure to the MMA sample solution. As we know, the ρ of the paper sensor is related to R and can be described by the Equation (1),

$$\rho = R \cdot \frac{A}{l} \quad (1)$$

where A is the cross-sectional area and l is the length of the PANI paper strip between the electrodes. Since A and l remain the same for each paper sensor before and after sample dispensing, the R is positively proportional to the ρ of the paper sensor. As a result, the resistivity change of the paper sensor is positively related to the resistance change of the paper sensor. The resistance change ratio (ΔR) after and before the sample exposure is normalized based on the MMA concentration at 0 mg/L by the following Equation (2).

$$\Delta R = \left(\frac{\rho_{after,\ sample}}{\rho_{before,\ sample}}\right) \Big/ \left(\frac{\rho_{after,\ DI\ water}}{\rho_{before,\ DI\ water}}\right) \quad (2)$$

2.5. Data Analysis

The limit of detection (LoD) of the MMA-MIP-PANI paper sensor is evaluated by the following method. First, the limit of blank (*LoB*) is determined by the equation [22],

$$LoB = \mu_b + \sigma_b \quad (3)$$

where μ_b and σ_b are the mean value and the standard deviation of blank samples, respectively. Then, the LOD is calculated by the equation [22],

$$LoD = LoB + \sigma_s \quad (4)$$

where σ_s is the standard deviation of the sample with a low concentration. By plugging in the mean value and standard deviation from the calibration curve into Equations (3) and (4), the LOD of the MMA-MIP-PANI paper sensor can be estimated.

Each paper sensor was dispensed with the sample solution and the impedance change of the sensor was recorded. Three repeating measurements (n = 3) on different paper sensors were conducted on each glucose concentration. Data analysis was conducted by the Origin software.

3. Results and Discussion

3.1. Resistance

Before measurement, the resistance of the as-prepared MMA-MIP-PANI paper sensors were evaluated. Figure 1 shows the measured resistance of each paper sensor normalized to the average value. This result shows that the resistance of the MMA-MIP-PANI paper sensor was stable with small variations.

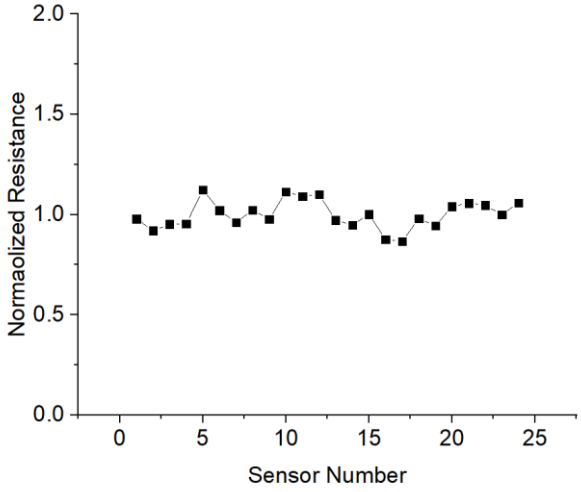

Figure 1. Normalized resistance distribution of the MMA-MIP-PANI paper sensors.

3.2. MMA Detection in Aqueous Solution

As seen in Figure 2, the resistance of the MMA-MIP-PANI paper sensor increases with the concentration of MMA, while the resistance of the NIP-PANI paper sensor kept almost constant. According to the linear fitting by the Origin software, a calibration curve as a function of MMA concentration in DI water was acquired with a correlation coefficient of 0.962. The resistance of the NIP-PANI paper sensor

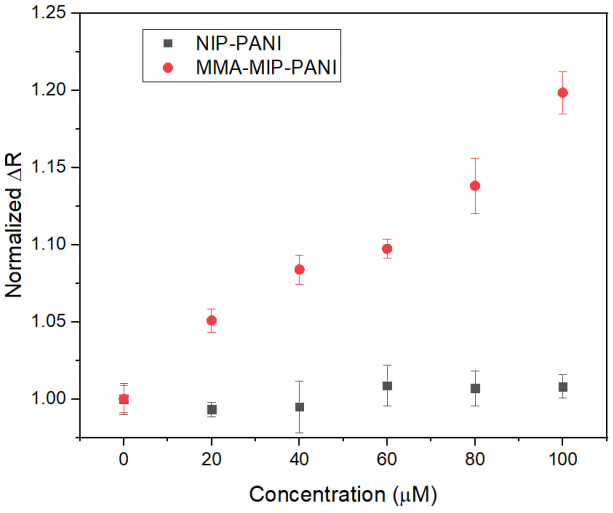

Figure 2. Normalized resistance ratio changes with the added MMA concentrations in DI water. Each point with error bar represents the average of three identical measurements with its standard deviation.

3.3. MMA Detection in Plasma

Figure 3 shows that the normalized resistance ratio of MMA-MIP-PANI paper sensor increases as the concentration of MMA increases. According to the linear fitting results, a

calibration curve as a function of MMA concentration in plasma was obtained with a correlation coefficient of 0.987. The standard deviation of normalized resistance ratio at 0 μM was 0.0065. The mean normalized resistance ratio at 20 μM was 1.0453 with standard deviation of 0.0066. Thus, the LOD is also estimated to be 0.197 μM by the equations mentioned in the methods part. This result demonstrated the detection of the added MMA in human plasma with a wide linear range from 0 to 100 μM.

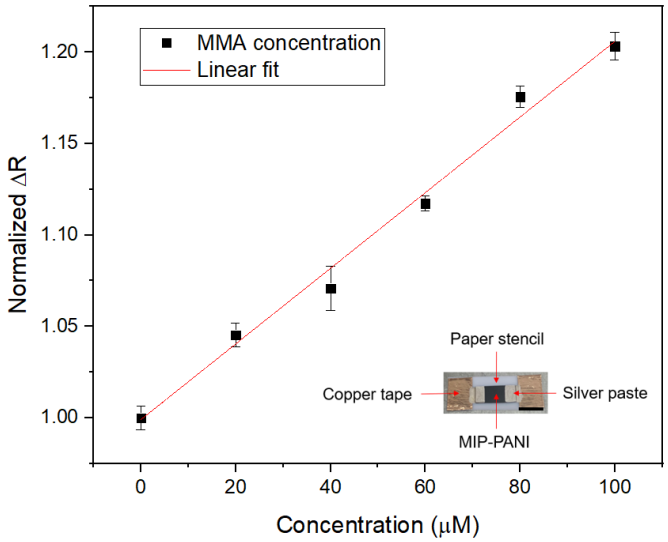

Figure 3. Normalized resistance ratio changes with the increased MMA concentration in plasma. Each point with error bar represents the average of three identical measurements with its standard deviation**.** Insert: a photograph of the MMA MIP-PANI paper sensor. Scale bar: 5 mm.

4. Conclusions

In summary, we proposed a simple and low-cost method for detecting MMA with a broad linear range by polyaniline paper sensor. The polyaniline paper strip was fabricated by a one-step solution process using MMA as the template by molecular imprinting technology. The concentration of MMA was determined by the resistance change of the paper sensor. A calibration curve as a function of MMA concentration in aqueous solution was acquired with a correlation coefficient of 0.962. We have demonstrated detection of the added MMA in plasma with a wide concentration range of 0 to 100 μM with a LOD of 0.197 μM. This disposable paper sensor is a promising alternative for MMA detection in cancer prognostics and diagnostics, especially for underserved communities.

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