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Preparation of a Novel Electrochemical Sensor for Isoniazid Determination

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This article introduces a new type of method for the content determination of isoniazid (INH) established on the principle of electrochemistry. Polyoxomolybdate, as the electroactive substance, was fixed on the surface of a glassy carbon electrode (GCE) with Nafion solution, so that the electroactive substance was tightly combined with the electrode surface and the electron transfer was enhanced. Based on this technique, a novel type of highly sensitive electrochemical sensor was established. The properties of the electrochemical sensor were characterized by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The results showed that polyoxomolybdate could contribute to electron transfer on the sensor and had a strong electrocatalytic activity for INH, which greatly improved the sensitivity of the sensor. Under optimized conditions, this method had a good linear range for INH (0.1 μ g/L to 0.3 μ g/L, R² = 0.9979), and the detection limit (LOD) was 0.024 μ g/L. The sensor showed excellent performance in terms of repeatability and lifetime. In addition, the stable crystal structure of polyoxomolybdate and the participation of Nafion solution contributed to the stability and reproducibility of the electrochemical behaviour. This method has been used for the successful determination of INH.

Keywords: Polyoxomolybdate; Electrochemical sensor; Detection method; Isoniazid

1. INTRODUCTION

Isoniazid (INH), since its clinical introduction in 1952, has achieved great efficiency in the treatment of tuberculosis. According to the 2019 World Health Organization report, approximately onequarter of the world's population are infected with Mycobacterium tuberculosis worldwide, so tuberculosis remains a serious global public health problem [1-3]. INH has high bactericidal activity against Mycobacterium tuberculosis, mainly by inhibiting the synthesis of mycobacterial acid, which in turn disturbs the construction of the cell wall, so INH is an indispensable main drug in the clinical treatment of tuberculosis [4,5]. Consequently, it is necessary to set up a fast, reliable and acute analytical method for the detection of INH to provide technical support for the quality control of pharmaceutical preparations.

A number of INH detection methods have been reported, including titration [6], capillary electrophoresis[7-9], performance liquid chromatography [10,11], liquid chromatography-mass spectrometry [12,13] and spectrophotometry [14,15]. Although these methods are highly sensitive and provide reliable analysis, the requirements for expensive instruments, skilled operators, and large time and cost-consumption, have limited their expanded use. For these reasons, researchers are working hard to develop simple, efficient and green INH detection technologies. Electrochemical technology has been widely used in pharmaceutical preparations in recent years due to its cost-effectiveness, uncomplicated operation, fast response and sensitive detection [16-18]. Nevertheless, the sensitivity of the bare electrode is low, which makes scientists searching for different electroactive substances to improve the performance of the sensor [19,20]. In addition, the sensitivity of the electrochemical sensor mainly depends on the unique properties of the modified materials. Therefore, the synthesis and selection of novel modified materials is a key step in the development of economical and stable electrochemical sensors. Researchers have developed many electrochemical methods to detect the INH content. It has been reported that B/N-doped mesopores can enhance the conductivity of electrodes. Based on this discovery, Balasubramanian et al. used a B/N-doped mesopores modified electrode to successfully determine the INH content of human serum and urine samples [21]. To determine the INH content in pharmaceutical preparations, scientists headed by Pankaj Kumar Rastog et al. used silver nanoparticledecorated copolymers to promote the oxidation of INH and improve its electrochemical behaviour [22]. In addition, MXene-modified screen-printed electrodes can also improve electrocatalytic activity [23]. Although the above methods offer higher sensitivity, the assembly processes of these sensors are relatively complicated, which presents a great challenges for the stability of the electrode. Hence, there is an urgent need to construct a novel sensor, that offers higher sensitivity, while maintains electrode stability.

Polyoxometalates (POMs) are a class of molecular components with high redox activity, and these components are anionic metal polymers based on high-value transition metals. In 1826, Berzerius successfully developed the first Keggin-type polyoxomolybdate ((NH₄)₃PMo₁₂O₄₀•nH₂O), the famous Keggin-type structure was first proposed by the British physicist J.F. Keggin in 1933. Their characteristics of accepting a large number of electrons made these components attractive in the preparation of modified electrodes and electrocatalysis [24-27]. In the Keggin-type system, the metal ions can directly interact through electron transfer and coordination with the bridge group and the end group ligand, demonstrating unique physical and chemical properties that allow them to be applied in catalysis, optoelectronic functional materials and medicine [28-30]. Keggin-type silicomolybdic acid could also be used as a nanomaterial because of its small particle size and good conductive effect, offering considerable potential in electrochemical sensors. In previous studies, it has been reported that a Keggin-type polyoxomolybdate anion was used to prepare a current-type glucose sensor by electrochemical polymerization. Moreover, researchers explored the modification of silicomolybdic acid to the traditional Pt/C catalyst, and found that silicomolybdic acid could improve the catalytic performance of Pt/C [31,32]. At the same time, we found that {SiMo₁₂} had electrochemical activity, which aroused our attention.

A novel concept of dipping the Keggin-type silicomolybdate acid-Nafion solution in layers into the surface of the GCE was adopted in this study. The Nafion film was not only conducive to the transmission of electrons, but could also fix the electroactive substances, thus successfully developing a simple assembly and high sensitivity electrochemical sensor and applying it for INH determination in standard solutions and tablets. The sensor could greatly promote the electrochemical reaction of INH

standard solutions and tablets. The sensor could greatly promote the electrochemical reaction of INH under acidic conditions (pH=1.0 HCl). The results showed that the current response value of the sensor was significantly improved, which might be related to the proton conductivity of polyoxomolybdate. In addition, as far as we know, this was the first application of polyoxomolybdate with a Nafion solution in INH electrochemical sensors. Compared with the pharmacopoeial method, the sensor equipment was inexpensive, easy to operate, accurate in result, and offered good stability, reproducibility, sensitivity, lifetime and low detection limit. This was due to the stable crystal structure of polyoxomolybdate and the fixing effect of the polymer film formed by Nafion solution, which also provided novel ideas for the rapid analysis of other similar drugs.

2. EXPERIMENTAL PROCEDURE

2.1 Materials

2.2.1 Reagents

We referred to existing methods in the literature and used the rapid evaporation method to obtain mentioned polyoxomolybdate. The chemical formula could be the expressed as [Himi]_v[H₃0]_x[SiMo₁₂O₄₀]•nH₂O, and it was called *HHSMOH* [33]. INH active pharmaceutical ingredients (purity \geq 99%) and INH tablets were provided by Meilun Biotechnology and Shanxi Yunpeng Pharmaceutical, respectively. HCl, H₂SO₄, HClO₄, HNO₃ and H₃PO₄ were purchased from Jinzhou City Chemical Reagent Co., Ltd., Dimethylformamide (DMF) (purity \geq 99.5%) was obtained from Tianjin Guangfu Technology (China). Nafion PFSA Polymer solution (5 wt%) was supported by the DuPont Company. All solutions were made with deionized water.

2.1.2 Instruments

In this paper, Fourier transform infrared spectroscopy (FTIR-920) was used to reveal characteristic bands corresponding to polyoxomolybdate (the scanning range was 4000 to 500 cm⁻¹, Shantou Keyi Instrument Equipment). X-ray photoelectron spectroscopy (XPS) and UV-vis were obtained from Thermo Fisher K-Alpha in the United States, and a Mipuda UV-1600 spectrophotometer was bought in Shanghai, China. In addition, a CHI 650D electrochemical workstation was used to quantify the INH based on CV and DPV (Shanghai Chenhua Co., Ltd.). The experiment used a traditional three-electrode system: modified GCE as the working electrode, platinum wire electrode as the auxiliary electrode and saturated calomel electrode (SCE) as the reference electrode. Atomic force microscopy (AFM) images and particle size distributions were collected from an AFM-6800 (Suzhou

Feishiman Precision Instruments) and BT-90 nanometer laser particle size analyser (Dandong Baxter Instrument Co., Ltd.).

2.2 Experimental method

2.2.1 Fabrication of the sensor

In previous studies, a large number of chemical methods have been used to prepare sensors [34,35]. In this paper, we introduced a dipping method to construct the sensor. Before the experiment, the GCE was polished in an orderly manner with 0.3 μ m and 0.05 μ m Al₂O₃ powder. To keep the electrode surface in a tidy and clean state, the polished electrode surface was ultrasonically cleaned with distilled water and absolute ethyl alcohol in sequence, and the cleaned electrode was dried with nitrogen before use. A certain amount of *HHSMOH* and the appropriate DMF solution were mixed together, and the mixture was called M1. Afterwards, M1 and 0.1% Nafion solutions were adhered to the GCE, and then the electrode was stored in a room environment for approximately 12 hours. Finally, the above dry electrode surface was briefly contacted with 0.1% Nafion solution and dried for approximately 10 minutes at room temperature.

2.2.2 Sample preparation

Before operating the experiment, 0.1% Nafion solution and different concentrations of acid solutions were obtained from the laboratory. To obtain the stable positive volt-ampere curve characteristics in 0.1 M HCl, a CV scan range was between 0 V and 0.4 V with negative scanning (scan rate: 100 mV/s). Quantitative drug analysis was performed using DPV in 0.1 M HCl (scan rate: 100 mV/s). All operational processes were carried out in a room-temperature environment.

3. RESULTS AND DISCUSSION

3.1 Characterization

3.1.1 FT-IR spectrum of HHSMOH

To identify the composition of *HHSMOH*, FT-IR was carried out. FT-IR spectra of the product clearly showed the characteristic absorption peaks, and they were compared with the positions reported in another paper (as shown in Table 1) [33,36,37]. As shown in Fig. 1, the absorption peak of the deformation vibration of Si-O was 1092 cm⁻¹ as measured by the experiment. The experimental measurements showed that the asymmetric vibration and symmetric vibration of Mo=O_t were 955 cm⁻¹ and 904 cm⁻¹, respectively, and the absorption peak positions of the symmetry and asymmetry vibration of Mo=O_{µ/v} were 793 cm⁻¹ and 863 cm⁻¹, respectively. These basic data were consistent with those in the literature, and the results data were basically consistent with the literature report. As a result, we believed that the substance synthesized in the laboratory was *HHSMOH*.



Figure 1. Infrared (IR) spectra of HHSMOH

Table 1. Comparison of characteristic absorption peak positions in HHSMOH and literature spectra

Characteristic group	Absorption peak positions in the literature/(cm ⁻¹)	Absorption peak positions in the experiment/(cm ⁻¹)	
δ(Si-O)	1092	1092	
v _s (Mo=O _t)	904	904	
$v_{as}(Mo=O_t)$	953	955	
$v_{s}(Mo=O_{\mu/\nu})$	862	863	
vas(Mo=O _{µ/v})	791	793	

3.1.2 UV-vis spectra of HHSMOH

HHSMOH was further verified by UV-vis spectroscopy. Fig. 2 (A) shows the UV-vis spectroscopy of *HHSMOH* in aqueous solutions at various concentrations. Fig. 2 (B) shows the monitoring of *HHSMOH* in various concentrations of HCl solution using UV-vis spectra. As shown in Fig. 2, as the concentration and acidity increased, the absorbance value of *HHSMOH* increased almost linearly. However, the absorption band had hardly changed, and it was consistent with the absorption band position of the reference, which demonstrated that *HHSMOH* was not affected by concentration and acidity. In other words, *HHSMOH* exhibited good stability [38].



Figure 2. (A) UV-vis spectra of the *HHSMOH* at different concentrations (from a to h:10, 20, 30, 40, 50, 60, 70 and 80 μg/mL). (B) UV-vis spectra of the *HHSMOH* at different acidities (from a to g: pH=1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0).

3.1.3 XPS spectrum of HHSMOH

XPS measurements were employed to determine the basic elements of the *HHSMOH*. In Fig. 3, although the XPS results depicted only the semi-quantitative fundamental composition, they still prove the subsistence of O, C, N, Mo and Si. We found that the peak intensities of Mo (3d_{5/2}) and Mo (3d_{3/2}) were 232.8 eV and 235.9 eV, respectively [39,40]. Moreover, the peak intensity of Si (2p) was 101.8 eV [41]. From the above data, we believed that the chemical composition of *HHSMOH* was not affected by other elements and was consistent with the electron energy spectrum of the central atoms in the Keggin-type polyoxomolybdate in the literature.



Figure 3. (A) XPS survey spectrum in the Si (2p) regions for *HHSMOH*. (B) XPS survey spectrum in the Mo (3d) regions for *HHSMOH*.

3.1.4 AFM images and particle size distribution of HHSMOH

To research the morphology and particle size of *HHSMOH*, we used AFM and Nano laser particle size distribution analyser to detect *HHSMOH*. Studies have noted that $PMo_{12}O_{40}$ nanoparticles are almost spherical in shape and monodisperse, with an average diameter of approximately 20-30 nm [42]. In this study, Fig. 4 is an AFM image of *HHSMOH*, which shows the small particle size and uniform distribution of *HHSMOH*. Fig. 5 shows a statistical diagram of the particle size distribution of *HHSMOH*. Fig. 5 shows a statistical diagram of the particle size was less than 20 nm, and this result was consistent with the AFM micrograph. The literature and experimental data indicated that Keggin-type polyoxomolybdate is a nanomaterial with a small particle size and uniform distribution, that can be used as an ideal electrode modification material and in the construction of electrochemical sensors.



Figure 4. Tapping mode AFM images and three-dimensional view of HHSMOH



Figure 5. The size distribution of *HHSMOH*

3.1.5 Preparation process of the sensor

CV was used to evaluate the electrocatalytic activity of the synthesized polyoxometalate for INH oxidation. INH does not produce a large oxidation current on the bare GCE, while on the Keggin-type polyoxomolybdate-modified electrode, an obvious current response can be observed, which indicated that the polyoxometalate-modified electrode had stronger electrocatalytic activity. Scheme 1 shows the assembly process of the modified electrode. At the same time, the electrocatalytic oxidation process of INH on the polyoxometalate-modified electrode was predicted [21,43].



Scheme 1. Schematic representation for fabricating the sensor and reaction of INH at the sensor

3.2 Electrode properties

3.2.1 Electrochemical behaviours of different modified electrodes

The electrochemical characteristics of different electrodes were investigated by CV. GCEs were evaluated by CV in 0.1 M HCl solution in the scan range of 0 V to 0.4 V (scan rate: 100 mV/s). It can be clearly seen that in the potential range of 0-400 mV, two reversible redox peaks appear, which was consistent with reference [44]. As shown in Fig. 6, the bare electrode (a) showed almost no peak current. Fig. 6 b shows only a slight peak current value gained from the GCE@Nafion@HHSMOH electrode (b), while curve (c) revealed that the peak current of the GCE@Nafion@HHSMOH@Nafion modified electrode increased significantly compared to the other two GCEs. In conclusion, it could be concluded that in 0.1 M HCl solution, the electrode modified by HHSMOH could significantly improve the performance of the electrode, and the addition of Nafion film on the sensor surface could keep the modified electrode stable in structure. In other words, HHSMOH did not detach from the electrode, and it was profitable to the research process. Namely, the modified electrode provided faster electron transfer

in a 0.1 M HCl solution. The results showed that the electron transfer rate on the electrode surface was improved when *HHSMOH* was applied as a modifier.



Figure 6. CVs of the bare electrode (a), the GCE@Nafion@*HHSMOH* electrode (b), and the GCE@Nafion@*HHSMOH*@Nafion modified electrode (c) in HCl (pH=1.0). Scan rate: 100 mV/s.

3.2.2 Effects of electrolyte

Electrolytes could affect the electron transfer speed and stability of the sensor. In an effort to compare the effects of different electrolytes in this experiment, the sensor was sited in H_2SO_4 , HCl, HClO₄, HNO₃ and H_3PO_4 at pH=1.0 and measured by CV on the electrochemical workstation (scanning rate was 100 mV/s). In Fig. 7, compared with other curves, curve (b) and curve (d) had two pairs of redox peaks, and the peaks were symmetrical. However, it was proven that the same electrode was more stable in HCl solution than in H_2SO_4 solution. Some scientists have also studied the influence of electrolytes on Keggin-type polyoxomolybdate, and their reports indicated that the response value current of Keggin-type polyoxomolybdate in HCl solution was relatively higher, which is consistent with our experimental data [45]. We speculated that this might be because the HCl solution was more beneficial to the electron transfer of the sensor. These results revealed that the mentioned sensor had great sensitivity and stability in HCl, and the current response value was also relatively high. Therefore, HCl was chosen as the most suitable electrolyte.



Figure 7. CVs of the sensor in different acids at pH=1.0 (from a to e: HNO₃, HCl, H₃PO₄, H₂SO₄ and HClO₄). Scan rate: 100 mV/s.

3.2.3 Effects of pH value

The pH value of the electrolytes also has a certain influence on the polyoxomolybdate compound, which may lead to a decrease in the performance of the sensor, so it is necessary to investigate the influence of the pH value on the polyoxomolybdate compound [46]. Hence, we investigated the effect of HCl with different pH values on the sensor. As shown in Fig. 8, when the electrolyte pH was 1.0, the peak current value was relatively maximum, but with the increase in the pH value of the electrolyte, the current response value of the sensor decreased gradually. This might be because the polyoxomolybdate more easily transferred electrons in highly acidic electrolytes. Finally, we chose pH=1.0 as the optimal reaction condition.



Figure 8. CVs of the GCE@Nafion@*HHSMOH*@Nafion modified electrode in HCl solution at various pH values (from a to g: 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0). Scan rate: 100 mV/s.

3.2.4 Effects of scan rate

To further acquire the reaction dynamics and the control step on the surface of the sensor, the CV of the sensor to a 0.1 M HCl solution under diverse scan rates from 50 to 300 mV/s was studied, as evidenced in Fig. 9 A. Fig. 9 B shows a linear dependence relation between the current response value (I_{pc}) and the scan rate (v), and the equation was acquired with a good correlation coefficient as follows: $I_{pc} = -0.3328 \text{ v} -80.762 \text{ (R}^2 = 0.998)$. Meanwhile, the linear dependence relation between the current response value (I_{pc}) and the square root of scan rate (v^{1/2}) was discussed, as shown in Fig. 9 C. The equation was designated $I_{pc} = -8.2040 \text{ v}^{1/2} -33.763 \text{ (R}^2 = 0.994)$. The slope value was 8.20, which denoted a scattering controlled process on the surface of the sensor. Another way to study the linear equations from the log I_{pc} versus log v is as follows: log $I_{pc} = -0.35 \log \text{ v} -1.36 (\text{R}^2 = 0.984)$, as shown in Fig. 9 D, which demonstrated that the proposed sensor surface was controlled by diffusion.



Figure 9. (A) Various scan rates (from a to i: 50, 80, 100, 130, 160, 200, 230, 260 and 300 mV/s) of the GCE@Nafion@*HHSMOH*@Nafion electrode in 0.1 M HCl solution. (B) Peak currents (I_{pc}) versus scan rates. (C) The plot of $v^{1/2}$ versus I_{pc} . (D) The plot of log v versus I_{pc} .

3.2.5 Standard curve and LOD

Through the optimization of the conditions of the sensor, we determined the best experimental conditions for the performance of the sensor. Under optimized conditions, we made a standard curve of INH and studied the connection between the I_{pc} and concentration of INH by the DPV method. Fig. 10 and Fig. 11 show that there was a good linear dependence relation between the concentration of INH (0.1 µg/L to 0.3 µg/L) and the peak current. The linear equation of peak currents (I) and INH concentrations can be expressed as $I_{pc} = 39.22 \text{ c} + 39.12 \times 10^{-6} (R^2 = 0.9979)$. The LOD could reflect

whether an analytical method has sufficient detection sensitivity. The LOD was the minimum concentration or minimum amount of the substance to be detected from the sample within a certain range. The results showed that the detection limit was 0.024 μ g/L (S/N=3).



Figure 10. DPV responses of different concentrations of the INH in the range of 0 V to 0.4 V at a scan rate of 100 mV/s (from a to e: 0.1, 0.15, 0.2, 0.25 and 0.3 μg/L).



Figure 11. The plot of I_{pc} versus concentrations of INH

Table 2 shows a comparison of several common INH detection methods with this experiment. There is no doubt that the polyoxomolybdate-modified electrode sensor is comparable to highly sensitive high-performance liquid chromatography and UV spectrophotometry. However, the method established in this experiment is simple to operate. Compared with capillary electrophoresis and other electrochemical detection methods, the use of polyoxomolybdate-modified electrodes had a lower detection limit. This may become an ideal alternative to INH detection. Numerous studies shown that polyoxometalates have many excellent properties, such as chemical stability and thermal stability.

Among them, the reversible multi-electron transfer property has been applied to the preparation of sensors in electrocatalytic activity. In this experiment, the strong electrocatalytic activity of keggin-type polyoxometalate on INH was used to detect the content of INH [47,48].

Technique	Detection range	LOD	Reference	
UV spectrophotometry	5 to 25 μg/mL	5 µg/mL	[49]	
High-performance liquid chromatography	0.1 to 20 $\mu g/mL$	0.023 µg/mL	[50]	
RP-LC	18.75 to 150 µg/mL	3.5 μg/mL	[51]	
Capillary electrophoresis	0.7 to 30 $\mu\text{g/mL}$	0.3 µg/mL	[52]	
Polyoxomolybdate modified electrode	0.1 to 0.3 $\mu g/L$	0.024 µg/L	This work	
Rhodium modified electrode	70 to 1300 µM	13 µM	[53]	
Ordered mesoporous carbon modified electrodes	0.1 to 370 μM	84 nM	[54]	
Carbon paste electrode	0.5 to 40 µM	0.17 μM	[55]	
Metal-organic framework compound- derived	etal-organic vork compound- 0.8 to 800 μM derived		[56]	
NiO@ZnO/GCE MWCNT–chitosan modified electrodes	0.1 to 10 µM	55 nM	[57]	

Table 2 Contrast of various detection techniques of INH

3.2.6 Repeatability, stability and lifetime

Table 3 Repeatability of the Keggin-type silicomolybdic acid-modified electrode (n=5)

C/(g/L)	Current response average/µA	SD/µA	RSD/%
1.0×10^{-7}	39.69	0.61	1.54
1.5×10^{-7}	40.60	1.03	2.50
2.0×10^{-7}	43.75	0.83	1.90
2.5×10^{-7}	46.31	0.53	1.15
3.0×10 ⁻⁷	50.95	0.14	0.28

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Repeatability is an essential factor to estimate electrochemical sensors. Under the best conditions, INH was tested five times with DPV. The modified electrode was tested in INH solution with variations of different concentrations (from 0.1 μ g/L to 0.3 μ g/L), and the relative standard deviation (RSD) was below 5% (Table 3), indicating that the modified electrode had appropriate repeatability.

The lifetime of the modified electrode was also a factor affecting the stability of the electrode. During the experiment, the electrode response values of the electrochemical sensor became increasingly stable with an increasing number of days. However, the electrode response value decreased after 6 days, which might be related to the increased amount of polyoxomolybdate consuming and falling off the electrode with increasing scanning times.

3.2.7 Analysis of actual samples

To verify the practicability of this sensor, it was applied to the evaluation of INH tablets. To further improve the accuracy of detection, spike and recovery experiments were performed. In Table 4, the percent recoveries ranged from 97.8% and 103.3%, explaining that this method can be used to detect INH tablets.

Table 4. Determination of the recovery rate of INH tablets

Sample	Original	Added	Found	Recovery	RSD
	(µg/L)	(µg/L)	$(\mu g/L)$	(%)	(%,n=5)
1	0.10	0.10	0.18	97.8	1.62
2	0.15	0.10	0.26	103.3	1.02
3	0.20	0.10	0.30	99.0	0.17

4. CONCLUSION

In the current work, a novel type of electrochemical sensor using Keggin-type silicomolybdic acid as an electroactive substance was studied and used to analyse INH in actual samples. Under the best conditions, the proposed electrochemical sensor showed a linear INH range of 0.1 μ g/L to 0.3 μ g/L (R²=0.9979), and the LOD was 0.024 μ g/L. The sensing platform showed many advantages, such as a large specific surface area, strong catalytic performance and great stability, which provided a method reference for the rapid determination of INH. We have also considered the reasons for the improvement in the performance of the electrode, which may be mainly related to the stable crystal structure of the polyoxomolybdate and the appropriate participation of two-layer Nafion films. Polyoxomolybdate and Nafion film not only improve the stability of the sensor but also facilitate electronic transfer during the experiment. However, there are still some drawbacks compared to other methods, especially on the detection limit. Therefore, further research on how to improve the detection range of the sensor needs to be addressed in future work.

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