Research Article

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Molecularly imprinted electrospun fiber membrane for colorimetric detection of hexanoic acid

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Abstract: An imprinted electrospun fiber membrane was developed for the detection of volatile organic acids, which are key components of human body odor. In this study, hexanoic acid (HA) was selected as the target, polymethyl methacrylate (PMMA) was used as the substrate, and colorimetric detection of HA was achieved by a bromoresol purple (BCP) chromogenic agent. The results showed that the morphology of the fiber membrane was uniform and continuous, and it showed excellent selectivity and specificity to HA. Photographs of the color changes before and after fiber membrane adsorption were recorded by a camera and quantified by ImageJ software by the difference in gray value (ΔGray). This method is simple, intuitive, and low cost and has great potential for application in human odor analysis.

Keywords: molecular imprinting, electrospinning, PMMA, bromoresol purple, colorimetric detection

1 Introduction

The liquid secreted by human skin glands is odorless. Volatile organic compounds (VOCs) are produced after interacting with bacteria, such as Brevibacterium, Micrococcus luteus, Propionibacterium acne, and Staphylococcus epidermidis, on the skin surface, which is the origin of human body odor (1). The main factors causing the variance in the VOCs composition in human body odor are age, gender and genetic differences, followed by lifestyle, physical and emotional state, as well as atmospheric factors (temperature, humidity) (2–4), etc. Therefore, human body odor has received extensive attention and has been successfully applied in the following fields: biometrics, medical diagnosis, and forensic identification (5–8). Through the unremitting efforts of many scientific researchers, different types of VOCs in human body odor have been discovered, including carboxylic acids, aldehydes, alcohols, ketones, hydrocarbons, amines, sterols, and sulfur compounds (9–12). Among them, carboxylic acids are considered crucial components of body odor, for example, acetic acid, propionic acid, isovaleric acid, hexanoic acid (HA), octanoic acid (OA), and other low molecular weight acids (13,14), which are of vital significance for detection.

The most common detection methods of human body odor are gas chromatography with mass chromatography (GC/MS) (15,16), but its application is limited by factors, such as complicated technology, large equipment, and apparatus costs (17). Therefore, it is necessary to develop a more convenient and efficient method. Molecularly imprinted polymers (MIPs) are functional materials that can specifically recognize target molecules by imprinted cavities that highly match the shape and size of the target molecule (18). Owing to its easy preparation, low cost and excellent selectivity, and physical and thermal stability (19), it is frequently used in chemical sensors (20,21). Jha and Hayashi (22) developed a MIPs-coated QCM sensor array for rapid testing of the main aldehydes in human odor using polyacrylic acid (PAA) as functional monomers and three organic acids (propenoic acid, HA, and OA) as template molecules. A similar method was proposed by Liu et al. (23) based on molecularly imprinted sol-gel materials for selective detection of hexanal, nonanal, and benzaldehyde.

However, the sensing methods mentioned above still have problems, such as unintuitive results and complicated data processing (24). The optical sensing method was developed in this situation, which can detect and identify various chemical substrates through digital imaging with quantitative colorimetric or fluorescence changes (25).
Ying et al. (26) prepared molecularly imprinted hydrogels using different molecular structural analogs of putrescine as virtual templates. After the hydrogel with ninhydrin solution added encountered putrescine molecules, purple stains appeared and deepened as the putrescine molecular adsorption quantity increased, and the detection of putrescine could be visualized. Iwata et al. (27) reported that MIP micropowders incorporating the fluorescent dye quinine sulfate were coated on glass substrates and used as sensing films for the selective visualization of odorant flows.

Electrospinning is an economical, effective, and widely used technology for preparing micro/nanofiber membranes with controllable morphology. The prepared fiber membranes have a large specific surface area and high porosity (28–30). Electrospinning technology was applied to prepare molecular imprinting materials that can significantly improve the permeability and accessibility of imprinting sites of imprinting materials, which is a very attractive method and has attracted widespread study by numerous researchers (31). Dhawane et al. (32) used chitosan and polyvinyl alcohol nanofibers with immobilized cholesterol oxidase and peroxidase enzymes to establish a biosensor for colorimetric detection of cholesterol. Double-layer nanofiber membranes were synthesized (33) to identify putrescine in which one layer was used for filtration and another was used for chromogenesis with ninhydrin as a chromogenic agent.

In this study, MIP approaches were merged with electrospinning for the design of a reliable and efficient recognition system. A molecularly imprinted electrospun fiber membrane for the detection of HA was developed, and the colorimetric detection of HA was realized by adding a certain amount of BCP chromogenic agent (the entire process is shown in Figure 1). The fiber membrane is simple to prepare, inexpensive, and portable, which provides good development potential for real-time monitoring of human odor in the future.

2 Experimental

2.1 Reagents and chemicals

Poly(methyl methacrylate) (PMMA), HA, hexanol (HEL), hexanal (HAL), and bromocresol purple (BCP) were purchased from Aladdin. N,N-Dimethylformamide (DMF) and ethanol were purchased from Xilong Science Co., Ltd. Sodium dodecyl sulfate (SDS) was purchased from Macleans, OA was purchased from Tianjin Damao Chemical Reagent Factory, and sodium hydroxide (NaOH) was purchased from Guangdong Guanghua Technology Co., Ltd. All of the reagents and chemicals used were of analytical grade and without further purification.

2.2 Characterization

Electrospinning was performed on an electrospinning apparatus (WL-2C, The Beijing Ion Beam Technology Co., Ltd., Beijing, China). The morphology of the fiber membrane was viewed by scanning electron microscopy.
(SEM) (Pure+, Phenom Scientific Instrument Co., Ltd., Shanghai, China) at an accelerating voltage of 10–15 kV under a high vacuum.

2.3 Electrospinning of the fiber membrane

The polymer solution was processed as follows: first, a beaker was used to weigh different masses of PMMA on an electronic balance, then, 12 mL DMF and template molecule HA were added, and finally, the mixture was stirred at 10 rpm for 6 h at 35 °C to form a homogeneous solution.

The electrospinning procedure was carried out using electrospinning equipment, which included a syringe equipped with a needle (21G), a high voltage power supply, and a collection device. As shown in Figure 2a, some of the electrospinning solution prepared above was taken into the syringe and then placed in the slot of the electrospinning machine’s push position. The spinning process parameters were set as follows: the voltage was 15 kV, the feed rate was 0.8 mL h\(^{-1}\), the distance from needle tip to roller was 15 cm, and the drum speed was 600 rpm. For environmental parameters temperature and humidity, which were separately controlled at 25–35°C and 60–80%, the entire procedure lasted 4 h. The obtained molecularly imprinted fiber membrane (MIM) was dried in an oven for 12 h and then sealed and bagged. The nonmolecularly imprinted fiber membrane (NIM) was the same as the MIM, but no template molecules were added.

2.4 Fabrication of MIM-BCP

Before loading the chromogenic agent, we needed to prepare it first. First, 0.01 g BCP, 0.05 g NaOH, and 0.5 g SDS were mixed into a beaker, and afterward, 2 mL of ethanol and 8 mL of water were added to form a 0.1% (m/v) chromogenic agent solution.

The obtained MIM and MIN fiber membranes were cut into a size of 3 cm × 3 cm with a paper cutter, and then, they were placed in an oven for decompression elution. The oven temperature was 85°C, the vacuum was at 0.085 MPa, and the elution time was 6 h. After the elution process was complete, the MIM and NIM adhered to the petri dish, and 200 µL of color developer was collected using pipette and added dropwise on them. Then, the molecularly imprinted chromogenic fiber membrane (NIM-BCP) and a nonmolecularly imprinted chromogenic fiber membrane (NIM-BCP) were dried in an oven for further chromogenic adsorption.

2.5 Coloring procedure and photographing equipment

A photo of the petri dish with a chromogenic fiber membrane attached to it was taken, and the photographing equipment is shown in Figure 2b. At the same time, a certain amount of HA solution was added to another petri dish that included a small fixed plastic cover; then, the first petri dish was placed on top of it to form a closed environment. The dish was placed in an oven to incubate for a certain period of time. After the adsorption was complete, a camera was used to take pictures again, and then, ImageJ software was used to take the gray value of the photos before and after the color development. The difference was recorded to evaluate the adsorption.

2.6 Adsorption experiment

Dynamic adsorption experiments were studied with 20 mg mL\(^{-1}\) of HA. 40 µL of the solution was used for color development, and the incubation time ranged from 0 to 120 min with intervals of 10 min. The dynamic adsorption behavior of MIM-BCP was analyzed by pseudo first-order (PFO) (Eq. 1) and pseudo second-order (PSO) (Eq. 2) kinetic equations:

\[
Q_t = Q_e(1 - e^{-kt})
\]

\[
Q_t = \frac{Q_e^2k_t}{1 + k_2Q_e t}
\]

where \(Q_t\) is the HA adsorption capacity (µg cm\(^{-2}\)) at any time, \(Q_e\) is the equilibrium adsorption capacity of MIM-BCP (µg cm\(^{-2}\)); and \(k_1\) (min\(^{-1}\)) and \(k_2\) (cm\(^2\) µg\(^{-1}\) min\(^{-1}\)) are the PFO and PSO model rate constants, respectively.
To estimate the properties of the specificity of prepared MIM-BCP and NIM-BCP vs HA, three competitor molecules (HAL, HEL, and OA) were chosen as compounds having similar chemical structures. The imprinting efficiency (IE) of MIM-BCP is defined using Eq. 3:

\[
IE = \frac{Q_{\text{MIM-BCP}}}{Q_{\text{NIM-BCP}}}
\]

3 Results and discussion

3.1 Chromogenic principle

The chromogenic principle of BCP is shown in Figure 3. BCP exhibits a quinone structure, three substituted phenyl groups are connected to the central carbon atom, and one of the benzene rings is connected by double bonds in an alkaline environment. Its structure turns to a lactone ring structure along with increasing concentration of the acids with two benzene rings and sulfonic acid groups connected to it in the molecule. Meanwhile, the color of the solution changed from purple to yellow, as shown in Figure 4. This color change resulted from the structural change leading to the solution of the maximum absorption wavelength of the redshift from the initial 598 to 423 nm, which was roughly the same as described by Sukhanov et al. (34).

3.2 Characterization of MIM

According to Figure 5a and b, the overall morphology of MIM did not change significantly after adding template molecules compared with that of NIM, but the micropores on the MIP fibers reduced sharply, which may be explained by the template molecules successfully being embedded in the PMMA fibers. After vacuum elution, the micropores on the fiber surface were recovered again, and the fibers were still intact, as shown in Figure 5c, with only a few fibers broken, which indicates that vacuum elution can remove template molecules without destroying the general shape of the fiber. After the elution process, the chromogenic solution was added to the fiber membrane; thus, a certain amount of developer was deposited on the surface of MIM-BCP, as shown in Figure 5d, but it did not affect the pore structure of the fiber.

The FTIR spectra of NIM and MIM before elution and MIM after elution are shown in Figure A1 (in Appendix). The peaks of NIM were detected (35,36): 2,950 cm\(^{-1}\) (–CH\(_3\)), 1,725 cm\(^{-1}\) (C=O), 1,146 cm\(^{-1}\) (C–O), 1,435 cm\(^{-1}\) (–CH\(_2\)), and 1,481 cm\(^{-1}\) (–CH\(_3\)). The –CH\(_3\) stretching vibration, –CH\(_2\) stretching vibration, C=O stretching vibration, and C–O stretching vibration increased when HA was added, but there were no new functional groups, which showed that HA and PMMA were combined by noncovalent bond forces. After elution, the stretching vibration of the related group weakened, similar to that in NIM, which demonstrated successful imprinting and elution of the template molecule HA.

3.3 Influence of color development conditions on color development

3.3.1 Effect of the reaction temperature

Different temperatures were set to investigate the effect of temperature on color development. According to Figure 6, a lower temperature corresponds to a smaller ΔGray, indicating that the reaction cannot be fully carried out below 55°C. When the temperature increased to 55–65°C, the volatile HA reacted sufficiently with the chromogenic agent, and ΔGray increased and was steady. However,
when the temperature exceeded 65°C, ΔGray decreased dramatically, which suggests that the increase in temperature may aggravate the thermal motion of HA molecules. As a result, the binding of some HA molecules to the chromogenic agent was unstable, and the chromogenic effect was inferior to that of 55–65°C. If there were no special conditions, the chromogenic temperature of the follow-up experiments was 65°C.

3.3.2 Effect of elution time

The PMMA fiber membrane was not suitable for solution elution because of the weak mechanical strength; thus, vacuum elution was used to ensure that the fiber membrane structure was not considerably damaged. As shown in Figure 7, the ΔGray of the fiber membrane changed
slightly when the elution time was less than 6 h. This demonstrated that the HA molecules on the fiber membrane were only shed partially; thus, there was little adsorption of HA. Of note, ΔGray of the fiber membrane fell slowly at the beginning when the elution time exceeded 6 h; then, with further extension of the elution time, ΔGray declined rapidly and was kept at a low value, which means that as the elution time is prolonged, the produced microstructure of the fiber membrane may be destroyed (Figure A2), resulting in a decrease in ΔGray (24). Consequently, the optimal elution time was 6 h.

3.4 Coloring procedure and calculation of adsorption

To facilitate the application, the standard curve of the adsorption capacity of the HA solution relative to ΔGray was constructed. The concentration range of HA solution was 0–40 mg mL⁻¹, and the concentration gradient was 10 mg mL⁻¹. ΔGray was determined by ImageJ after full adsorption. The color change and standard curve obtained are shown in Figures 8 and 9. It illustrates that the higher the concentration of HA solution, the more obvious the color change, the greater ΔGray value, and there is a strong linear relationship between the adsorption amount of HA per unit membrane area and the change of ΔGray in this concentration range. The standard curve was \( y = 0.05236x + 2.79534 \), and the correlation coefficient was \( R^2 = 0.969 \).

3.5 Influence of the concentration of HA

The addition of template molecules affects the formation and number of imprinted sites. In Figure 10, the addition of template molecules was less than 0.025%, and the adsorption capacity of MIM-BCP relative to NIM-BCP made little difference, which revealed that the added amount of template molecules was small; thus, fewer

**Figure 7:** The effect of elution time on color development.

**Figure 8:** The photographs of NIM before and after adsorption of HA.

**Figure 9:** The standard curve line between adsorption and ΔGray value of the NIM-BCP.

**Figure 10:** The effect of elution time on color development.
imprinted sites were formed. However, when the template molecular weight was added to between 0.025% and 0.045%, the adsorption capacity decreased slightly; when the template molecular weight was higher than 0.045%, there was a significant decrease, which may have been because as the addition of template molecules increases, the number of imprinting sites increases, and the recognition space becomes disordered. This phenomenon becomes more obvious with the addition of template molecules. Of course, it may also be because as more template molecules are added, most of the template molecules cannot be completely eluted in 6 h, which promotes the decrease of adsorption capacity; thus, the added amount of template molecules was set at 0.025%, and the adsorption capacity of MIM-BCP was 185.6 μg cm⁻² (IE is 1.38).

### 3.6 Dynamic adsorption experiment

The adsorption kinetics curves of MIM-BCM and NIM-BCP are shown in Figure 11. The adsorption capacities of both materials grew slowly at 0–20 min, rapidly increased at 20–60 min, slowed down after 60 min, and gradually stabilized. The adsorption capacity of MIM-BCP was greater than that of NIM-BCP, which was obviously because the adsorption of MIM-BCP was specific adsorption and the adsorption sites were more abundant than in NIM-BCP. Nevertheless, the adsorption capacity increased slowly and showed no significant difference at 0–20 min, which was probably due to insufficient volatilization of the solution.

The adsorption mechanism was analyzed by the pseudo first-order (PFO) (Eq. 1) model and pseudo second-order (PSO) model (Eq. 2), and the results are shown in Figure A3 and Table 1. The results indicated that the PFO model was in accordance with the adsorption process of MIM-BCP and NIM-BCP because the correlation coefficient ($R^2$) of the PFO model was greater than that of PSO.

### 3.7 Specificity of MIM-BCP

To study the specificity of MIM-BCP, HA was mixed with HAL, HEL, and OA in a certain volume ratio and then diluted to a certain concentration. As shown in Figures 12–14,

| Sample    | PFO | PSO |
|-----------|-----|-----|
|           | Fitting parameters | Fitting parameters |
|           | $k_1$ | $Q_e$ | $R^2$ | $k_2$ | $Q_e$ | $R^2$ |
| MIM-BCP   | 0.01066 | 237.9225 | 0.94348 | 0.000016 | 397.1000 | 0.94016 |
| NIM-BCP   | 0.00924  | 215.3786 | 0.94132 | 0.000015 | 369.9825 | 0.9386 |

**Figure 10**: The influence of the addition amount of template molecule HA on the adsorption of MIM-BCP.

**Figure 11**: The dynamic adsorption of HA on the MIM-BCP and NIM-BCP.

**Table 1**: Parameters obtained for PFO and PSO models
MIM-BCP showed good specificity for HA in mixtures of different proportions. In the competitive adsorption process with HAL, the IEs of MIM-BCP for different volume ratios of the mixture were 1.38, 1.32, and 2.65. The trend may be that as the concentration of HA decreases, the reduction in the adsorption of HA by NIM-BCP is much greater than that of MIM-BCP. The same effect was present in the process of competitive adsorption with HEL and OA, and the IEs of MIM-BCP for different volume ratios of the mixture to HEL were 1.38, 1.15, and 1.98. Additionally, the IEs of MIM-BCP for different volume ratios of the mixture to OA were 1.38, 1.52, and 1.34, 1.11, respectively. Of note, the adsorption of pure OA by MIM-BCP was not significantly different from that of NIM-BCP, which also indicated that MIM-BCP could selectively adsorb HA.

### 4 Conclusion

In this study, we used electrospinning technology to prepare a PMMA molecularly imprinted electrospun fiber membrane for specific recognition and colorimetric detection of HA. The color of the material changed from violet to yellow and became more obvious with increasing HA adsorption. To achieve a better color effect, the chromogenic agent and chromogenic conditions, such as temperature and elution time, were optimized. A standard curve for the adsorption capacity of ΔGray was constructed to quantify the adsorption of HA. The experimental results showed that the coloring procedure occurred 60 min after contact with HA, the adsorption of MIM-BCP was 185.6 μg cm⁻², the imprinting efficiency was 1.38 when the added amount of template molecules was 0.025%, and the imprinted material showed stable specificity to HA among the mixed components. In sum, this method provides a relatively simple and intuitive idea for the detection of human body odor and has broad application prospects.

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Appendix

Figure A1: FTIR spectra of NIM (a), MIM before elution (b), and MIM after elution (c).

Figure A2: SEM of different elution time: 12 h (a) and 15 h (b).

Figure A3: The Dynamic fitting for PFO model and PSO model.