Molecularly Imprinted Membrane-Based Sensor for the Detection of Chloramphenicol Succinate Residue in Milk

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Abstract: Molecularly imprinted films (MIF) were prepared for the detection of chloramphenicol succinate (CAP-SC) by photopolymerization method on screen-printed electrodes and superficial characteristic of these films was analyzed using electron microscope scanning. A sensor based on MIF for the detection of CAP-SC was assembled after the screen printed electrode was connected with an electrochemical analyzer through an electrode slot, and the detection result was recorded by the recorder connected with an electrochemical analyzer. Standard curve for CAP-SC detection was established and analysis of CAP-SC in milk samples was carried out. Scanning election microscopic images of the imprinted films showed that there were numerous imprinted micropores with a diameter about 100 nm on the surface of MIF. The MIF-based sensor showed high sensitivity and specificity toward the target CAP-SC and the response of MIF-sensor to concentration of CAP-SC displayed a linear correlation over a range from 1 × 10⁻⁸ M to 1.2 × 10⁻⁵ M with a detection limit of 2 × 10⁻⁹ M. The recoveries reach 93.5%–95.5% based on milk samples.

Key Words: Molecularly imprinted films; Screen printed electrode; Chloramphenicol succinate; Photopolymerization

1 Introduction

The development of the technique based on molecularly imprinted films (MIF) for the isolation and detection of different substances is a novel trend in analytical chemistry. Up to now, the studies in this field have been mainly focused on the preparation of different MIFs and its specificity of recognizing template molecules including some hormones and drugs. In 1992, Piletsky et al.[1] connected the MIFs with the electrochemical analyzer for the first time and developed a novel sensor with high stability for detecting atrazine in the range of 3.0 × 10⁻⁵–1.0 × 10⁻³ M. Afterwards, they carried out further studies on the preparation of MIFs and polymers of L-phenylalanine and sialic acid. Since 1997, sensors using molecularly imprinted polymers (MIPs) have been successively developed for the detection of glucose, sulfite, fructose, ephedrine, trichloroacetic acid, uranium, tegafur and L-histidine[2–9]. However, only a few literatures have presented novel methods based on the preparation of MIPs and MIFs by thermal and electrochemical polymerization for the detection of chloramphenicol residues in biological samples[10,11]. The transducer parts using MIPs prepared by thermal polymerization exhibit the disadvantage of complicated assembling process, elaborate analyzing procedure and unsuitability for the multisample analysis. On the other hand, the manufacture of the sensors with the MIFs prepared by electrochemical polymerization is in a stage of laboratory improvement. Furthermore, as the preparation of this type of MIFs and detection are performed on the surface of the column metal electrodes, it is very difficult to scale up the manufacture of MIFs and carry out multisample analysis for the future generalization.

In this experiment, MIFs were directly synthesized on the one-touch screen-printed electrodes (SPE) by the in-situ photopolymerization methods using ultraviolet light. Then an electrochemical sensor for the detection of chloramphenicol residue was assembled through connecting the MIF-modified SPE with the electrochemical analyzer. Multisample detection
can be realized only by replacing the MIF-modified SPE. The sensor using MIF-modified SPE as the transducer showed the advantage of high sensitivity, specificity, low cost and short detecting time.

2 Experimental

2.1 Instruments and reagents

Chloramphenicol succinate (CAP-SC), Methylacrylic acid (MA) and ethylene glycol dimethacrylate (EGDMA) were purchased from Sigma Corporation (USA), Azobisisobutyronitrile (AIBN), Tetrahydrofuran (THF), 4-nitrobenzoic acid and all other reagents (analytical grade) were from Shanghai Reagent Corporation (China). Methacrylate-polyurethane galacta (MPG) was prepared in our laboratory according to the method reported by Qu et al [12].

SIRION-200 Scanning Electron Microscope (FEI Corporation, Holland), Ultraviolet lamp 400 W (Fuhui Electrical Equipment Co. Limited, Shanghai), 90-1 Timing Magnetic Stirrer (Minhang Hardware Factory, Shanghai, China), CHI1230 Electrochemical Workstation (Chenhua Equipment Limited, China), Ag/AgCl reference electrode (Leici Instrument Branch, Shanghai Precise Instrument Company, China), SC-6 Oscillator (IKA Works, China), Soxhlet’s Apparatus (Jiangsu Huasheng Laboratory Apparatus Co. Limited, China) and Disposable Screen Printed Electrodes (Newpro Co. Limited, Taibai) were used in this study.

2.2 Preparation of molecularly imprinted films (MIF) and nonmolecularly imprinted films (N-MIF)

MIF was prepared according to the optimized methods established in our laboratory. Briefly, 0.5 g CAP-SC (template molecule) was dissolved in 0.07 ml MA (functional monomer) and the mixture was agitated for 2 h for prepolymerization. Next, 0.48 ml EGDMA (cross linking agent), 0.02 g AIBN (initiator), 0.38 g THF (solvent) and 0.25 g MPG (adhesive material) was added into the prepolymerization solution, followed by agitation for proper time to get good homogeneity. A total of 10 ᵪ of the mixture, which had been degassed for 5 min with N₂, was added dropwise into the shallow well of the SPE, on which a PVC chip (thickness, 75 nm) with a round hole (diameter, 6 mm) was glued on. After that, the SPE was covered by a thin glass slide and exposed to UV light (400 W, 365 nm) for 6 h for complete polymerization. The MIF-modified SPE was cooled down in a refrigerator and then subjected to extraction for 24 h to remove the unpolymerized compounds and the template using methanol and acetic acid (8:2, V:V) in the Soxhlet’s Apparatus after the slide had been carefully displaced. The SPE from the final step was washed and stored in distilled water at ambient temperature. The N-MIF modified SPEs were prepared using the same methods described above and the only difference is that the template (CAP-SC) was not added during the photopolymerization.

2.3 Analysis of MIF/N-MIF by electron microscope

The studies on the superficial characteristics of the MIF and N-MIF were conducted using a SIRION200 scanning electron microscope. Electron microscopic scanning pictures were taken and observed under different magnifications.

2.4 Establishment of testing method

As Fig.1 shows, the analytic system is mainly consisted of the following parts: electrochemical analyzer, electrode slot used for attaching the SPE to the electrochemical analyzer, MIF-modified SPE (transducer of the sensor), recorder (a computer connected with the electrochemical analyzer), magnetic stirrer and testing cell. During the electrochemical analysis, a MIF-modified SPE is attached to the electrochemical workstation through the electrode slot and electrochemical signals can be recorded and analyzed on the computer connected with the sensor. The detection of CAP-SC residue in multiple samples can be easily performed only by changing the one-touch MIF-modified SPE.

In this study, 2 M perchloric acid was used as the supporting solution for the voltammetric analysis. Stock solution of CAP-SC was added into the supporting solution to prepare the standard CAP-SC solution with the final concentration at 2, 4, 6, 8, 10 and 12 μM. After the sensor was assembled, the MIF-modified SPE and the reference electrode were inserted into the electrochemical cell containing 5 ml of standard CAP-SC solution. Cyclic voltammetry was performed after UV-initiated Polymerization

![Fig.1 Schematic structure of analytic system](image-url)

1. Electrochemical analyzer; 2, electrode slot; 3, screen printed electrodes; 4, reference electrode; 5, recorder; 6, molecularly imprinted membrane (MIF); 7, testing cell; 8, magnetic stirrer
the testing solution was agitated on the magnetic stirrer for 10 min and allowed to stand for 20 s. The optimum instrumental conditions for the detection were selected as follows: scanning range, −0.6–0.6 V; scanning speed, 100 mV s\(^{-1}\). The MIF-modified SPE was changed each time and the electrochemical detection was repeated five times for each standard solution to establish the calibration curve for the determination of CAP-SC residues.

### 2.5 Interference experiment

Some antibiotics and substances, based on their availability in clinic use and structural similarity, were chosen for the interference experiment. In this study, chloramphenicol (CAP), thiamphenicol, florfenicol and 4-nitrobenzoic acid were used as interfering materials. 2 M perchloric acid was used to prepare 10 ml 0.5 \(\mu g\) l\(^{-1}\) CAP-SC solution, into which the same amount of the interfering substance as that of CAP-SC in the solution was added. The electrochemical analysis was conducted on the sensor described above. The interference experiment was repeated 5 times for each interfering substance.

### 2.6 Determination of CAP-SC in field samples

For further investigation of the reliability of this technique, six different brands of milk were randomly selected as the field samples from a Shanghai supermarket. Three samples of each brand was collected and pretreated according to the methods described by Agüí et al\(^{[13]}\). The pretreated milk samples were then analyzed on the sensor according to the methods described above. High-efficiency liquid chromatography was also performed as the confirmatory method to verify the agreement of the two methods.

To determine the recovery, the blank milk samples confirmed by HPLC was spike respectively with standard CAP-SC solution in different concentrations. The electrochemical detection was performed under the same experimental conditions described above. Five replications were carried out for each concentration.

### 3 Results and discussion

#### 3.1 Characteristics of MIF and N-MIF

The MIF prepared in this experiment has pale yellow color and the thickness is about 80–100 \(\mu m\). The layer of MIF exhibits moderate tenacity and is tightly affixed to the SPE since proper amount of adhesive material has been added into the solution for photopolymerization. From the view of naked eye, the surface of MIF and N-MIF is smooth. Figure 2 shows the electron microscopic scanning images of the MIF and N-MIF. There are several dints on the surface of MIF and N-MIF. Small amounts of micropores with diameter of 2–3 \(\mu m\) are distributed into some dints of the N-MIF. Compared with N-MIF, more dints can be found on the surface of MIF and N-MIF. Small amounts of micropores with smaller diameter of 50–200 nm are detected in most of the dints. Moreover, many micropores are also distributed in the areas around the dints of MIF. These superficial characteristics of CAP-SC MIF are coincident with that of atrazine MIF described by Sergeyeva et al\(^{[14]}\). The only difference between the methods for the preparation of MIF and N-MIF is whether the template molecules are added into the solution for photopolymerization, so we conclude that these micropores are formed after the template molecules (CAP-SC) accumulated during the polymerization are eluted away. A considerable amount of bigger dints may be shaped after some micrones attached to the surface of MIF and N-MIF, are eluted (as shown in Fig.2, the white particle that arrow directs in picture B\(_{3}\) refers to the micrones).

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*Fig.2  Superficial characteristics of molecularly imprinted membrane (MIF) and nonmolecularly imprinted membrane (N-MIF) by electron microscopic scanning

A: electron microscopic scanning photographs of N-MIF, where \(A_{1} = \times 2500\), \(A_{2} = \times 10000\), \(A_{3} = \times 100000\), respectively. B: electron microscopic scanning photographs of MIF, where \(A_{1} = \times 2500\), \(A_{2} = \times 20000\), \(A_{3} = \times 100000\), respectively*
It is very difficult to elute these micrones if they are affixed to the surface of MIF too tightly. However, the superficial structure of MIF and N-MIF would be damaged and some relatively big pores will be formed once the accumulated micrones are eluted from the surface (as shown in Fig.2, the black hole that arrow directs in picture A 2 refers to the damage formed on the surface of MIF and N-MIF). The reason these macropores are more intensively distributed in the superficial dints of MIF may be that the micrones are eluted away together with the template molecules due to the “dragging effect” of the chemical bond between the macromoles and template molecules (including other relatively smaller polymers).

3.2 Establishment of standard curve for CAP-SC detection based on the MIF

The cyclic voltammogram (CV) was obtained from the standard CAP-SC solutions according to the methods described in Section 2.4. The reduction peak current appeared at 0.3 V and the absolute value of the peak current was increased with the concentration of CAP-SC. This method also demonstrated a good linear response of reduction peak current to CAP-SC concentration over the $1 \times 10^{-8}$–$1.2 \times 10^{-5}$ M range with a detection limit of $2 \times 10^{-8}$ M. Figure 3A showed the calibration curve for the determination of chloramphenicol succinate. The equation of the linear regression is as follows: $Y = 5.216 + 0.162X$ ($R = 0.987$, $S.D. = 0.068$, $n = 5$, $P < 0.001$). Compared with the voltammogram obtained from MIF, the changes of the reduction peak currents from the N-MIF was not significant when different concentration of CAP-SC over the same range as above was applied. Figure 3B shows the difference of the CAP-SC voltammetry between MIF and N-MIF. It is concluded that the interaction between the MIF and the template molecules changed the electrochemical activity of the films on the SPE. CAP-SC bound onto the MIF can be electrochemically catalyzed at the potential of 0.3 V due to the synthetic effect of the interaction between the SPE and MIF. There is a positive correlation between the peak current and the amount of CAP-SC bound to the MIF. The higher the concentration of the template in the testing solution is, the more

the amount of CAP-SC absorbed onto the MIM would be. Accordingly, the peak current is increased with the concentration of CAP-SC in the sample. As described above, the voltammetric response of N-MIF to the changes of the CAP-SC concentration is not significant, indicating that the N-MIF does not possess the characteristics of specific absorbent effect toward molecular template. To further investigate the specifically absorbent abilities of MIF toward the template molecules, the chromatography was performed to determine the changes of the CAP-SC concentration in the testing solution where the electrochemical detection had been conducted. The typical Scatchard equation was used to calculate the adsorption capacity of MIF. The equation is expressed as follows: $Q/C = (Q_{\text{max}} - Q)/K_d$, where $Q$ represents the adsorption capacity, $C$ is the concentration of the template molecules at the adsorption equilibrium, $Q_{\text{max}}$ stands for the maximum adsorption amount of the MIF, and $K_d$ for the dissociation constant. The result showed that the $Q_{\text{max}}$ and $K_d$ of the MIF in this study was $11.85 \mu$mol g$^{-1}$ and $4.37 \times 10^{-3}$ M respectively. However, the $Q_{\text{max}}$ and $K_d$ of the N-MIF is only $1.24 \mu$mol g$^{-1}$ and $2.27 \times 10^{-4}$ M, respectively.

3.3 Interference experiment

On the basis of the methods described in Section 2.4, the electrochemical analysis was conducted on the sensor described above after 10 ml of $0.5 \mu$g l$^{-1}$ CAP-SC standard solution prepared with 2 M perchloric acid was supplemented with the same amount of the interfering substance as that of the CAP-SC (Fig.4). The results showed that the amount that was detected in the testing solution spiked with same amount of CAP was close to the summed amount of the CAP-SC in the original solution and supplemented CAP (Table 1). However, the amount detected in the solution, which was supplemented respectively with thiamphenicol, Florfenicol and 4-nitrobenzoic acid, was nearly equal to the CAP-SC content in the original solution without the interfering material. The content of CAP-SC detected in the testing solution spiked with thiamphenicol, florfenicol was a little higher than its true quantity in the solution, whereas the content of CAP-SC in the testing solution spiked with...
Fig. 4 Molecular structure of interfering material

Table 1 Effect of interfering substance on detection of CAP-SC on MIF based sensor

<table>
<thead>
<tr>
<th>Amount of CAP-SC (µg l⁻¹)</th>
<th>Concentration of interfering substance (µg l⁻¹)</th>
<th>Detected (µg l⁻¹)</th>
<th>SD</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>CAP/0.5</td>
<td>0.87 ± 0.009</td>
<td>0.0215</td>
<td>2.5</td>
</tr>
<tr>
<td>0.5</td>
<td>Thiamphenicol/0.5</td>
<td>0.543 ± 0.007</td>
<td>0.0371</td>
<td>6.8</td>
</tr>
<tr>
<td>0.5</td>
<td>Florfenicol/0.5</td>
<td>0.537 ± 0.004</td>
<td>0.0329</td>
<td>6.1</td>
</tr>
<tr>
<td>0.5</td>
<td>4-nitrobenzoic acid/0.5</td>
<td>0.478 ± 0.003</td>
<td>0.0712</td>
<td>14.7</td>
</tr>
</tbody>
</table>

4-nitrobenzoic acid was lower than its true quantity. These data showed that the substance with similar structure to CAP-SC could interfere with the detection. The more similar the structure of the substance with CAP-SC is, the stronger the interfering effect would be. As reported in the published literatures, the recognizing capacity of MIF is mainly based on the interaction of the predominant groups and the whole molecules of the template with MIF. The CAP-SC MIF demonstrated some recognizing ability toward thiamphenicol and florfenicol because both of them possess some molecular groups similar to CAP-SC. The MIF recognize not only some predominant molecular groups but also the whole molecule of the target substance, so the interfering effect caused by thiamphenicol and florfenicol is limited extremely. On the basis of the fact that the amount which was detected in the testing solution spiked with same amount of CAP was about two folds of the CAP-SC in the original solution, it is concluded that this methods designed for the detection of CAP-SC can also be used to analyze CAP. Because both CAP and CAP-SC (the derivative of CAP) are banned in edible animals, it is of great significance in the field detection. The data shown in Table 1 also indicate that the detection was hardly interfered by 4-nitrobenzoic acid since its structure is not similar to CAP-SC.

3.4 Recovery analysis

The results from the field samples demonstrated that CAP-SC residue was found in two out of 30 samples (5 samples per brand) and the two positive samples were respectively from two different brands. The concentrations of CAP-SC in the two samples are 0.127 and 0.042 µg l⁻¹, respectively. The data obtained from the technique based on MIF show good agreement with the results from HPLC. The only difference is that the amount of the CAP-SC detected by HPLC is slightly higher compared with the method established in this experiment.

The recovery analysis based on milk samples was performed according to the method described above after the blank milk samples was spiked respectively with standard CAP-SC solution with different concentrations. The recovery is 93.5%–95.0% (Table 2).

3.5 Sensor performance

The detection of CAP-SC in each sample can be achieved within 12 min on the sensor designed in our laboratory, which showed the advantage of short detecting time. After the detection was performed successively by changing the MIF-modified SPE for five samples spiked with same amount of CAP-SC, the results showed that this methods exhibited good reproducibility with the relative standard deviation (RSD) at 2.6%. To investigate the stability of the transducer, 10 MIF-modified SPEs were stored at -4 ºC and determination was performed for the same sample containing a certain amount of CAP-SC at the time of 60 and 120 d, respectively. The results showed that the RSD of the CAP-SC amount detected before the storage and on the 60th day is less than 3.7%. After four months, the RSD is still less than 4.9%. It was concluded that this kind of transducer have a service life of more than four months since its service life mainly depends on the activity of the MIF on the SPE. In addition, the activity of the MIF did not change significantly even after a year of storage.

Table 2 Recovery of CAP-SC in milk samples

<table>
<thead>
<tr>
<th>Group 5 replication per concentration</th>
<th>Concentration of CAP-SC (µg l⁻¹)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Added</td>
<td>Found</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>94.0</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>95.0</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>94.7</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>93.5</td>
</tr>
</tbody>
</table>
storage at 4 ºC. The MIF-modified SPE is disposable and it is very convenient to replace it with a novel one, so the sensor to which the transducer is attached can be designed for long-term use. In summary, the sensor based on the MIF in our laboratory demonstrates the advantage of ideal reproducibility, stability, durability and quick response.

4 Conclusions

In this study, MIF was prepared directly on the surface of the SPE and an electrochemical sensor was assembled for the successive determination of CAP-SC in multiple samples by collecting the MIF-modified SPE with the potentialstat. This analytical system shows the advantage of high stability, low cost, short detecting time and low detection limit ($2 \times 10^{-9}$ M). We predicted that this type of sensor would have great prospect in practical use.

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References

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