Self-assembled Imprinted Polymer Film-Based Sensor System for Spermidine on Screen Printed Electrodes

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Abstract

Spermidine (SPD) is an important signal molecule relate to protein food safety, but vulnerable to interference during detection. Here, an electrochemical sensor system for the spermidine detection was developed by a self-assembled molecularly imprinted polymer (MIP) film coated on disposable screen-printed electrodes. The modification imprinted polymer was prepared with template molecules SPD and functional monomer meth acrylic acid (MA) with molar ratio 1:4 through ultraviolet polymerization. The cyclic and differential pulse voltammetry (CV and DPV) scanning showed that the best adsorption equilibrium time of the modified electrodes was 360 s. The electrodes exhibited good linear relationship ranging from 1.0 × 10⁻⁶ mol/L to 5.0 × 10⁻⁶ mol/L (y = −1.22x+7.82, R² = 0.9952), and the detection limit was 1.7016 × 10⁻⁷ mol/L. When take high concentration of histamine and tyramine as interfering components, the modified electrodes detection relative standard deviation was < 5% and thus proved good anti-interference ability. In addition, the density function theory (DFT) calculation explored the intera-molecular interaction of SPD-MA imprinted complex, and the simulation showed that the prepared device can keep recognition stability under the potential difference stress range from 0 to 0.005 a.u. Therefore, the prepared system has the potential for rapid detection spermidine in the place.

Keywords

Spermidine, Molecular imprinting, Modified electrode, Screen-printed, Detection

Introduction

Spermidine (SPD) was biosynthesized by putrescine and methionine in vivo [1], which involved multifunction in life activities, promotes cell growth and lower blood pressure. It was widely present in a variety of foods (e.g., ripe cheese, pulses, mushrooms, and whole grains) [2-4], and Figure 1 shows structure schematic. In vivo, spermidine act as physiological blockers of inward rectifier K+ channels [5], increase autophagy-related gene transcription to expand the lifespan of model creatures [6, 7]. More importantly, as a common biogenic amine, spermidine related many to food safety [8]. The spermidine levels in meat products can reflect the freshness and spoilage degrees [9, 10], microbe species and count [11]. During food processing, the nitrite compounds in meat can form carcinogenic nitrosamines with spermidine [12]. Many studies provide that spermidine accumulated in rats has subacute toxicity (600 mg/Kg) and a strong stimulating effect on the mucosa [13], and high concentration of spermidine...
in the patient body fluids related to various cancer potential [14]. Hence, the accurate and rapid detection of spermidine concentration in vitro and in vivo samples is necessary.

At present, the biogenic amines detection strategies including gas chromatography (GC), thin layer chromatography (TLC), ultra-high-performance liquid chromatography (UHPLC), ion chromatography, mass spectrometry (MS), and optical sensors [15–19]. However, most of these methods have disadvantages in rapid detection in place. Electrochemical sensors with the advantage of efficient and convenience has applied to trace food risk substance detection, which transform the concentration response changes into sensitive and sharp electrical signals. The advanced electrochemical techniques, such as Au or carbon nanoparticle-modified electrodes, pulsed amperometric detection, capillary electrophoresis electrochemiluminescence [20, 21]. And the latest methods such as the carbon dots (CDs), fluorescent sensor, smart visual detection and enzymatic biosensors [22–24]. However, these methods were insufficient to recognition target substance specificity [25]. The molecularly imprinted polymer (MIP), which can specifically adsorb target molecules, was obtained by the polymerization between the template molecule and functional monomer with the aid of a crosslinking agent [26]. And there exist many MIP modified electrode sensors devices, such as carbon electrode [27, 28], functioned graphene electrode [29], coated silica [30]. But these sensor systems also have the shortage of rapid synthesis efficiency. Screen printing is a kind of stencil printing, “probe molecules” can transfer to the substrate “holes” under pressure, the electric probe can induce oxidation reduction reaction and current change on the electrode surface. Screen printed imprint modified electrode was widely used in many rapid detection fields [31].

In order to improve the spermidine detection above, we prepared a disposable biosensor based on the screen-printed electrodes, the electrodes modified with imprinted polymer can specific identification spermidine in the presence of interferences. The polymer was directly polymerized on the electrode surface with functional monomer meth acrylic acid assist by the crosslinking agent ethylene glycol dimethacrylate, and the electron acceptor K₃[Fe(CN)₆] was used as a typically probe substance because it was a good electrical conductor. Moreover, considered the potential difference occurs periodically at the electrode cyclic voltammetry detection response process [32, 33], and the induced electric field effect may impact the structural stability of the imprinted complex, the density function theory (DFT) calculation was used for electronic structure parameters and intra-molecular weak interaction analysis. Over all, our work can provide a certain reference for the preparation of spermidine rapid detection devices (Figure 1).

![Spermidine structure](image)

**Figure 1: Schematic of spermidine structure.**

**Materials and Methods**

**Reagents and instruments**

Spermidine and histamine (purity 98%; Sigma–Aldrich, USA), tyramide and meth acrylic acid (purity 99%; Sigma–Aldrich, USA), ethylene glycol dimethacrylate (purity 98%; Sigma–Aldrich, USA), azobisisobutyronitrile (analytical grade; Chengdu Gresia Chemical Technology Co., Ltd. China), potassium ferricyanide (analytical grade; Tianjin Kemico Chemical Reagent Co., Ltd. China), nitrogen (Guizhou Yong-sheng Industrial Gas Company, China), and screen printing electrode (Kim Kun Biological Engineering Co., Ltd., Wuxi City, China).


**Methods**

**Screen printed electrodes pretreatment**

The electrode was first immersed at 20% H₂O₂ solutions for 20 min and then at 20% H₂SO₄ for 20 min to clean the dust and oxidation on the surface. Then, the electrode was continuously soaked with deionized water for 20 min, and then dried and preserved at 4°C for further use.

**Electrodes buffer preparation**

The K₃[Fe(CN)₆] phosphate buffer solution with the concentration of 0.5 mmol/L and pH of 7.0 was prepared using 1.646 g potassium ferricyanide standard and 0.2 mol/L phosphate buffer. The 0.2 mol/L phosphate buffer solution was prepared by adding 39 ml of 0.2 mol/L Na₂HPO₄ and 61 mL of 0.2 mol/L Na₃PO₄ in a beaker.

**Spermidine molecularly imprinted polymer film preparation**

Based on our previous work [34], choose the theory calculated optimum molar ratio of template molecules spermidine to the functional monomer methacrylic acid 1:4 as the experimental polymerization ratio. Accurately weighed 217.5 mg spermidine and 100 mg azobisisobutyronitrile, accurately measured 510 mL of meth acrylic acid and 2 mL of ethylene glycol dimethacrylate, mixed in a beaker. Then, 50 mL of carbon tetrachloride solution were added, and the solution was placed in 100 W ultrasonic cleaners for dissolution, nitrogen was bubbled to remove dissolved oxygen within the solution system. For polymerization, take 1.5 µL polymerization solution and dropped into the working area of the screen-printed electrode covered with cover glass to isolate oxygen and form a uniform film. Note that the cover glass should be used for covering to ensure that the polymerization solution cover the reference electrode and prevent impact, and the entire dropping and glass slide covering steps must be performed as quickly as possible to avoid rapid evaporation of carbon tetrachloride. Then, placed modified electrode in an incubator at 4°C and irradiated with 365 nm UV lamp at a distance of < 10 cm to polymerization for 96 h. After
polymerization was completed, a waxy surface layer was formed on the electrode surface. The prepared modified electrodes were stored at 4 °C for next steps.

**Spermidine-imprinted modified electrode elution time**

The prepared spermidine-imprinted electrode was soaked into 10% methanol acetic acid solution (v/v = 9:1), and stirred gradually for elution spermidine molecules. In order to avoid excessive elution MIP layer, the stirring intensity should not be too strong. The DPV scanning method was used every 60 s after 60 s of elution in the range of -0.6 V~0.8 V.

**Spermidine-imprinted modified electrode adsorption time**

Equilibrium adsorption time can reflect the electrode detection speed. The specific adsorption of spermidine on imprinted modified membrane electrodes was dynamic adsorption equilibrium process through intermolecular weak intermolecular interactions [35]. The spermidine-imprinted modified electrode was placed into the buffer solution for adsorption test, and the adsorption time interval was 60 s. After adsorption, the spermidine remaining on the surface was washed with distilled water. Then, the electrode was inserted into the spermidine-free buffer to perform DPV scan at range of -0.8~1.0 V, record the peak current and plot it versus time.

**Spermidine-imprinted modified electrode detection limit**

The lower detection limit of the electrode, the more detection sensitivity of the electrode. After elution, the imprinted modified electrodes were placed in adsorption solution at series concentration 1, 2, 3, 4, 5 µmol/L. The saturated adsorption time was referenced with the adsorption equilibrium time (360 s), and the current changed was detected by DPV scanning in the range of -0.8~1.0 V. Plot the standard curve with the response values and spermidine concentration. The detection limit was determined by formula: DL = 3δb/K, where δb is the standard deviation (SD) of blank parallel interference experiment

Molecularly imprinted polymers can adsorb the target substance by geometric and structural matching principles [36]. Tyramine and histamine, similar in shapes and groups of spermine, were selected as interfering components. First, a buffer solution containing 1.0 × 10~6 mol/L SPD was prepared in 2 beakers, and 50-fold molar tyramine and histamine concentrations were added. The eluted spermidine imprinted polymer modified electrode was placed in a beaker for adsorption. The adsorption time was referenced with the adsorption equilibrium time. For check the occupied conditions of eluted holes on SPD, after adsorption was completed, the buffer solution was subjected to DPV scanning, and the results were compared with the current changes.

**DFT theory calculations**

The DFT calculation was used to simulation and explore the electrode modification material molecular imprinted complex (SPD and MA with best polymerization ratio 1:4) structural stability under the external electric field. The geometry optimization with frequency vibration analysis of the SPD-MA complex were performed on the B3LYP/6-311G (d, p) theory level at gas phase, and the keywords nosymm fixed the SPD-MA complex oriented at the external electric filed direction. The external electric field was set at SPD-MA complex cartesian coordinates X axis with intensity values 0, 0.001, 0.002, 0.003, 0.004, 0.005 a.u (1.a.u = 51.423V/angstrom), which range from 0V/angstrom to 0.25V/ angstrom. The single point energy was calculated at M062X/def2TZVP level based on the optimized structure with on imaginary frequency. For thermodynamics data, the vibrational zero-point energies (ZPEs) scale factors was 0.988.

Bader's Atoms in Molecules (AIM) theory states that the electron density distribution is related to the chemical bond type. The bond critical point (BCP) occurs between attractive atom pairs, the most important was hydrogen bond donor and acceptor atoms. For small interacting monomers, the strength of hydrogen bonds (EHB) can be reasonable characterized by binding energies (BEs). And the BEs of neutral hydrogen bond has the fitted equation with electron density at the corresponding bond critical point (BCP).

\[
BE \approx -223.08 \times \rho(BCP) + 0.7423
\]

where the unit of \(\rho\) is a.u., the unit of BE is kcal/mol [37].

In order to visualization the intermolecular weak interactions at SPD-MA complex BCPs, the independent Gradient Model (IGM) for non-covalent interaction (NCI) analysis was also performed. All the DFT theory calculations were performed on the Gaussian 16 (Revision A. 03) package support by the Guizhou university High performance computing cluster [38]. The BCP and IGM analysis of SPD-MA complex was finished by Multiwfn 3.8 suites on the personal computer [39].

**Results and discussion**

**Spermidine-imprinted modified electrode elution time and stability**

When elution imprinted polymers with buffer solution, it generated “holes” that allow the probe electric substance pass through. When detection, the film-modified electrode absorbed spermidine immediately and blocked the “holes”, thus the probe K3[Fe(CN) 6 ] cannot pass through [40]. The electrode elution was at 10% methanol acetic acid solution (v/v = 9:1). In order to measure the current changes of the modified electrode elution process, the DPV scan was performed at the scan range of -0.8 ~1.0 V, the result is shown in figure 2.

The stability of modified electrode was also crucial to the subsequence results. Many studies show that the electrode surface was not absolutely smooth [41], thus make the MIP films coated on the surface tightly during the UV polymerization process. Furthermore, after elution, a three-dimensional network porous structure formed on the MIP modified electrode surface. And the network porous, which cross-linked each other and adhered to the rough electrode surface through molecular force interaction [42]. The electrode potential stability of the bare electrode, eluted electrode, un-
Self-assembled Imprinted Polymer Film-Based Sensor System for Spermidine on Screen Printed Electrodes

Huang et al.

eluted electrode in buffer solution (contain the probe substance K₃[Fe(CN)₆]) was also measured at the same conditions by CV scan, and the result was at figure 3.

As shown in figure 2, the response current was only almost 3.0 µA at the begin of elution. In this imprinting stage, the polymer film was assembled on the electrode surface, and the spermidine wrapped in the pores that formed by the functional monomer and crosslinking agent. Only few holes present on the electrode surface were still open, thus make probe substance K₃[Fe(CN)₆] cannot pass through because eluted cavities were occupied by spermidine molecules, and the electrodes cannot specifically detect spermidine because the current transportation pathway was obstructed. When eluted the template molecular spermidine, the movable K₃[Fe(CN)₆] probe and the electrode surface process redox reaction and exhibited electrical signals. From figure 2, As the elution time increase, the current upped significantly from 1000 s to 4000 s, indicated the spermidine was gradually eluted from the pores. During the stage, the imprinted modified electrodes also can adsorption spermidine from the mixed solution by specific recognition ability, and the reabsorbed spermidine molecules binding the pores against with the K₃[Fe(CN)₆] molecule to keep a dynamic equilibrium. Given that spermidine was not electrostatic substance, the current flow generated on the electrode surface is reduced [43]. As shown in figure 2, the increased current tends to stabilize at 4200 s, which may point to the end of elution. Considered that the current of the entire system ranging from 4200 s to 5700 s only increased 0.2 µA, the elution termination time was set at 4200 s.

In figure 3, bare electrodes possessed the largest scan peak because the surface was uncovered with the imprinted polymer, and the K₃[Fe(CN)₆] in solution contacted abundantly on the electrode surface. When the electrode work area surface was covered by molecularly imprinted polymer, the un-eluted polymer blocked the pores by spermidine on the electrode surface. Hence, K₃[Fe(CN)₆] cannot contact the electrode surface to undergo redox reaction and electron transfer, causing the smallest peak current generated. The peak current of the eluted electrode was smaller than the bare electrode because the imprinted polymer itself coated on the electrode working area. In above, the electrode peak current sequence satisfied: bare electrode > eluted electrode > un-eluted electrode. This illustrated that the un-eluted electrode passed through the smallest amount of K₃[Fe(CN)₆], and the eluted electrode passing through K₃[Fe(CN)₆] was still smaller than the bare electrode.

Spermidine imprinted modified electrodes adsorption time

The current changes of the electrode adsorption spermidine was in figure 4. The equilibrium adsorption time of the imprinted modified electrodes on spermidine was shown in figure 5.

From figure 4, after adsorption, the current of the modified electrode was lower than before and reached maximum peak values when the potential values near zero, which indicates the adsorption status has become saturated. The spermidine concentration in the sample solution can measured by the relationship between the scanned current and the spermidine adsorption gradient concentrations. As shown in the curve in

Figure 2: Elution time of the imprinted modified electrodes.

Figure 3: CV scan of the three electrodes (Bare electrode, Uneluted electrode, Eluted electrode).

Figure 4: DPV scan of SPD imprinted modified electrodes before and after adsorption.
At first, the current value decreased rapidly and then gradually increased until reached equilibrium. This indicated that the nonspecific adsorption played an important role at the beginning of the adsorption process, and the specific adsorption occurred afterward. This was consistent to the adsorption and recognition of spermidine by molecularly imprinted polymer at polymerization ratio of 1:4. And from figure 5, the response current value was decreasing along with the adsorption time because the spermidine molecules re-adsorbed subsequently after the probe substance $K_3[Fe(CN)_6]$ eluted from the imprinted polymer. The response current was close to equilibrium at 360 s, and in order to shorten the detection time, we defined the electrode adsorption time of spermidine sample solution as 360 s. Figure 5 shows that the current values decreased obviously after adsorption completely, this relate to the decrease of the electrode surface cavities and the amount of moveable $K_3[Fe(CN)_6]$.

### Interference experiment

The 50-fold spermidine concentrations ($50.0 \times 10^{-6}$ mol/L) interference components tyramine and histamine were added to the buffer solution, and the solutions was scanned by DPV to obtain the current value changes. The result was shown in table 1.

<table>
<thead>
<tr>
<th>Number</th>
<th>No interfering substances ($\mu$A)</th>
<th>With interfering substances ($\mu$A)</th>
<th>Relative standard deviation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spermidine/tyramine</td>
<td>7.71</td>
<td>7.85</td>
<td>1.83</td>
</tr>
<tr>
<td>Spermidine/histamine</td>
<td>7.71</td>
<td>7.35</td>
<td>2.06</td>
</tr>
</tbody>
</table>

Table 1 shows the relative standard deviation (RSD) was < 5%, indicated the presence of interference components effect little on the current change. As reported, the RSD of a surface plasmon resonance histamine sensor based on molecularly imprinted polymer film was range from 2.48% to 4.66% [47]. Moreover, the lowest RSD of imprinted fluorescent spermidine chemosensor at spiked serum was 3.5% [48]. Therefore, our prepared imprinted modified electrode possessed a good selective adsorption capacity for the target substance spermidine, because the eluted molecularly imprinted polymer specific adsorption sites cannot recognize the interfering substances. Hence, the interference substance cannot block the cavities. Furthermore, due to the specificity adsorption equilibrium of modified electrode only occurred on the target substance spermidine during the adsorption process. Therefore, the response current value of the interfering components group was very similar to the blank control group.
**Structural stability of SPD-MA imprinted complexes**

As many reports show [49, 50], the hydrogen bond binding energy ($\Delta E_{\text{binding}}$) donated the main intera-/molecular weak interaction in MIP system, which was crucial for the stability of self-assembled supramolecular system. The atom in molecules (AIM) theory and independent Gradient Model (IGM) was widely used in intera-/molecular non-covalent interaction (NCI) analysis. Similar to reduced density gradient function, the IGM can analyze NCI based on the electron density topology, and when define the isosurfaces as function $S$, there has the equation as follows [51]:

$$ S = \frac{1}{2(3\pi^2)^{1/3}} \frac{|\nabla \rho(r)|}{(\rho(r))^{4/3}} $$

where $\rho(r)$ represents the electron density (ED) and $|\nabla \rho(r)|$ stands for the norm of the ED gradient vector. The basis of the Independent Gradient Model (IGM) is also ED gradient in terms of atomic components. When SIGM based on the $|\nabla \rho(r)|$ IGM, the largest deviation of SIGM and $S$ occurring at the bond critical point (BCP). Set the difference as function $\delta g$, $\delta g(r) = |\nabla \rho(r)|_{\text{IGM}} - |\nabla \rho(r)|$, $\delta g$ can divided into intramolecular interaction ($\delta g\text{intra}$) and intermolecular interaction ($\delta g\text{inter}$). The figure 7 was the BCP combined IGM analysis of the SPD-MA complex, the green colours are identified as Van der Waals interaction and blue colours identified hydrogen bond.

**Figure 7:** Non-covalent interaction (NCI) and BCP between SPD-MA complex (0 a.u, at gas phase). Bond critical points are shown as orange spheres. Bond paths are drawn as yellow paths. SPD-MA complex structure is represented as CPK model. Carbon, hydrogen, oxygen, nitrogen atoms is colored by cyan, white, red and blue, respectively. The dark blue oval region between the SPD and MA is isosurface of reduced density gradient (RDG) of 0.015 mapped by sign ($\delta g$) function.

In general, the charge redistribution was related to bonding properties [53]. And the external electric field can impact the molecular structure by changing the charge distribution and molecular dipole. Combined the IGM analysis results, the intermolecular hydrogen bond at the BCP contributed most to the MIPs specific recognition ability. Therefore, we discuss focused on the hydrogen strength or binding energy here, and the results was list at table 2.

```
<table>
<thead>
<tr>
<th>Hydrogen bond</th>
<th>0 a.u</th>
<th>0.001 a.u</th>
<th>0.002 a.u</th>
<th>0.003 a.u</th>
<th>0.004 a.u</th>
<th>0.005 a.u</th>
</tr>
</thead>
<tbody>
<tr>
<td>$O_6-H_63...O_{10}$</td>
<td>-70.42</td>
<td>-71.74</td>
<td>-72.58</td>
<td>-73.81</td>
<td>-75.89</td>
<td>-78.23</td>
</tr>
<tr>
<td>$O_6-H_62...O_{10}$</td>
<td>-67.88</td>
<td>-65.63</td>
<td>-63.88</td>
<td>-62.6</td>
<td>-61.27</td>
<td>-59.62</td>
</tr>
<tr>
<td>$N_{10}-H_40...O_{21}$</td>
<td>-19.31</td>
<td>-19.36</td>
<td>-19.35</td>
<td>-19.14</td>
<td>-18.86</td>
<td>-18.45</td>
</tr>
<tr>
<td>$N_1-H_24...O_{75}$</td>
<td>-20.05</td>
<td>-19.78</td>
<td>-19.3</td>
<td>-18.42</td>
<td>-17.58</td>
<td>-16.64</td>
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<tr>
<td>$N_10-H_8...O_{65}$</td>
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<td>-32.5</td>
<td>-32.43</td>
<td>-32.54</td>
<td>-32.62</td>
<td>-32.65</td>
</tr>
<tr>
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<td>-65.63</td>
<td>-63.88</td>
<td>-62.6</td>
<td>-61.27</td>
<td>-59.62</td>
</tr>
<tr>
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<td>-32.62</td>
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<td>-71.74</td>
<td>-72.58</td>
<td>-73.81</td>
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<td>-19.78</td>
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<td>-18.42</td>
<td>-17.58</td>
<td>-16.64</td>
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<td>$O_6-H_65...N_{10}$</td>
<td>-67.88</td>
<td>-65.63</td>
<td>-63.88</td>
<td>-62.6</td>
<td>-61.27</td>
<td>-59.62</td>
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<tr>
<td>$O_{20}-H_{22}...O_{15}$</td>
<td>-32.61</td>
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<td>-32.43</td>
<td>-32.54</td>
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</tr>
<tr>
<td>Total (kJ/mol)</td>
<td>-242.95</td>
<td>-242.36</td>
<td>-241.76</td>
<td>-241.83</td>
<td>-242.26</td>
<td>-242.05</td>
</tr>
</tbody>
</table>
```

Form the table 2, all the SPD-MA molecular imprinted complex formed six hydrogen bonds under the external electric field range from 0 to 0.005 a.u. The hydrogen bond strength of $O_6-H_63...O_{10}$, $N_{10}-H_40...O_{21}$ and $N_1-H_24...O_{75}$ was little decreased with the external electric field intensity increasing. While the $E_{\text{HB}}$ values of $O_62-H_63...N_1$ and $O_{20}-H_{22}...O_{15}$ was little increased with the external electric field intensity decreasing. Compared with the 0.05 a.u results to 0 a.u outfield conditions, the $E_{\text{HB}}$ maximum changes was 8.26 kJ/mol, which was only occupied 3.34% of total $E_{\text{HB}}$ values at 0 a.u conditions. This indicated that electric field impact little on the SPD-MA molecular imprinted complex binding ability at the field intensity range from 0 to 0.005 a.u. Moreover, the effect of the external electric field on the whole complex system was not reasonable, because sum of the six hydrogen bonds strength was very similar at range of 0 to 0.005 a.u electric field, and the maximum changes was only 1.12 kJ/mol.
The equilibrium time was 360 s. The modified electrodes possessed a good linear relationship ranging from 1.0 × 10⁻⁴ mol/L to 5.0 × 10⁻⁴ mol/L (y = -1.22x + 7.82, R² = 0.9952), and the detection limit was 1.7016 × 10⁻⁷ mol/L. The interference components showed the modified electrodes exhibited good anti-interference ability. The DFT calculations showed that the structural stability of SPD-MA imprinted complex changes little under the external electric filed at intensity range from 0 to 0.005 a.u., which indicated the prepared device can resistant to a certain level of potential difference stress. With the advantage of convenient, stable, and sensitive, the prepared spermidine detection electrodes avoid the complex sample processing steps, and provide a reference method on protein food safety evaluation.

**Conflict of Interest**

The authors declare no conflict of interest.

**References**


