Application of Molecularly Imprinted Polymer-Modified Potentiometric Sensor for Quantitative Determination of Histamine in Serum

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Abstract

A molecularly imprinted polymer-modified potentiometric histamine (HIS) sensor was prepared and used for quantitative determination of HIS in bovine serum. The calibration curve using the potential responses measured in $1 \times 10^{-3}$ mol L$^{-1}$ phosphate buffer (pH 7.4) showed good linearity in the HIS concentration range of $3 \times 10^{-4}$ to $1 \times 10^{-2}$ mol L$^{-1}$ ($r = 0.92$), with a detection limit of $1.6 \times 10^{-4}$ mol L$^{-1}$. In bovine serum samples, the HIS sensor showed good recovery values of 91%–104%. Therefore, this HIS sensor successfully determined the HIS concentration in bovine serum samples.

Keywords
Potentiometric sensor, Molecularly imprinted polymer, Histamine, Serum, Quantitative determination, Selectivity
**Introduction**

Chemical sensors determine the presence and concentration of analytes using techniques such as electrochemical measurement,\textsuperscript{1,2} surface plasmon resonance,\textsuperscript{3} and quartz crystal microbalance.\textsuperscript{4-5} Chemical sensors have advantages including the real-time monitoring, the lack of skilled technology required. Biosensors utilizing biological reactions are a class of chemical sensors that have been widely used owing to their high recognition ability.\textsuperscript{6-8} However, their drawbacks include limitations regarding available analytes, a lack of long-term stability, and high cost. The development of recognition elements is a key factor in chemical sensors.

Our research has focused on molecularly imprinted polymers (MIPs) as recognition elements. MIPs show the specific recognition ability, because they possess an analyte template within the polymer and memorize the functional groups and three-dimensional structure of the analyte.\textsuperscript{9} MIP-modified sensors are expected to be alternatives to biosensors because MIPs can be easily manufactured from commercial materials using any isolable chemical as the template molecule.

Histamine (HIS; Fig. S1A (Supporting Information)) is a biological amine that is involved in immune responses and allergy symptoms,\textsuperscript{10,11} and various chronic inflammation.\textsuperscript{12,13} HIS blood levels provide information to help elucidate the mechanism of diseases concerning HIS. Although HPLC and enzyme immunoassays are used clinically as the HIS determination methods,\textsuperscript{14,15} they require expensive instruments and reagents. Therefore, a facile and low-cost analytical method for HIS determination would be clinically useful.

We previously reported a preliminary MIP-modified potentiometric sensor manufactured using HIS as a template molecule (denoted as the HIS sensor), which
showed good responsivity and selectivity for HIS and exhibited a near-Nernstian response.\textsuperscript{16,17} However, these results were obtained from experiments performed in distilled water. This study aimed to apply this MIP-modified potentiometric sensor to the quantitative determination of HIS in serum. First, we investigated the responsivity, selectivity and quantification of the HIS sensor in phosphate buffer, and the effects of bovine serum on quantification. Finally, we attempted to determine HIS spiked in bovine serum using the HIS sensor.

**Experimental**

*Potentiometric measurement of HIS sensor*

The potential response of the HIS sensor using a MIP-coated graphite electrode was measured against the Ag/AgCl reference electrode by transferring 1-mL aliquots of different concentrations of HIS aqueous solution or HIS spiked in bovine serum into phosphate buffer (100 mL, pH 7.4). All measurements were conducted at room temperature.

**Results and Discussion**

*Responsivity, quantification and selectivity of HIS sensor in phosphate buffer*

First, the effect of the salt concentrations in phosphate buffer (pH 7.4) on the responsivity of the HIS sensor was investigated. The potential change clearly became smaller as the salt concentration increased (Fig. S2 (Supporting Information)). Although
the responsivity of the HIS sensor reduced to 67% compared with that in distilled water, 1 mmol L\(^{-1}\) phosphate buffer was used for the following determinations.

Next, the selectivity of the HIS sensor was investigated. The potential changes by four substances (Fig. S1B-E (Supporting Information)) were all much smaller than that achieved by HIS (p < 0.05) (Fig. S3 and S4 (Supporting Information)). Therefore, these results showed that the HIS sensor using the MIP template had better recognition for HIS than other substances.

To quantify the HIS sensor, the potential responses after adding HIS aqueous solutions of various concentrations were determined. The potential change became larger with increasing HIS concentration (Fig. S5 (Supporting Information)). The potential change values at 25 min reached an enough stable potential as seen in Fig. S5 (Supporting Information) were plotted versus the logarithm of HIS concentration to obtain a calibration curve in Fig. 1. The potential change was proportional to HIS concentration in the range of 3\(\times\)10\(^{-4}\) to 1\(\times\)10\(^{-2}\) mol L\(^{-1}\) (r = 0.92). The regression equation of the calibration curve was \(y = 22.7x + 86.3\). Furthermore, the detection limit was 1.6\(\times\)10\(^{-4}\) mol L\(^{-1}\) at a signal-to-noise ratio of 3.

**Effect of bovine serum**

To investigate the effect of serum in the sample solution on the HIS sensor responsivity, the potential response of the HIS sensor was measured by adding HIS stock solution to phosphate buffer (pH 7.4) containing different ratios of bovine serum. The potential responses in phosphate buffers containing bovine serum showed similar behavior to that in phosphate buffer alone (Fig. S6 (Supporting Information)), but decreased gradually with increasing bovine serum ratio.

Figure 2 shows the relative potential changes of the HIS sensor calculated by
dividing the potential change after 25 min measured in each phosphate buffer containing bovine serum by that in phosphate buffer alone. No significant differences were observed between the potential changes for phosphate buffers in the absence and presence of 1 or 3 vol% bovine serum. In contrast, the relative potential change significantly decreased in phosphate buffer containing more than 5 vol% bovine serum. This might be attributed to interference from matrix components of serum, such as the adsorption of serum proteins on the electrode surface of the HIS sensor. These results indicated that the HIS sensor clearly responded to HIS in the phosphate buffer containing 1% bovine serum.

Quantitative determination of HIS spiked in bovine serum

To demonstrate the application to biological samples, the determination of HIS spiked in bovine serum was tested. The potential response of the HIS sensor was measured when bovine serum samples (1 mL) with different HIS concentrations were added to phosphate buffer (100 mL) to obtain a bovine serum ratio of 1 vol%, with the results shown in Fig. 3. The potential response curves observed for HIS spiked in bovine serum were the same as those for HIS aqueous solutions, with the potential change increasing with increasing HIS concentration spiked in the bovine serum samples. In contrast, the potential change of the HIS sensor showed almost no increase when the bovine serum sample without HIS was added to the phosphate buffer. These results also supported that the HIS sensor responsivity was not influenced by the 1 vol% bovine serum–phosphate buffer system, as mentioned above, indicating that the HIS sensor specifically responded to HIS spiked, even in bovine serum samples.

Using the potential change values at 25 min in Fig. 3 and the regression equation obtained from Fig. 1, the recovery (%) of HIS concentration spiked in bovine serum
samples was calculated, with the results shown in Table 1. In the measured HIS concentration range, the recovery values were 91%–104%, indicating good accuracy. The HIS sensor recognized and responded to free form of HIS. In serum proteins, albumin is a major protein that binds with various substances. HIS has been reported to bind with bovine serum albumin (BSA), with HIS showing two binding models in the HIS–BSA system, in which the low affinity binding model is more dominant than high affinity binding model. This result indicated that spiked HIS existed in its free form in sample solutions, because HIS was spiked in bovine serum and their solutions were diluted to be the 1 vol% bovine serum–phosphate buffer system for measurements. Therefore, close to 100% recovery values were considered to be obtained at any spiked HIS concentration. This study showed that the HIS sensor could be applied to the quantitative determination of HIS in serum samples without pretreatment, such as solid phase extraction and deproteination.

The reported HIS blood concentrations for normal adults and patients with acute coronary syndrome (occlusive type) are 44.87±1.09 ng mL\(^{-1}\) (4.04×10\(^{-7}\) mol L\(^{-1}\)) and 127±6.34 ng mL\(^{-1}\) (1.14×10\(^{-6}\) mol L\(^{-1}\)), respectively. As HIS is mostly stored in mast cells and basophils, the HIS concentration in plasma and serum is much lower than in blood. For future clinical application to the quantitative determination of HIS blood concentration, further improvement of our proposed HIS sensor might be required, such as the necessity of dilution for measurement and enhanced sensitivity at lower HIS concentrations.

**Conclusions**
This was the first report that we have proposed an MIP-based potentiometric sensor for the quantitative determination of HIS concentration in bovine serum samples. This HIS sensor allowed satisfactory measurement of the accurate HIS concentration with recovery values of 91%–104% in a 1 vol% bovine serum–phosphate buffer system. However, the current HIS sensor remains challenges, e.g., the HIS determinations at lower HIS concentrations and in intact serum samples. Toward to future clinical application, we have been gradually going success to enhance the potential response of the HIS sensor by improving the binding affinity between HIS and the MIP through functional group modification on the MIP surface.

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Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.
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Table 1  Recovery (%) of histamine (HIS) concentration in bovine serum samples

| Spiked (mol L\(^{-1}\)) | Found (mol L\(^{-1}\)) | Recovery (%) |
|-------------------------|------------------------|--------------|
| 1.0×10\(^{-3}\)       | 9.07×10\(^{-4}\)      | 91           |
| 3.0×10\(^{-3}\)       | 2.89×10\(^{-3}\)      | 96           |
| 5.0×10\(^{-3}\)       | 4.70×10\(^{-3}\)      | 94           |
| 1.0×10\(^{-2}\)       | 1.04×10\(^{-2}\)      | 104          |
**Figure Captions**

Fig. 1  Calibration curve of HIS sensor as a function of varying HIS concentration. Each bar represents the mean ± standard deviation for three different samples.

Fig. 2  Effect of bovine serum ratio in phosphate buffer on the relative potential change of the HIS sensor. Each bar represents the mean ± standard deviation for three different samples. *$p < 0.05$ and **$p < 0.01$ vs. phosphate buffer alone.

Fig. 3  Potential response curves of HIS sensor in $1\times10^{-3}$ mol L$^{-1}$ phosphate buffer (pH 7.4) for bovine serum spiked with various HIS concentrations. HIS concentrations (mol L$^{-1}$): (A) 0, (B) $1\times10^{-3}$, (C) $3\times10^{-3}$, (D) $5\times10^{-3}$, and (E) $1\times10^{-2}$. 
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