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Determination of histamine in canned tuna by molecularly imprinted polymers-surface enhanced Raman spectroscopy

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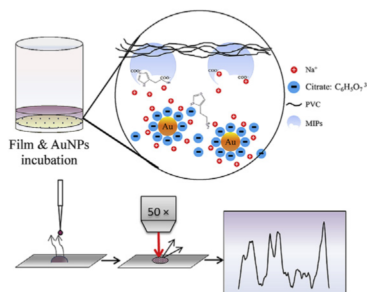
HIGHLIGHTS

- A MIPs-PVC-SERS approach was developed for the first time to determine histamine in tuna.
- This approach is high-throughput and can be finished in 20 min, including sample preparation, separation and detection.
- The generation of this MIPs-PVC-SERS approach is simple and cost effective.

GRAPHICAL ABSTRACT



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ABSTRACT

We introduce a rapid, cost effective and reliable approach to determine histamine level in canned tuna. Molecularly imprinted polymers (MIPs) were synthesized as artificial antibodies towards histamine by utilizing the interaction between histamine and a functional monomer (methacrylic acid) to impress specific binding sites on polymer particles after polymerization. Polyvinyl chloride (PVC) was used to immobilize the MIPs, yielding a MIPs-PVC film that functioned as a recognition element to specifically separate histamine from tuna extract. A gold colloid solution served both as an eluting solvent to extract histamine from MIPs-PVC film and furnish a substrate for surface enhanced Raman spectroscopy (SERS) detection of histamine signals. Principal component analysis together with a partial least square regression (PLSR) model (R^2 = 0.947, RMSECV = 3.526) verified the reliability of MIPs-PVC-SERS approach for the detection and spectral analysis of histamine. Linear regression models were also constructed to relate the intensity of different histamine SERS bands with the corresponding spiking levels. One such model (using a band at 1576 cm^{-1}) performed slightly better in predicting histamine content in tuna than the PLSR model. We conclude that our new MIPs-PVC-SERS approach can rapidly and reliably determine histamine at levels from 3 to 90 ppm in canned tuna meat.

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1. Introduction

Histamine is a biogenic amine that acts to transmit signals from cell to cell in the skin, gut, and other organs of the immune system. Structural differences in receptors on cell membranes account for different histamine responses among individuals [1]. The interaction between histamine and H1 receptors can cause a drop in blood pressure and muscle contraction. H2 receptors to histamine are associated with the secretion of acid by stomach [2]. The ingestion of histamine-rich foods can trigger histamine toxicity manifested as nausea, headache, diarrhea, and asthma. Reactions such as these

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are known as histamine poisoning or scombroid poisoning [3]. Among foods containing histamine, tuna is one of the most extensively consumed. To prevent the potential risk provoked by histamine in foods, the European Community has directed that the average concentration of histamine in fish must be lower than 10 mg/100 g. For canned tuna, the limit of histamine level is normally 3 mg/100 g [4].

Studies have established the principal mechanism of histamine formation in tuna. Tuna contains large amount of free histidine, and the enzyme, histidine decarboxylase, efficiently converts histidine to histamine. The common histamine-producing bacteria, *Morganella morganii*, *Klebsiella pneumoniae*, *Hafnia alvei*, *Citrobacter freundii*, and *Escherichia coli* all possess histidine decarboxylase [5]. High temperature supports the rapid growth of these histamine-forming bacteria [4,6]. When the temperature is above 20 °C, histamine rapidly forms in several tuna species (*e.g.* bigeye, skipjack and yellowfin) and reaches an extremely high level within one or two days, accompanied by the appearance changes in tuna meat (*e.g.* muscle color, texture and odor) [4,6]. Guizani et al. reported that yellowfin could be stored for 17 days at 0 °C without exceeding the safety level of histamine [6]. In another study, the amount of histamine produced in bigeye reached the toxic level after six days of storage at 4 °C [4]. Accordingly, tuna fish must be chilled immediately after harvest to prevent histamine formation. However, low temperature alone is not sufficient to completely suppress bacterial growth and the continuing accumulation of histamine. Once formed, histamine in tuna meat is not destroyed by freezing, canning or cooking [6,7]. Thus, assured food safety demands continuous monitoring of histamine level in tuna.

Conventional methods to determine the histamine level in tuna include high-performance liquid chromatography (HPLC), fluorometry, and enzymatic detection [*e.g.* enzyme-linked immunosorbent assay (ELISA)] [8–11]. HPLC and fluorometry entail time consuming protocols to derivatize histamine by o-phthalaldehyde or dansyl chloride, [9–11]. Fluorometric assay requires methanol extraction, anion exchange column purification, and derivatization as pre-treatment. Because of the structural similarity of histidine to histamine, fluorescence measurement has a poor selectivity for histamine [12], even when aided by HPLC separation [13]. In contrast, enzyme based methods offer a rapid means of detection, but necessitate the use of unstable enzymes and expensive test kits, and tend to overestimate histamine [12].

In addition to the aforementioned conventional methods, recent studies have identified several other effective analytical approaches. Cohen et al. reported a good correlation between HPLC detection and rapid, simple and inexpensive ion mobility spectrometry for the quantification of histamine in tuna [14]. Previous work has explored molecularly imprinted polymers (MIPs), testing their affinity and selectivity for histamine [15–18]. Horemans et al. have employed MIPs as the separation element of sensors relying on electrochemical impedance spectroscopy using a quartz crystal microbalance (QCM) to analyze histamine in aqueous media [19]. Another MIPs-QCM sensor was used to determine histamine in canned fish [18], yielding a limit of detection in the nano-molar range. In these studies and others, MIPs function as a "plastic antibody" featuring thermal and chemical stability and low cost. During the synthesis of MIPs, functional monomers imprint for an interaction with analyte molecules (*e.g.* covalent interaction, H-bonding, electrostatic and hydrophobic interaction), while cross-linking monomers contribute to the rigid structure of MIPs [20].

Surface enhanced Raman spectroscopy (SERS) has attracted a great deal of attention owing to its high sensitivity [21]. A SERS substrate (*i.e.* noble metal nanoparticles or nanoscale roughened surface) amplifies the intensity of normal Raman signal to a remarkable degree. Enhancement arises from both electromagnetic and chemical (charge transfer) effects. Photo-excitation of a noble metal in a nanostructured format generates a localized surface plasmon resonance, increasing the field that inelastically scatters from the sample [22]. Raman enhancement factors can surpass 10^{14} times [22–24]. The SERS technique has been applied in the detection of trace components in foods [25], but the food matrix contributes interfering features to the SERS spectrum, which presents a major challenge to its analytical utility. For this reason, several studies have developed and discussed an integrated MIPs-SERS approach to study the binding between analytes and polymers in a micro- or nano-scale as well as simultaneously separate and detect chemical hazards in the environmental and food samples. For example, Kostrewa and coworkers applied SERS to record the processes of template release and adsorption in MIPs layer and executed it by fabricating MIPs layer on top of a metallic film serving as a SERS substrate [26]. In another two studies, the size of a "two-layer" sensor consisting of a MIPs layer and a metallic layer has been reduced to microscale by employing nano-fountain pen to disperse the MIPs pre-polymer solution onto the SERS substrate surface for polymerization [27,28]. In addition, Xue et al. have developed MIPs-Au nanoparticles in a core-shell structure to detect bisphenol A in water and soft drinks [29]. Other studies have also been conducted to develop a "Ag core-MIPs shell" complex structure for the determination of Rhodamine B [30] and theophylline in green tea drinks [31]. In addition, MIPs were tested as components of solid phase extraction (SPE) cartridge for the separation of racotomine [32–35] or thin layer chromatography (TLC) plates [36] to separate chemical hazards in foods, followed by SERS detection.

The current study introduces a novel approach, combining MIPs with SERS to detect histamine in canned tuna. Dissolved polyvinyl chloride (PVC) serves to distribute MIPs fine particles and produce a MIPs-PVC film. Gold colloid suspended in the eluting solvent is used to desorb histamine off the MIPs and provides SERS enhancement. Methacrylic acid acts as the functional monomer for MIPs synthesis. Cations, such as sodium ion in gold colloid aid elution by disrupting the interaction between the MIPs carboxylic group and histamine (Fig. 1). We have confirmed that the SERS signal obtained by this approach rapidly and accurately quantifies histamine in canned tuna.

2. Materials and methods

2.1. Reagents and materials

Histamine (HA), methacrylic acid (MAA), ethylene glycol dimethylacrylate (EGDMA), azobisisobutyronitrile (AIBN), PVC, chloroauric acid ($HAuCl_4$), trisodium citrate dihydrate, and acetonitrile in HPLC grade were purchased from Sigma Aldrich (St. Louis, MO). L-histidine, hexane, acetic acid, and ammonium acetate (all in HPLC grade) were obtained from Fisher Scientific (Waltham, MA). Tetrahydrofuran (THF) for HPLC was purchased from VWR (Wayne, PA) and ethanol was purchased from Commercial Alcohols (Toronto, ON). Canned tuna fish products were obtained from a local grocery store in Vancouver BC. Deionized (DI) water (18.2 MΩ/cm) was prepared by the Millipore system (Billerica, MA).

2.2. Synthesis of MIPs, fabrication and characterization of MIPs-PVC film

Histamine (0.13 mmol), MAA (0.65 mmol), and EGDMA (3.28 mmol) were dissolved in 20 mL acetonitrile, and AIBN (20 mg) was then introduced. The mixture was incubated at 55 °C for 24 h to achieve polymerization. Afterward, the resulting polymer particles were washed with ethanol and acetic acid (9:1, v/v), followed by pure ethanol to remove any residual histamine captured by

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