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# Multipoint recognition of domoic acid from seawater by dummy template molecularly imprinted solid-phase extraction coupled with high-performance liquid chromatography

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ABSTRACT

Due to the high cost of domoic acid (DA), different carboxylic acid compounds including indole-3-acetic acid (IAA), pyrrole-2-formic acid (PFA), pyridine-2,3-dicarboxylic anhydride (PDA), trimesitinic acid (TA) and citric acid (CA) were investigated as dummy templates for the molecularly imprinted solid-phase extraction (MISPE) for selective isolation and pre-concentration of an amnesic shellfish poison (ASP), DA. The highest binding amount of the polymers towards DA was obtained when CA was used as dummy template owing to its high hydrophilicity. In addition, the "four-point" recognition site constructed by three COOH groups and a OH group in CA was also speculated to be the reason for the high binding amount of CA-MIPs and the rebinding of DA can be depend on the three COOH groups and a NH group with conformational change in the recognition process. Finally, the CA-MISPE column was chosen for DA isolation and pre-concentration and effective result was obtained with recoveries higher than 90% and relative standard deviation (RSD) less than 5% (n = 3). This new polymer can be effectively applied to the monitoring and predicting the existence of trace DA.



## 1. Introduction

Domoic acid (DA), an excitatory and neurotoxic amino acid, is a kind of amnesic shellfish poison (ASP). The first isolation of DA was from red algae, followed by the finding in microalgae of genus *Pseudonitzschia*. It can be accumulated in shellfish, birds and mammals by direct filtration or by feeding on contaminated organisms [1], which would result in many serious symptoms such as nausea, temporary amnesia, persistent memory loss, coma, and ultimately even death [2]. Realizing the seriousness of the problem, many countries have imposed actions with a limitation of 20 $\mu g\,g^{-1}$ DA in wet tissues of aquatic products which can avoid many serious health problems caused by this kind of ASP.

After the first reported outbreak of DA [3], several analytical methods including enzyme-linked immune sorbent assay (ELISA) [4], thin-layer chromatography (TLC) [5], surface plasmon resonance (SPR) [6], capillary electrophoresis (CE) [7], radio immunoassay (RIA) [8] and liquid chromatography-mass spectrometry (LC–MS) [9] were developed. High-performance liquid chromatography-diode array detector (HPLC-DAD) is the most commonly used analytical tool available in the majority of certification and testing authorities for the rapid and effective quantitation of DA.

Owing to the low concentration and complex environmental matrix, it is rather difficult to detect DA directly. In order to prevent its uncontrollable adverse effect on human health, highly effective and practicable methods for the selective recognition, isolation, determination and prediction of trace residues of DA are urgently required. Solid-phase extraction (SPE) owing to its advantages over other pre-treatment methods is the most commonly applied technique for sample clean up and enrichment [10]. However, lack of selectivity and low recoveries [11,12] limited the spread of commercially available SPE cartridges.

Molecularly imprinted polymers (MIPs), artificially tailor-made materials with high selectivity towards the target molecule in preference to other closely-related compounds, have attracted much more attention. Due to the high specific recognition ability, stability and ease preparation, they have been applied to a wide range of fields [13–15]. The combination of MIPs with SPE, so called molecularly imprinted solid-phase extraction (MISPE), combing the specific selectivity of MIPs with the high enrichment efficiency of SPE.

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The use of MIPs for the recognition of DA has been reported in recent years. Since the high cost and unavailable of DA, it became difficult to prepare MIPs using DA as template. Dummy template using other template structural analogues as the substitutes can resolve this problem [16]. Kubo et al. [5] found that *o*-phthalic acid as the template can achieve the best recognition ability towards DA. While his following experiment proved that 1,3,5-pentanetricarboxylic acid (1,3,5-PeTA) can achieve a better result [17]. Both Lin et al. [18] and Zhou et al. [19] prepared MIPs using 1,3,5-PeTA as the dummy template to detect DA from shellfish samples and reached the detection limits of 0.17 $\mu g\,g^{-1}$ and 0.1 $mg\,L^{-1}$, respectively.

In order to investigate the effect of template on the adsorption properties, experiments were carried out using dummy templates with different acidity and water solubility including indole-3-acetic acid (IAA), pyrrole-2-formic acid (PFA), pyridine-2,3-dicarboxylic anhydride (PDA), trimesitinic acid (TA) and citric acid (CA) in this study. Adsorption experiments were performed to optimize the template and to obtain the adsorption capacity of MIPs. Structural calculation and selective experiment were performed to investigate the recognition mechanism of the polymer. Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR) were performed to characterize the morphology and organic group composition of the optimal MIPs. The application of the optimal MIPs as SPE adsorbent for extraction and enrichment of trace DA in seawater samples has been investigated.

## 2. Materials and methods

### 2.1. Reagents and chemicals

DA, IAA, PFA, PDA, TA, kainic acid (KA), CA and trifluoro acetic acid (TFA) were purchased from Aladdin Reagent Company (Shanghai, China). Acetonitrile and methanol of high performance liquid chromatography grade were obtained from Merck (Darmstadt, Germany). Methacrylic acid (MAA) and 2, 2-azoisobutyronitrile (AIBN) were obtained from Kermel Chemical Company (Tianjin, China). Ethyleneglycol dimethacrylate (EGDMA) was obtained from Alfa. All of the water used in the experiments was produced by a Millipore Milli-Q purification system (Milli-pore, Bedford, MA, USA). All reagents were at least HPLC grade or analytical grade unless specified. Stock solution of DA was initially prepared at 100 $mg\,L^{-1}$ in the mixture of acetonitrile and water (v:v, 1:9) and stored at −20 °C in the dark. Working solutions at various concentrations were obtained daily by diluting stock solution of DA using acetonitrile and water.

### 2.2. Instrumentation and conditions

The chromatographic analysis was performed by HPLC-DAD using a Hitachi L-2000 series HPLC system, equipped with a L-2130 Binary Pump, a L-2200 Auto Sampler, a L-2300 Column Compartment and a L-2455 diode-array detector. The analytical column was performed by a LaChrom C18 reverse-phase column (250mm × 4.6mm × 5 μm, Hitachi, Japan) in a thermostated oven at 30 °C. The elution solvent consists of $CH_3CN$ and 0.1% TFA solution with a volume ratio of 10:90. The injection volume was 20 μL with a constant flow rate of 1.0 $mL\,min^{-1}$ and the analytical wavelength of the diode-array detector was set at 242 nm.

A 6210 time-of-flight mass spectrometer (Agilent, USA) equipped with an electro spray ionization (ESI) interface was employed for the certification of DA. An Agilent 1200 series LC system with a degasser, a binary pump, a column oven and an auto sampler was coupled to the MS instrument. Conditions for the detection of DA were optimized as follows: fragmentation voltage, 100 V; spray voltage, 4.0 kV; drying gas velocity, 11.0 $L\,min^{-1}$; drying gas temperature, 350 °C. The mass spectrometer was scanned from *m*/*z* 150 to 650 in full scan mode. Due to the strong polarity of TFA which may attract positive charge and inhibit the ionization efficiency of compounds, formic acid was employed instead of TFA as the mobile phase in the MS detection leading to the difference in retention time.

### 2.3. Preparation of dummy template molecularly imprinted polymers

Five DA molecularly imprinted polymers (MIPs) were prepared using DA structure similar chemicals IAA, PFA, PDA, TA and CA as dummy templates respectively to probe into the effect of template type on the adsorption amount of the polymer. 0.5 mmol of dummy template and 2 mmol of functional monomer MAA were mixed in 15 mL of methanol in a three-neck flask. The mixture was agitated by ultra-sonicate for 15 min to form the pre-polymerization product and then 10 mmol of EGDMA and 30.0 mg of AIBN were added into the solution. After purging with $N_2$ to drive away $O_2$ in the flask, the mixture was thermal-initiated in a 60 °C water bath under $N_2$ atmosphere for 24 h. The obtained polymer was washed by methanol and Milli-Q water and extracted with the mixture of methanol:acetic acid (v:v, 9:1) using a Soxhlet apparatus for 48 h until no template remains. After washing and extraction, the polymer was dried under vacuum at 50 °C. The obtained polymer was ground into particles and sieved with diameters between 150 and 200 μm. For comparison, the corresponding Non-imprinted polymers (NIPs) were prepared using the same formulation without the addition of template.

### 2.4. Characterization of the polymer

The surface and inner morphology of the MIPs and NIPs were observed by a Hitachi S-4800 cold field-emission SEM (Tokyo, Japan). FT-IR was employed to investigate the formation of the organic groups in the polymer by an Avatar-360 Spectrometer (Nicolet Instrument Corporation, USA) using the KBr pellet method in the range of 4000–500 $cm^{-1}$ with a resolution of 4 $cm^{-1}$.

### 2.5. Adsorption experiments

20.0 mg of the MIPs (the optimized polymer obtained in 3.1) and NIPs were mixed with 1 mL of various concentrations of DA standard solution (0–50 $mg\,L^{-1}$) respectively. The mixture was agitated at 25 °C for 24 h and the obtained supernatant was filtrated with 0.22 μm membrane followed by HPLC detection. The adsorption amounts ($Q$, $\mu g\ g^{-1}$) of the MIPs and NIPs were calculated by binding experiments according to Eqs. (1) [20]. Three repeated measurements were performed for each sample, and the mean value was recorded.

$$Q = (C_0 - C_e)V/m \quad (1)$$

In Eq. (1), $C_0$ ($mg\,L^{-1}$) and $C_e$ ($mg\,L^{-1}$) are the initial and equilibrium concentration of DA. $V$ (L) is the total volume of the sample. $m$ (g) is the mass of MIPs or NIPs.

The selectivity experiment of CA-MIPs was conducted using KA (the structure of KA was shown in Fig. 1) as DA structural analogue. 1 mL of 2 $mg\,L^{-1}$ mixture standard of DA and KA was equilibrated with 20.0 mg of MIPs and NIPs, followed by the same treatment as the adsorption experiment above. The imprinted factor (*IF*) and the selectivity factor (α) were employed for the evaluation of the selectivity of CA-MIPs according to Eqs. (2) and (3).

$$IF = Q_{MIPs}/Q_{NIPs} \quad (2)$$