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# Selective enrichment of ochratoxin A using human serum albumin bound magnetic beads as the concentrating probes for capillary electrophoresis/electrospray ionization-mass spectrometric analysis

Chen-Yu Hong [a], Yu-Chie Chen [a,b,*]

[a] *Department of Applied Chemistry, National Chiao Tung University, Hsinchu 300, Taiwan*
[b] *Institute of Molecular Science, National Chiao Tung University, Hsinchu 300, Taiwan*

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**Abstract**

Ochratoxin A (OTA) is a toxicant commonly present in many food products. Conventionally, immuno-affinity analysis is applied to rapidly screen the presence of OTA in food. However, antibodies are expensive. In this study, we present a new approach for selectively enriching OTA from aqueous samples using human serum albumin (HSA) bound magnetic beads as the affinity probes, followed by the analysis of CE/ESI-MS. In addition to demonstrating the feasibility of using the affinity probes to concentrate OTA, we also propose a rapid concentration and elution method for extraction, that is, OTA are extracted from aqueous samples by pipetting the samples in and out of a sample vial for 1 min followed by elution with pipetting for another minute. On the basis of the magnetic property, the affinity magnetic probe–target species could be rapidly isolated from the solution during the process of extraction and elution by magnetic separation. CE/ESI-MS, coupled by the electrodeless/sheathless interface, is used for the analysis of the samples. As this method features speed and cost-effectiveness, it is suitable for the purpose of rapid screening. In fact, the lowest detection limit for OTA is $\sim 4 \times 10^{-3}$ mg/L.


*Keywords:* CE–ESI-MS; Albumin; Magnetic beads; Ochratoxin A

## 1. Introduction

Ochratoxins generally contaminate food such as cereals, coffee, and wine. Ochratoxin A (OTA) and ochratoxin B (OTB) are secondary metabolites of several strains of *Aspergillus* and *Penicillium* [1–9]. OTA is significantly more toxic than OTB by about one order of magnitude [10]. Moreover, it has been demonstrated that OTA may result in several severe diseases [11–14]. However, no methods can remove OTA completely from food products. Thus, developing appropriate analysis methods to monitor the presence of OTA in food products is significant.

Conventionally, sample pre-treatment using immuno-affinity technique [9] and solid phase microextraction [13] to remove interferences are carried out prior to analysis by thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography (GC) [15]. Though solid phase microextraction can concentrate analytes with similar polarities, it lacks selectivity. Meanwhile, immuno-affinity techniques have quite good selectivity for their corresponding target species; however, antibodies are expensive.

We herein propose an alternative approach by using serum albumin as the probe molecules to selectively concentrate OTA from sample solutions because it has been demonstrated that serum albumin can bind with OTA with a high affinity [14–17]. Furthermore, serum albumin can be obtained much easier to be obtained and is cheaper than antibodies. It was Chu [15] who first investigated the binding of OTA with bovine serum albumin using various spectroscopic methods. In addition to bovine serum albumin, several reports have also demonstrated the affinity interactions between human serum albumin (HSA) and OTA [16–18]. Using magnetic beads as affinity probes is quite suitable because it becomes very easy to isolate the

* Corresponding author at: Department of Applied Chemistry, National Chiao Tung University, Hsinchu 300, Taiwan. Tel.: +886 3 5131527; fax: +886 3 5131527.
*E-mail address:* yuchie@mail.nctu.edu.tw (Y.-C. Chen).

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magnetic bead–target species conjugates from sample solutions after extraction by magnetic separation. We previously proposed several approaches using magnetic particles as the affinity probes for target species such as proteins [19,20], peptides [19,20], phosphopeptides [21,22], and pathogenic bacteria [23,24] followed by characterization of matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. Capillary electrophoresis (CE) combined with electrospray ionization-mass spectrometry (ESI-MS) is an alternative analysis method to characterize the trapped species from magnetic affinity probes [25]. As a result, a straightforward electrodeless/sheathless interface combining CE with ESI-MS was demonstrated [25,26]. A pulled bare-capillary tip was used as the ESI emitter, which was not coated with any electrically conductive materials. A high external voltage was not applied on its outlet as well. This interface is very easy to operate. In this study, we employed HSA bound magnetic particles as the affinity probes to trap OTA from sample solutions followed by the analysis of CE/ESI-MS interfaced by the electrodeless/sheathless capillary emitter. A rapid enrichment approach that pipetted the sample solution for just 1 min to vigorously mix and to enrich target species from complex sample solutions could obtain OTA in sufficient quantities for CE/ESI-MS analysis. As a result, the combination of the use of magnetic affinity probes and rapid pipetting approach for OTA extraction followed by electrodeless/sheathless CE/ESI-MS was demonstrated.

## 2. Experimental

### 2.1. Reagents and materials

Acetic acid and iron(III) chloride 6-hydrate were obtained from Riedel de Haën (Seelze, Germany). Acetonitrile, hydrochloric acid, sodium hydroxide, and trifluororacetic acid (TFA) were purchased from Merck (Darmstadt, Germany). Human serum albumin, bradykinin, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), 2-(*N*-morpholino)ethanesulfonic acid hydrate (MES), and 3-aminopropyl triethoxysilane (APTES) were obtained from Sigma (Steinfein, Germany). 3-(2-Aminoethylamino)propyl trimethoxysilane (EDAS), and iron(II) chloride tetrahydrate were obtained from Aldrich (Milwaukee, WI). Ammonium hydroxide and 2-propanol were obtained from J.T. Baker (Phillipsburn, NJ). Ethanol and methanol were obtained from Tedia (Fairfield, OH), while ochratoxin A and tetraethoxysilane were obtained from Fluka (Buchs, Switzerland). The fused silica capillary (50-μm i.d. × 365-μm o.d.) was obtained from Polymicro Technologies (Phoenix, AZ). All wine was purchased from a local grocery store.

### 2.2. Preparation of functional magnetic beads

$FeCl_3$ (5.4 g) and $FeCl_2$ (2.0 g) were dissolved in an aqueous hydrochloric acid (2 M, 25 mL) at room temperature under sonication. After the salts were completely dissolved in solution, the mixture was degassed using a pump followed by filling with nitrogen. The mixture was slowly injected with ammonia (28%, 40 mL) while stirring under nitrogen protection. After stirring for 1 h, the generated particles were collected by aggregating the particles on the edge of the flask through applying the application of a magnet outside of the flask. The particles were rinsed with deionized water five times until the smell of ammonia totally disappeared, while the particles remaining in the flask were re-suspended in methanol (100 mL). A flask containing the iron oxide particle suspension (2.0 mg/mL, 100 mL) was injected with EDAS (2 mL) while stirring in a nitrogen-protected condition, and the mixture was refluxed in an oil bath at 110 °C. After 24 h, the supernatant was removed after aggregating the particles on the edge of the flask through an external magnet. The particles were rinsed three times with methanol and three times with deionized water followed by re-suspension in deionized water (10 mL), which gave the final concentration of this particle suspension ∼11.1 mg/mL.

The silanized magnetic beads obtained above were further covalently bound with HSA. The beads isolated from 2.8 mL of the suspension (11.1 mg/mL), HSA (10 mg), and EDC (100 mg) were mixed in MES buffer (100 mM, 10 mL, pH 6.8) through vortex mixing. After 24 h, the beads were isolated from the solution by using magnetic separation to remove the supernatant. The supernatant (1 mL) was measured by absorption spectroscopy. The absorption change of HSA at the wavelength of 280 nm before and after reaction was used to estimate the binding amount of HSA on the beads. The isolated beads ($Fe_3O_4$@HSA) were rinsed three times with deionized water, followed by re-suspension in deionized water (5 mL). The final concentration of the suspension was 6.9 mg/mL.

### 2.3. Fabrication of the capillary emitter

Either direct ESI-MS analysis or CE/ESI-MS was carried out using a pulled bare capillary as the emitter [25,26]. The inner surface of the capillary was modified by APTES to reverse the direction of electro-osmotic flow. The capillary was sequentially flushed with NaOH (0.1 M) and deionized water for 30 min using a pump. An APTES (10 mM) solution was flushed into the capillary for 10 min followed by successive flushes with deionized water (for 10 min) and air (for 10 min) using a pump (pressure: 18 mmHg). The modified capillary was placed in an oven at 110 °C for 90 min to strengthen the cross-linking of APTES on the capillary wall. The capillary was flushed with deionized water for 10 min using a pump. After letting it stand at room temperature overnight, the capillary was fabricated by applying a small weight (50 g) on the lower end of a vertical capillary. The lower part of the capillary was heated and then quickly drawn to form a narrow capillary tip. After cooling to room temperature, the capillary tip was immersed in HF solution (24%) for 10 min. The capillary was ready for the use in CE/ESI-MS after rinsed by methanol.

### 2.4. Enrichment of OTA by $Fe_3O_4$@HSA magnetic beads followed by CE/ESI-MS analysis

OTA is a photosensitive chemical, so it is required to wrap the sample vials with aluminum foil during the sample treatment