学 位 論 文 審 査 の 要 旨

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Development of Microfluidic Paper-based Devices for Food Analysis
（食品分析のためのマイクロ流体ペーパー分析デバイスの開発）

Food and water contamination cause safety and health concerns to both animals and humans. Conventional methods for monitoring food and water contamination are often laborious and require highly skilled technicians to perform the measurements, making the quest for developing simpler and cost-effective techniques for rapid monitoring incessant. Since the pioneering works of Whitesides ' group from 2007, interest has been strong in the development and application of microfluidic paper-based analytical devices (μ PADs) for food and water analysis, which allow easy, rapid and cost-effective point-of-need screening of the targets. Several methods of detection using μ PADs have been developed so far including colorimetric, electrochemical, fluorescence, chemiluminescence, and electrochemiluminescence techniques for food and water analysis. In this research, we have developed μ PADs for the detection of aflatoxin B1 (AFB1), a highly toxic and carcinogenic foodborne substance and the most toxic aflatoxin produced by Aspergillus fungi, via a simple colorimetric competitive immunoassay. AFB1 is a common contaminant in a variety of agricultural as well as processed food products including peanuts, corn and other grains, cottonseed meal, as well as animal feeds. The maximum permissible levels set by several food agencies are 5 μ g/kg for AFB1 and 20 μ g/kg for total aflatoxins. However, more rigorous regulations for AFB1 and total aflatoxins in groundnuts, nuts, dried fruits and cereals have been set to 2 μ g/kg and 4 μ g/kg, respectively, by the European Union. Hence, it is highly necessary to device a practical method to detect AFB1 for food safety and monitoring.

In this context, the general introduction including the research theme and objectives are described in Chapter 1.

In chapter 2, the development of a simple, portable assay system using μ PADs coupled with colorimetric detection for rapid measurements is described. The properties of different paper substrates are investigated first to determine which type of paper would be the most suitable for the fabrication of the μ PADs. Simultaneous detection of horseradish peroxidase (HRP) is demonstrated using a single μ PAD, which is fabricated through photolithography. The test regions are immobilized with 3,3 ' ,5,5 ' -tetramethylbenzidine for HRP assay. The detection range obtained with the proposed system covers HRP concentrations from 0.37 to 124 fmol (or 3 to 1000 ng/mL). The detection limit (blank + 3 σ) for HRP is calculated to be 0.69 fmol (or 5.58 ng/mL) through a 4-parameter logistic nonlinear regression. The findings obtained using the developed system suggest that μ PAD assay systems for simple but highly sensitive measurements can be designed to give on-site determinations of target compounds using peroxidase-conjugated molecules.

Chapter 3 describes the development of a competitive immunoassay system on a μ PAD platform. The photolithographically fabricated μ PADs consist of a sample introduction zone, control and test zones located at the other end of the μ PAD and are opposite to the sample introduction zone, and a capture zone wherein a capture reagent is immobilized allowing competition during immunoassay. The colorimetric detection similarly involves TMB-H2O2 reaction to produce the blue colored TMB/dimiine complex in the presence of peroxidase enzyme conjugated to antibody. The color intensity generated at the test zone after TMB oxidation increases with increasing target concentration introduced at the sample zone, but remains constant at the control zone. The developed competitive immunoassay system using μ PADs is tested first using biotin as the model compound. In the present work, the detection limit for the competitive immunoassay of biotin on μ PADs is 0.10 μ g/mL. To demonstrate the versatility of the developed competitive immunoassay system further for the detection of target compounds on μ PADs for practical applications, AFB1 has been detected as well. The detection limit obtained for AFB1 using the developed μ PAD immunoassay system is 1.31 ng/mL.

In chapter 4, two competitive immunoassay (CI) systems are described for the detection of AFB1. Using a different μ PAD platform from the one used in the previous chapter, the μ PAD immunoassay system similarly consists of a reaction zone, a sample introduction zone, and a capture zone. In both CI systems, detection is performed at the reaction zone via TMB oxidation by hydrogen peroxide in the presence of peroxidase conjugate. However, in the first CI system (CI-S1), competition occurs at the capture zone and signal intensities at the reaction zone increases with increasing target AFB1 concentration. In CI system 2 (CI-S2), on the other hand, competition takes place prior to sample introduction. Similarly, signal intensity increases with increasing target AFB1 concentration. In all sections of the manuscript, images of the μ PADs are captured and colorimetrically analyzed via ImageJ software for quantification.

The final chapter is the summary of the findings in the present research. In addition, several prospects on μ PAD analysis for future research are described in this chapter.

Based on the review and interview of the doctor thesis, this study can be judged to be very significant and valuable from the viewpoints of scientific research and application potential. Ph.D. degree in Chemical Sciences and Engineering should be awarded to the candidate.