CHAPTER 1 INTRODUCTION

1.1 Research Background

Developing countries are often reported frequently occur outbreak of food borne disease. In particular, gram negative bacterial pathogen accounts for approximatrely 63% cases in Thailand in 2005 [1]. Gram-negative bacteria that often cause outbreaks is *Salmonella*. They are harmful to human health and can lead to death. *Salmonella* infections are associated with consumption of undercooked meat and dairy product because they are commonly found in gastrointestinal of animal and contaminated through animal feed. Animal feeds can potentially become contaminated with *Salmonella* either during harvesting, processing at the feed mill or during storage [2, 3].

Several methods used for detection of *Salmonella* include polymer chain reaction (PCR) [4], multiplex PCR [5], real time PCR [6], immunomagnetic separation (IMS) [7], electrochemistry [8], and enzyme-linked immunosorbent assay (ELISA) [9]. However, these methods have several drawbacks such as sophisticated equipment needed, high operational skill requirement, expensive and time consuming [10]. Detection assay need not only fast and cost effective methodologies but also high sensitivity and selectivity. Accordingly, optical biosensors have gained increasingly interest due to their speed, readily multiplexed, and samples can be interrogated with many wavelengths simultaneously without interfering with one another. The optical biosensor format may involve direct detection of the target of interest or indirect detection through optically labeled probes [11].

Various methods for optical technique have been developed. One of the most well-known approaches for detecting DNA or protein is a scanometric detection developed by Mirkin and co-worker [12]. Gold nanoparticles (AuNPs) functionalized with DNA probe were hybridized with target DNA and the complex of DNA functionalized AuNPs and DNA target were cohybrized to DNA that was immobilized on a substrate. Then, silver enhancer was used to facilitate visualization of nanoparticle labels hybridized on the array surface. Silver ions are reduced by hydroquinone to silver metal at the surfaces of the AuNPs. These methods allowed target DNA to be detected in low concentrations by either the naked eye or with a scanner (so-called scanometric detection) without expensive detection systems. This method has high sensitivity and a limit of detection as low as 50 fM of target DNA [12].

AuNPs have been applied as probes in many studies for protein detection using a scanometric detection strategy. Amplification signal employing silver enhancement allowed the target spot to be visible. Silver ions were reduced at the surface of gold nanoparticle resulting in enlargement of particles [12]. Gupta and co-worker reported on sandwich immunoassay using a combination of gold nanoparticle labels and silver amplification technique. Primary antibodies (monoclonal goat anti-mouse (GAM IgG) and goat anti-rabbit (GAR IgG) antibodies) were immobilized on solid substrate to bind the target (mouse (M IgG) and rabbit (R IgG) antibodies) in solution. Secondary antibody (goat anti-mouse (GAMg IgG) and goat anti-rabbit (GARg IgG) antibodies) as labeling with AuNPs was bound to target and enhancement the signal by silver enhancer. This method

have limit of detection as low as 0.1 $\mu g/cm^3$ [13]. Kim and co-worker developed a scanometric immunoassay that uses the light scattering of antibody-oligonucleotide AuNP conjugates that developed with silver or gold enhancing solution for signal readout. This method was capable of detecting 300 aM of prostate specific antigen [14].

Other nanomaterial that has a wide range of applications in biosensor is carbon nanotubes (CNTs). CNTs have high surface area that allow accessible to immobilization of biomolecules or other nanomaterials. In addition, their unique chemical, electrical, and mechanical properties have led to a varieties of applications, such as nanoelectronics, scanning probes, and electrocatalytic activity [15]. CNTs have been applied in many studies including using CNTs to immobilize enzyme to enhance ELISA signal for *S. enterica* serovar Typhimurium detection and could achieve limit of detection (LOD) of 10^3 and 10^4 CFU/mL for direct and sandwich ELISA, respectively [16]. Yang and co-workers develop an optical CNT immunosensor for the detection of Staphylococcal enterotoxin B (SEB) in food. Anti-Staphylococcal enterotoxin B antibodies were immobilized onto the CNTs surface through electrostatic interaction and was bound onto a polycarbonate film. SEB was detected by a sandwich-type ELISA assay on the polycarbonate film. The signal was approximately 6 – 8 times larger than the standard immunosensor for Staphylococcal enterotoxin B detection in food with limit of detection as low as 0.1 ng/mL [17].

Recently, combination of CNTs and AuNPs has provoked great interest in the area of sensors due to the combination of unique properties of these materials. Huang et al. reported the combination of multiwalled carbon nanotube-gold nanoparticle (MWCNT-AuNP) composites and chitosan presented a novel sensitive molecularly imprinted electrochemical sensor for selective detection of tyramine. MWCNT-AuNP composites were introduced for the enhancement of electronic transmission and sensitivity. In this case, tyramine was used as the template molecule, silicic acid tetracthyl ester and triethoxyphenylsilane as the functional monomers on molecularly imprinted polymer (MIP). The molecularly imprinted film displayed excellent selectivity towards tyramine [18]. Chauhan and Pundir fabricated an amperometric uric acid biosensor by immobilizing uricase onto gold nanoparticle (AuNPs)/multiwalled carbon nanotubes (MWCNTs) layer deposited on Au electrode via carbodiimide linkage. In this work, AuNPs were dispersed on the surface of MWCNTs that provide a large available surface and enhance the electrocatalytic activity for H_2O_2 electrooxidation [19].

The particles that traditionally used for concentration, separation, purification and identification of molecules are magnetic particles. The magnetic properties of some nanoparticles have been used as labels in biosensing [20, 21]. Several immunomagnetic biosensors have been developed for detection for example Liébana et al. reported magneto immunosensing for *Salmonella* detection in milk. Magnetic beads was used to capture and preconcentrate bacteria from milk samples. Peroxide functionalized secondary polyclonal antibody was used as serological confirmation with electrochemical detection based on magneto-electrode. With the electrochemical measurement this method gave limit of detection as low as 0.108 CFU/mL (2.7 CFU in 25 g of milk) [22]. Alefantis et al. developed a method for Staphylococcal enterotoxin B (SEB) detection. Two-antibody systems were used where one antibody was attached to a magnetic bead and the other was labeled with Alexa fluor 647. The fiber optic spectrometer was used for measurement with

fluorescence intensity at 665 nm, and this method gave limit of detection as low as 100 pg of SEB [23]. Fan et al. reported sensitive chemiluminescent (CL) immunoassay using magnetic separation and colloidal gold label. Primary antibody was immobilized on the surface of magnetic beads, and second antibody was labeled with colloidal gold. Gold particle that attach on surface of magnetic bead was oxidized and a large number of Au^{3+} from each gold particle determined by luminol CL reaction. This method gave a limit of detection 3.1×10^{-12} M human IgG [24].

This research aimed to combine great properties of CNTs, AuNPs, magnetic beads and specificity of antibody to construct a new type of immunoassay based on a scanometric detection to detect *Salmonella enterica* serovar Typhimurium. Gold nanoparticles (AuNPs) were attached on the surface of multiwalled carbon nanotubes (MWCNTs) to form MWCNTs/AuNPs nanocomposite. The high surface areas of MWCNTs would allow a large number of AuNPs deposition which could result in improvement of the sensitivity of scanometric detection. Magnetic beads were used for separation of the bound from the unbound MWCNTs/AuNPs nanocomposite to provide precise detection.

1.2 Objectives

The objective of this research is to improve sensitivity and selectivity of *Salmonella enterica* serovar Typhimurium detection using scanometric assay based on MWCNTs composited with AuNPs.

1.3 Scope of Work

1. Fabrication of MWCNT/ AuNPs nanocomposite.
2. Immobilization of antibody onto MWCNT/AuNPs.
3. Optimization conditions of sandwich immunoassay such as concentrations of capture antibody, MWCNTs/AuNPs and magnetic beads and silver enhancer development time.
4. Evaluation of sensitivity and selectivity of immunoassay.

1.4 Expected Output

MWCNT/AuNPs nanocomposite can detect *S. enterica* serovar Typhimurium with high sensitivity and selectivity based on scanometric detection.