CHAPTER I

INTRODUCTION

1.1 Statement of Problem

Shrimp diseases due to viral and bacterial infections have caused major economic losses in Thai shrimp farming during the past decade. Conventional immunoassay methods for the determination of bacteria include enzyme-linked immunosorbent assays (ELISA), radio immunoassay, and fluorescence-labeled antibody assays. However, they are expensive, use hazardous materials and complex procedures during operation, and require skilled labor and long analysis time.

Recently, piezoimmunosensors namely quartz crystal microbalance (QCM), which use a quartz piezoelectric (P/Z) crystal detector as the transducer, have been developed for the assay of biological substances and microorganisms in the food industry, for the monitoring of environmental pollutants and for clinical diagnostics. Unlike previous enzyme-linked immunoassay or radio immunoassay procedures, which require an enzyme label, QCM provides a direct non-labeling method for the assay of bacteria.

Procedures to deposit functionalized monolayers for the immobilization of biological compounds onto metal surfaces using the self-assembled monolayer (SAM) technique have been reported in the literature for studies in electrochemistry, quartz crystal balances, and immunosensors. There are several advantages using the SAM techniques to develop *Vibrio harveyi* piezoimmunosensor. First, an organized monolayer offers the least resistance for the entrance of analyte to the active sites of the coating material, as compared to conventional coating using interconnecting channels, and hence, it could reduce the response time of the piezoimmunosensor to attain equilibrium. Second, the assembly of a SAM is relatively easy and less dependent on special technique and equipment, because a SAM layer is formed spontaneously by the immersion of an appropriate substrate into a solution of an

active surfactant in an organic medium. Third, a stable and ordered film can be formed by a SAM in an ambient environment.

In this study, a piezoelectric immunosensor is developed for detection of *Vibrio harveyi*. The procedure includes: (1) a formation of self-assembled monolayer (SAM) of carboxyl-terminated alkanethiol on gold-coated quartz crystal surface, (2) an activation of carboxyl groups by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) and *N*-hydroxysuccinimide (NHS), (3) an attachment of the monoclonal antibody to the activated carboxyl groups, and (4) a detection of the target bacteria. Condition for each step is optimized. Stepwise modification is mainly followed by QCM.

1.2 Objectives

1. To determine an appropriate condition for immobilization of monoclonal antibody against *Vibrio harveyi* on gold-coated substrate.
2. To detect *Vibrio harveyi* using gold-coated substrate immobilized with monoclonal antibody by quartz crystal microbalance.

1.3 Scope of Investigation

The stepwise investigation was carried out as follows.

1. Literature survey for related research work.
2. To form self-assembled monolayer (SAM) of carboxyl-terminated alkanethiols on gold electrodes of quartz crystal microbalance.
3. To activate carboxyl groups of the monolayer with coupling reagent, *N*-hydroxysuccinimide/1-ethyl-3-(3-dimethylaminopropyl) -carbodiimide (NHS/EDCI).
4. To immobilize monoclonal antibody onto the activated carboxyl groups.
5. To detect the target bacteria, *Vibrio harveyi*.