Chapter 1
Introduction

1.1 Background and Rationale

The determination of trace amount of antigens, bacterial antigens, drugs, viruses, and many other proteins is in need of an analytical device that is specific and sensitive to target analyte. Especially when the samples contain very low concentration of target analyte and are with the presence of interfering substances. In recent years several research groups have turned their attention to the development of analytical devices which combine the principle of immunology with electrochemistry that is called immunosensors.

Immunosensors belong to a class of biosensors called affinity biosensors. They are based on binding interactions between the immobilized biomolecule and the analyte of interest (Mattiasson, 1984; Taylor, 1991). Immunosensors are defined as analytical devices that detect the binding of an antigen to its specific antibodies or antibody fragments by coupling the immunochemical reaction to the surface of a device known as a transducer (Gizeli and Lowe, 1996; Luppa *et al.*, 2001; Wang, 2000). Among the many types of transducers electrochemical transducers offer good possibilities for sensitive detection of target analyte.

Detection of immunointeraction using electrochemical principles can be performed directly without any label or indirectly using label elements. Indirect immunosensors are derived from the immunoassay technology, where signal generation is significantly facilitated. However, this is expensive, time-consuming and makes real-time measurements impossible (Berggren *et al.*, 2001). Therefore, many electrochemical immunosensors capable of direct and specific measurement of very low protein concentration have been investigated.

Direct electrochemical immunosensors have been developed using several types of transducers such as potentiometric (Fu *et al.*, 2004; Tang *et al.*, 2004b; 2004c; Taylor *et al.*, 1991), amperometric (Jung *et al.*, 2005; Ramanaviciene

and Ramanaviciene, 2004; Stefan and Bokretsion, 2003; Zhang *et al.*, 2005), conductimetric (Hianil *et al.*, 1999; Kanungo *et al.*, 2002; Yagiuda *et al.*, 1996) and impedemetric (Katz and Willner, 2003; Kharitonov *et al.*, 2000; Tang *et al.*, 2004a). Capacitive transducer has also been investigated as a highly sensitive approach (Berggren and Johansson, 1997; Berggren *et al.*, 1998; Bontidean *et al.*, 1998; 2000; Hedström *et al.*, 2005; Hu *et al.*, 2002; 2005; Jiang *et al.*, 2003). These immunosensors have advantages such as, specific and sensitive to target analyte, can detect the analyte directly without the need for a label, short analysis time, can be developed for various substrates and simplification (Berggren, 2001; Ghindilis *et al.*, 1998; Luppa *et al.*, 2001; Rogers, 2000). The work presents in this thesis describes the investigation of direct, label-free capacitive immunosensors for selected analytes.

For biosensors, immobilization is also an important part, especially in capacitive immunosensor since the electrode surface has to be electrically insulated. Capacitive biosensors often used self-assembled monolayers (SAMs) of sulfur compounds on gold for immobilization (Berggren and Johansson, 1997; 1998; Hedström *et al.*, 2005). SAMs that are widely used include thioctic acid (TA; $S_2C_7H_{13}$-CO_2H) (Berggren and Johansson, 1997; Berggren *et al.*, 1998; Disley *et al.*, 1998; Hedström *et al.*, 2005; Liu *et al.*, 1999) and 3-Mercaptopropioic acid (MPA; $HSC_2H_4CO_2H$) (Disley *et al.*, 1998; Sawaguchi *et al.*, 2001; Vaughan, *et al.*, 1999). Amine modified SAM, such as 2-Mercaptoethylamine (MEA; HSC_2H_4-NH_2), was also an effective grafting material to immobilized the protein (Jiang *et al.*, 2003). Since, TA, MPA and MEA are rather expensive part of this work is to develop a procedure for a new self-assembled monolayer for capacitive immonosensor using cheaper thiol solution, that is, thiourea. To our knowledge no one has applied it to immunosensors.

1.2 Objectives of the research

The aims of this study are to develop and evaluate the performances of capacitive affinity biosensors, to use these biosensors to directly analyze antigens concentration in real samples, and to validate the results with conventional methods. To reach these objectives four subprojects using the capacitive detection principle were carried out as follows;

1. initial study of the system by investigation direct detection of protein affinity reaction of two affinity binding pairs, human serum albumin (HSA) and anti human serum albumin antibody (anti-HSA), and crystallizable fragment (Fc-fragments) from IgG (anti-HSA) and protein A.
2. monitoring of endotoxin in fermentation liquid
3. preparation of a new self-assembled monolayer for capacitive immonosensor using thiourea compared to thioctic acid and 3-mercaptopropioic acid

and

4. development of a reusable immunosensor for carcinoembryonic antigen (CEA) detection using thiourea modified gold electrode.

1.3 Benefits

It is expected that this capacitive biosensor technique, with all its advantages, *i.e.*, direct detection, reusability, high sensitivity, accuracy and precision, and low analytical time will become an alternative approach to detect trace amount of affinity binding analytes.