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Preparation and characterization of ethylenediamine and cysteamine plasma polymerized films on piezoelectric quartz crystal surfaces for a biosensor

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Abstract

This paper describes a method for the modification of quartz crystal surfaces to be used as a transducer in biosensors that allow recognition and quantification of certain biomolecules (antibodies, enzymes, proteins, etc). Quartz crystal sensors were modified by a plasma based electron beam generator in order to detect the level of the toxin histamine within biological liquids (blood, serum) and food (wine, cheese, fish etc.). Cysteamine and ethylenediamine were used as precursors in the plasma. After each modification step, the layers on the quartz crystal were characterized by frequency measurements. Modified surfaces were also characterized by contact angle, X-ray photoelectron spectroscopy and atomic force microscopy to determine the physical and chemical characteristics of the surfaces after each modification. Finally, the performance of the sensors were tested by the response to histamine via frequency shifts. The frequency shifts of the sensors prepared by plasma polymerization of ethylenediamine and cysteamine were approximately 3230 Hz and 5630 Hz, respectively, whereas the frequency change of the unmodified crystal surface was around 575 Hz.

Keywords: Quartz crystal; Electron beam; Glow discharge; Cysteamine; Plasma polymerized films; Histamine

1. Introduction

An immunosensor is a tool, which incorporates an immunologically active sensing element with a transducer to perform the assay of antigen/antibody binding. Potentiometer, optical, amperometric and pieozoelectric crystal devices can be used as physical transducers in an immunosensor and this has brought about a new generation of immunoassay devices. Such devices are small, self contained, specific, cheap and robust. The transducers of these devices convert signals from the different physical changes resulting from the immunological reaction into an electrical output which indicates that an interaction between antigen and antibody has occurred.

Piezoelectric crystal devices provide the advantage of a direct measurement of biologically active molecules where there is no need for labelling and use of additional chemicals. Antigen–antibody affinity reactions can be determined directly by measuring the frequency shift, which corresponds to a mass change on the quartz crystal surface [1,2]. In such devices, a quartz crystal wafer is sandwiched between two electrodes. The electrodes provide the connection between the reaction surface and the external oscillator circuit, which drives the quartz crystal at its resonant frequency. This device known as the quartz crystal microbalance (QCM) was first introduced as a mass sensor in gas phase and in vacuum. The relationship between the frequency change and the mass loading on the crystal surface is described by the Sauerbrey equation [3]

$$\Delta f = -2.3 \times 10^{-6} f^2 \Delta m / A$$

where, Δf is the frequency change of the crystal resonance, f is the fundamental frequency of the crystal (in Hz), A is the surface area (in m^2) and Δm is the deposited mass (in g).

The construction of the recognition layer on the quartz crystal surface is very important; a sensitive procedure is needed to incorporate the biological element to the transducer. In

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general, the crystal surface is modified with a polymer layer to obtain an active surface interacting with a biomolecule. The conventional methods of preparing such a layer is by pre-coating with polyethylenimine, cross-linking with bovine serum albumin, silanization with 3-aminopropyltriethoxysilane or gold-protein A complex formation [4–6]. The immobilization of antibodies for the bioactive layer is carried out by various methods such as passive adsorption, covalent attachment, polymer or gel entrapment, cross-linking, electropolymerization, and photo-immobilization. In studies related with the modification of the quartz crystal surface, a large number of compounds have been employed for example, simply Protein A or thin saline layer [7] to *o*-phenylenediamine [8], polyethyleneoxide, polypropyleneoxide triblock copolymers [9], alkanethiol and amino-alkoxysilane monolayers, polyethylenimine-glutaraldehyde and plasma coatings [10], copolymer coating of hydroxyethylmethacrylate and methylmethacrylate [11] and ethylenediamine plasma polymerized film matrices [12]. Preparation methods of the polymer layer and also the antibody immobilization methods given in these papers include some drawbacks to define the sensitivity and the reliability of the sensor.

The functional layer in quartz crystal immunosensors is usually achieved by using amine-functionalized coatings of thiols, silanes and some polymers [13]. Recently, the coatings of thiols for surface plasmon resonance or quartz crystal microbalance (QCM) device were mostly studied due to its simplicity and versatility. The method was applied by the deposition of alkanethiols on gold surface. This application gives a self-assembled monolayer, which is obtained by immersion of an appropriate substrate into the solution of a surfactant in an organic solvent [14–16]. There are several types of self-assembled monolayers, e.g. organosilicon on hydroxylated surfaces, alkanethiols, dialkylsulphides and disulphides on gold and other noble metals, carboxylic acids on aluminium oxide [17,18]. The dip coating method reported in these studies requires a drying stage in air and a washing stage to remove excess unbound material after the quartz crystal is immersed in a reagent solution. So, the applied washing and drying stages after the interaction may cause a change in the quantity of the immobilized antibody which may result in variability in the mass of polymeric film on the quartz crystal [19]. Thiol substituted monolayer films, which involve sulphur–gold linkages, can used for the immobilization of antibody. However, as reported in literature, some drawbacks exist such as the desorption of thiols which implies instability of the obtained layer [20]. To overcome these drawbacks, thin and stable polymeric films for quartz crystal modification may be superior to self-assembled layer techniques.

The plasma polymerization technique, providing a thin and adherent layer may be considered as an attractive method. In this technique, plasma, which can be regarded as the fourth state of matter is composed of highly excited atomic, molecular, ionic, and radical species. It is typically obtained when gases or vapors are excited into energetic states by radio frequency (rf), microwave, or electrons from a hot filament discharge [21]. Plasma is a highly reactive chemical environment in which many plasma-surface reactions occur. Plasma-surface modification as an economical and effective materials processing technique is gaining popularity in the biomedical field. It is possible to change in continuum the chemical composition and properties such as wettability, metal adhesion, dyeability, refractive index, hardness, chemical inertness, lubricity, and biocompatibility of material surfaces. Several plasmas can be created within the glow-discharge apparatus, which modify the surfaces by depositing films having functional groups. These plasma polymerized films covering crystal surfaces are extremely thin and homogenous. The obtained layer generally adheres strongly to the crystal, and is highly resistant to chemical and physical treatments. The sensors produced by using this method are more reproducible from sample to sample and exhibit lower noise than sensors made by using the conventional immobilization methods.

In our previous studies, the radio frequency (13.56 MHz) plasma polymerization technique was used for surface modification of various materials such as membrane, particles and quartz crystals. The modified surfaces were used for biosensor, separation and adsorption studies [22–25]. In the biosensor group of these applications, ethylenediamine was generally used as a monomer to modify quartz crystal surfaces [22–24]. In this study, quartz crystal surfaces were modified by plasma based electron beam generator using cysteamine and ethylenediamine to obtain a functional crystal surface to which the antibody was bound. The advantage of the electron beam generator over the conventional plasma devices is that the plasma medium can be obtained from solid, liquid or gas phase materials. Cysteamine in solid state was directly placed into the reactor, but the ethylenediamine, in liquid state was fed from the monomer tank to the reactor under vacuum condition. The polymerization of cysteamine and ethylenediamine carried out by this technique gives a good approach for the application of the plasma polymerization of thiols on crystal surfaces. In the final step of the quartz crystal surface preparation, anti-histamine was immobilized in order to detect histamine. Histamine is a biogenic amine, which causes poisoning by ingestion of food which contains high levels of histamine. Consequently, the detection of histamine was chosen as a target because of the importance of the estimation of the levels of biogenic amines in food [27–29].

In this paper, we describe the preparation of a quartz crystal sensor surface by plasma polymerization technique employing thiol and amine compounds, and characterization of the modified surfaces by X-ray photoelectron spectroscopy (XPS), contact angle and atomic force microscopy (AFM) for each step. Finally, the prepared mass sensitive immunosensor was checked for the detection of histamine.

2. Experimental details

2.1. Materials

The quartz crystals (AT-cut, 10 MHz, with gold electrode) were supplied by ICM (International Crystal Manufacturing Co., Inc., USA). Etylenediamine (EDA) was supplied by BDH (UK), and glutaraldehyde was purchased from Aldrich (UK). Cysteamine (CS) and anti-histamine was purchased from Sigma (UK). All other reagents (Na_2HPO_4, $KHPO_4$, NaOH, HCl) used