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Analytica Chimica Acta 365 (1998) 147–149

ANALYTICA CHIMICA ACTA

Monitor of antibodies in human saliva using a piezoelectric quartz crystal biosensor

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Received 30 May 1997; received in revised form 20 September 1997; accepted 13 October 1997

Abstract

A piezoelectric quartz crystal immunosensor has been developed for the detection of antibodies in diluted human saliva. The system consists of the quartz piezoelectric crystal, oscillator and frequency counter. The antibodies against human immunoglobulin A are immobilized onto the gold electrode surface on the quartz crystal. The immobilization techniques used in this work are the adsorption method and the covalent bond method. The latter gives more stability in repetitive assays.

Keywords: Saliva; Antibody; Immunoglobulin; Piezo electric quartz crystal; Biosensor

1. Introduction

The linear relationship between the frequency change of an AT-cut piezoelectric quartz crystal and the mass deposited on the crystal [1] is very useful in a micro-gravimetric immunosensor [2,3] and DNA sensor [4,5]. Human immunoglobulin A (IgA) in saliva in the range of several hundred microgram quantities has been determined by enzyme-linked immunosorbent assay (ELISA). The method using piezo electric quartz crystal is simple for the direct detection of an immunoreaction in real time but with low repeatability. In this study, immobilization of the antibodies against human immunoglobulin A (anti-IgA) onto the electrode surface is examined and the immunoreaction between anti-IgA and antibodies in diluted human saliva are monitored.

2. Experimental

Fig. 1 shows the structure of the quartz crystal biosensor. Mouse monoclonal antibodies against human IgA were immobilized on the gold electrode of the quartz crystal. Immobilization methods of anti-IgA were adsorption or the covalent bonding onto the surface of the crystal.

Immobilization by adsorption used a solution of anti-IgA (2 mg Bio-ZyMED) in 10 mmol sodium acetate buffer. The solution (20 μl) was dropped on one side (liquid phase) of a quartz crystal and stood for 24 h at 37°C. After the solution was removed, the electrode surface with immobilized anti-IgA was washed with phosphate buffer solution (PBS pH 7.4).

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0003-2670/98/$19.00
PII S0003-2670(97)00596-5

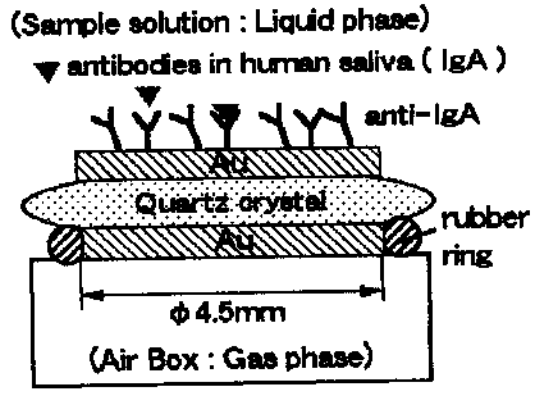

Fig. 1. Structure of a piezoelectric quartz crystal biosensor and antibodies.

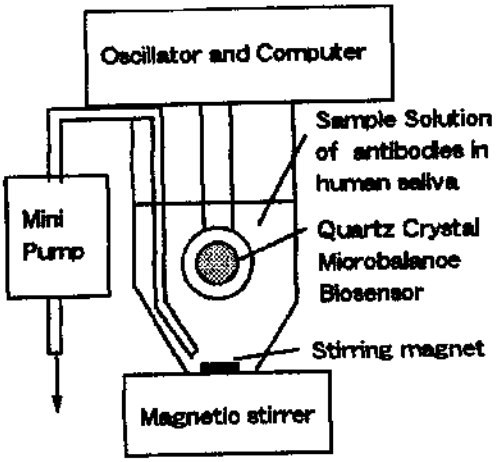

Fig. 2. Monitoring system of antibodies in human saliva.

Immobilization by covalent bonding was carried out by the reaction between the gold electrode and cysteamine. After reaction with an ethanol solution of cysteamine(1 mmol, 11.3 mg/100 ml pH, 6.4) for 1 h at room temperature, 15 μl of anti-IgA solution (1 mg ml^{-1}) and 5 μlof 1-ethyl-3-(3-dimethylamino-propyl)carbodiimiide HCl solution (5 mg ml^{-1}) in sodium acetate were mixed and stood for 24 h at 4°C.

One side of the quartz crystal was set on the O-ring sealed air box. On the other side of the crystal for sample liquid, anti-IgA was immobilized.

Fig. 2 shows the measuring apparatus for immunoreaction between immobilized anti-immunoglobulin and immunoglobulin in a sample solution. The crystal was oscillated by an oscillator (Sogo Pharmaceutical, SF-105) at a basic frequency of 9 MHz. The oscillation frequency was measured and fed into a computer (Biopac System, MP100A-Macintosh 5300 and PC9800). Sample solution (10 ml) with standard human IgA (Seikagaku Kogyo: IgA from human saliva) was prepared in the range of 1 to 3 μg ml^{-1}

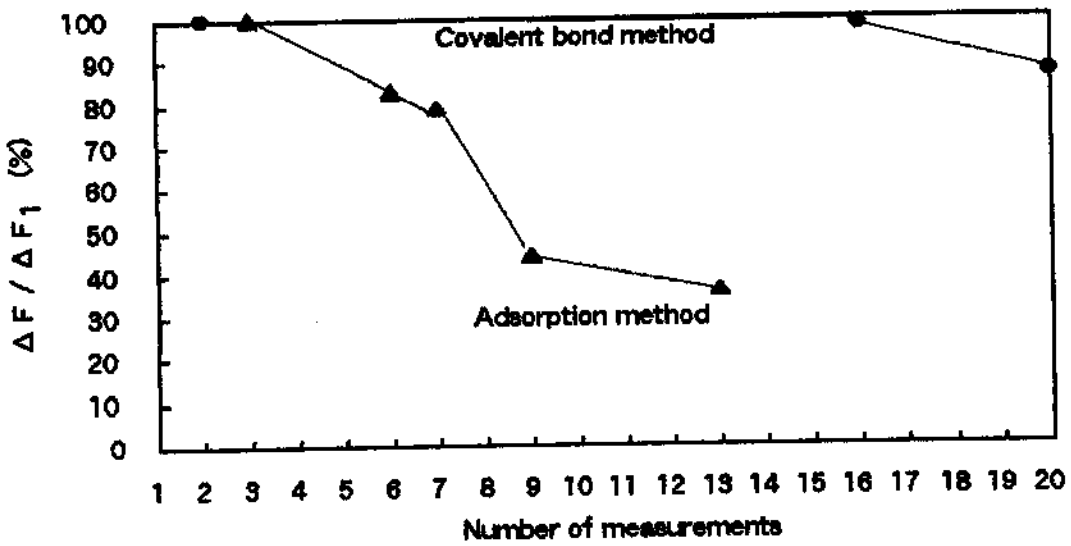

Fig. 3. Repeatability on the frequency change (ΔF) of quartz crystal biosensor immobilized by the adsorption method or covalent bond method. The first frequency change is ΔF1.

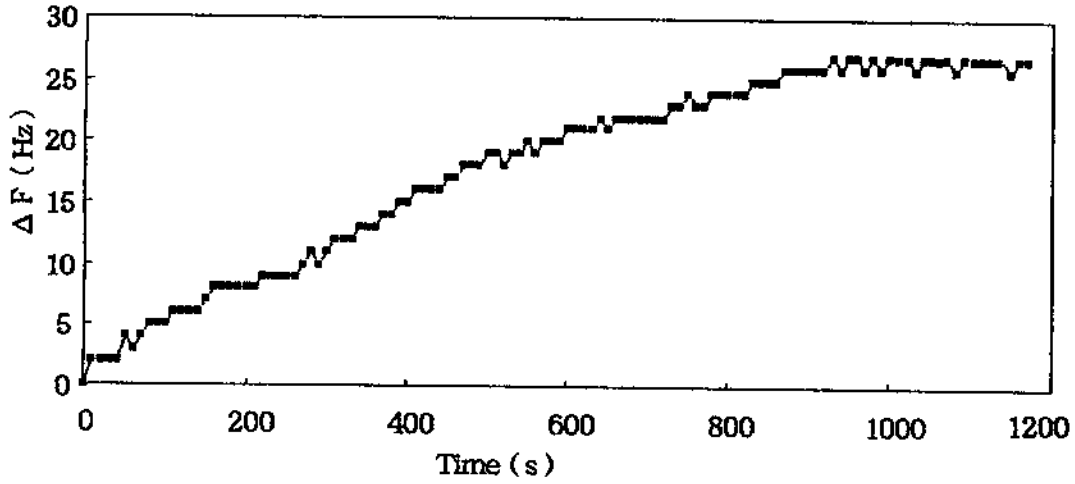

Fig. 4. The frequency change of the quartz crystal biosensor in 100 times diluted human saliva.

with PBS or 100 time diluted human saliva with PBS. The sample solution was stirred and wasted by bio-minipump. The dissociation buffer after the immuno-reaction was 10 mmol HCl aquaeous.

3. Results and discussion

Fig. 3 shows the repeatability on the frequency change in immunoreaction after dissociation with HCl. Immobilizaton of anti-IgA onto the electrode surface on the quartz crystal by covalent bonding method has much more repeatability (more than 15 measurements) compared with that by adsorption (only 3 times). It is considered that gold and sulfur covalent bonds formed by cysteamine maintain more stable immobilization of anti-IgA onto the electrode in repetitive assay.

Fig. 4 shows the time course of the frequency change of the quartz crystal biosensor with anti-IgA immobilized onto the electrode by covalent bonding method. The immunoreaction between the immobilized anti-IgA and 100 times diluted human saliva (IgA) is monitored by the frequency change in real time. The maximum frequency change is detected in about 1000 s.

The relationship between the maximum frequency change of human saliva and the concentration of standard human IgA was linear. The frequency changes were 26 Hz (1 $\mu g\ ml^{-1}$), 50 Hz (2 $\mu g\ ml^{-1}$) and 80 Hz (3 $\mu g\ ml^{-1}$). Antibodies in a human saliva sample were measured by an ELISA to be 100 $\mu g\ ml^{-1}$ quantities. A 100 times diluted solution of this same human saliva gave a ΔF value of 27 Hz at 1000 s as shown in Fig. 4 is in agreement with the standard 1 $\mu g\ ml^{-1}$ IgA solution, confirming the accuracy of the method.

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