

Design considerations for the acoustic waveguide biosensor

Electra Gizeli

University of Cambridge, Institute of Biotechnology, Tennis Court Road, Cambridge CB2 1QT, UK

Received 14 February 1997, accepted for publication 30 June 1997

Abstract. Love waveguide devices utilize shear-horizontal waves which propagate on the top layer of a coated SSBW acoustic device. Initially, attention was focused on the selection of a material which would effectively guide the Love wave. Silica and polymethyl methacrylate were used as guiding layers and the mass sensitivity of the corresponding sensors was tested in air. Low-shear-acoustic-velocity polymer overlayers were found to guide the Love wave most effectively with a maximum sensitivity of $430 \text{ cm}^2 \text{ g}^{-1}$. The polymer waveguide sensor was further used to detect protein adsorption on the polymer surface from IgG solutions within the concentration range of $1\text{--}400 \mu\text{g ml}^{-1}$. Radiolabelled IgG was applied to the device surface in order to calibrate the wave response to protein surface mass density. Finally, the effect of the acoustoelectric interaction on liquid-based applications was studied by utilizing a three-layer waveguide geometry. It was found that the evaporation of a 50 nm gold layer on the polymer overlayer can be used to eliminate acoustoelectric interactions without interfering with the Love wave propagation. After activation with protein A and IgG, the above system was used successfully to detect the direct binding of 400 ppb atrazine.

1. Introduction

Acoustic devices have been used extensively during the last thirty years for chemical sensing [1–6]. However, the majority of work concerns the detection of gas or volatile species in gaseous environment, while application to liquid phase measurements has only recently received more serious attention. Acoustic devices, often referred to as piezoelectric devices based on the nature of the substrate used for the fabrication of the device, are mainly one of the two following types; bulk acoustic wave (BAW) or surface acoustic wave (SAW) devices. BAW devices are acoustic resonant structures where the wave travels from the top excitation surface to the opposite detection surface and, thus, the energy is mainly confined in the bulk of the piezoelectric substrate. SAW devices utilize interdigital transducers (IDTs) to generate and detect a wave on the same surface of the piezoelectric substrate; in general, these systems result in high energy confinement on the surface of the piezoelectric solid. The principle of operation of acoustic sensors is the same for both devices: changes occurring at the piezoelectric solid/sample interface result in a change of the propagating characteristics of the wave, i.e. the amplitude and velocity or frequency. In the case where the detection medium is gas, changes to the propagation characteristics of the wave are related to the mass of the deposited gas analyte on the device surface. When the detection medium is liquid, the wave/matter interactions depend on a number of parameters which are the mass,

viscosity, electric and dielectric properties of the interface. In both media, the magnitude of the wave perturbation due to matter interaction, in other words the sensitivity, will depend on the concentration of acoustic energy on the sensing surface; obviously, the greater the latter the larger the perturbation.

Although both types of device can be used for gas analysis, their application in liquid is limited to those devices utilizing a pure shear wave. Common BAW devices utilize shear bulk acoustic waves; of the SAW type devices, the most common shear operating systems are the acoustic plate mode (APM) [7] and the surface skimming bulk wave (SSBW) [8]. The sensitivity of the BAW devices is limited substantially by both, design and frequency limitations. In order to maximize the acoustic energy present on the sensing surface and reduce the energy in the bulk of the piezoelectric substrate, extremely thin crystals would be required which would be difficult to handle without affecting the durability of the device. Problems related to the design durability also limit the operating frequency to the lower megahertz range (up to 20 MHz). Similar problems are encountered with the APM devices where the wave travels through multiple reflections between the top and bottom surface. Although APM devices can operate at much higher frequencies than BAW devices (above 100 MHz), acoustic energy is distributed between the top and lower surface of the device and cannot be concentrated at the sensing surface unless very thin plates are used. SSBW devices utilize a shear-horizontal (SH) wave which

propagates just below the surface of the device. A major drawback of the SSBW device is the radiation of energy into the bulk of the crystal, which is limiting the sensitivity of the systems. There are two ways to circumvent this problem. A corrugating surface deposited between the IDTs can be used to confine the SH wave to the surface [9, 10]. The surface transverse wave (STW) device utilizing a corrugating guiding layer has been shown to couple the SH wave efficiently and give high surface sensitivities. STW devices have been used as indirect immunosensors for the detection of low concentrations of low-molecular-weight analytes [11]. The second approach involves the deposition of a dielectric overlayer on the entire surface of the SSBW. The effect of the layer is to convert the SSBW into a guided, surface SH wave, where the wave is now located predominantly in the overlayer and in that part of the substrate close to the interface [12, 13]. This composite acoustic waveguide geometry is known as the Love waveguide geometry and can occur only if the shear acoustic velocity in the overlayer (V_2) is smaller than that in the piezoelectric substrate (V_3), i.e. $V_2 < V_3$. The major advantage of the Love waveguide device is that it is an extremely versatile system offering enormous design flexibility. This is due to the fact that, for a certain frequency, energy confinement is purely determined by the acoustic properties and thickness of the deposited overlayer and is independent of the thickness of the piezoelectric substrate. This device has been shown both theoretically [14–16] and experimentally [17, 18] to be the most sensitive acoustic structure for liquid sensing. Love waveguide devices have been used successfully for the binding of biomolecules on the surface of the device [17, 19].

This work is concentrated on the effect of the acoustic properties of the overlayer on developing sensitive and robust biosensors. Initially, attention is focused on the selection of a layer which will guide most effectively the Love wave and result in high surface mass sensitivity. Studied overlayers involve elastic layers of amorphous silica and inelastic ones of polymethylmethacrylate (PMMA). The surface mass sensitivity of the two Love waveguides is tested in order to select the most suitable system for biosensing applications. Furthermore, the best-performing system is used to detect protein adsorption from liquid samples. A complementary technique, utilizing ^{125}I -labelled protein is also applied in order to quantify the exact amount of protein adsorbed on the surface and calibrate the liquid-based Love waveguide biosensor. Finally, a three-layer system is reported, utilizing a thin gold layer on the polymer surface. The effect of the metal layer is to eliminate the acoustoelectric interaction and, thus, develop a sensor which is closer to a pure liquid mass sensor.

2. Experimental details

2.1. Device and instrumentation

Single-crystal Y-cut (42.5°), z -propagating quartz was used as a piezoelectric substrate. The input and output interdigital transducers comprised a 100 \AA chromium flash followed by a 2000 \AA gold overlayer. The IDTs consisted

of 80 pairs with a periodicity of 45 \mu m . Devices were patterned by conventional photolithographic techniques in the Southampton Microelectronics Centre. A Hewlett–Packard 4195A network analyser was used to monitor the phase and frequency of the wave. The temperature was kept constant at $22.2 \pm 0.1^\circ\text{C}$ by utilizing a Peltier plate.

2.2. Surface cleaning of the device

Before depositing a polymer layer on the device surface, the quartz surface was cleaned of any contaminants in order to obtain a reproducible chemical surface. A routine cleaning procedure involved 10 min in a chromic acid solution in an ultrasonic bath, followed by nanopure water rinse and another 10 min in the ultrasonic bath in a sodium hydroxide solution (NaOH, 0.2 M). After a final rinse with isopropyl alcohol, the device was left in the oven for 10 min at 100°C .

2.3. Polymer coating

PMMA solutions of 5.0, 7.5, 10.0, 15.0, 20.0, 22.5 and 25.0% (w/v) were prepared by dissolving PMMA (Aldrich) in 2-ethoxyethylacetate (2-EEA) (Sigma). A layer of PMMA was applied over the clean device surface by spin coating the PMMA solutions at 4000 rpm for 40 s. The adhesion of the film was improved by heating the device for 30 min at 150°C . The actual thickness of each film was determined by mechanical film thickness measurements, using a Talysurf instrument with an accuracy of $\pm 0.01 \text{ \mu m}$. Each PMMA film was removed by sonicating the device in 2-EEA for 10 min, before the new polymer solution was applied to the surface. The photoresist 'Novolac', provided by Plessey GEC Marconi, was diluted 50% (w/w) in 2-EEA. A Novolac layer was applied over the entire surface of the device by spin coating the polymer solution at 4000 rpm for 40 s. The film was further cross-linked by heating the device in a conventional oven for 2 h at 180°C .

2.4. Silica–gold deposition

Silica overlayers were deposited using an Edwards evaporator. Initially, acoustic devices were plasma cleaned in the evaporator chamber for approximately 10 min before thin-film deposition. Silica films were deposited at about 3 \AA s^{-1} and at a pressure below 3×10^{-5} Torr. A Talysurf profilometer was used to measure the thickness of each film with an accuracy of $\pm 0.01 \text{ \mu m}$. A gold layer of thickness 50 \AA was deposited on the polymer surface in the area between IDTs in a similar way.

2.5. Deposition of Langmuir–Blodgett films

Bilayer assemblies of *Langmuir–Blodgett* (LB) films were prepared by dissolving a small quantity of a solution of ω -tricosenoic acid ($\text{CH}_2=\text{CH}-(\text{CH}_2)_{20}-\text{COOH}$) (TCI, Fluorochem) in chloroform in the nanopure water filling the LB trough. After waiting for few seconds in order for the chloroform to evaporate, the monomolecular layer was carefully compressed until it formed a quasi-solid one molecule thick. The device was then repeatedly dipped and raised through the compact monolayer, under controlled

conditions. In all cases each dip and raise run resulted in a bilayer deposited on the surface.

2.6. IgG adsorption

IgG (Sigma, I4506) solutions were prepared by diluting human IgG in adsorption PBS buffer (pH 7.3). The sample was applied on the device surface using a Perspex flow cell, a peristaltic pump and polyvinylchloride (PVC) tubing. A rubber sleeve was used to seal the cell to the Love plate device. The cell was placed between the IDTs exposing an area of about 23 mm² to liquid. Buffer was first applied on the device and left for 10 min, until a stable reading was obtained. This was followed by the protein solution until the equilibrium was reached and then by a final buffer rinse. During the experiment protein solutions were kept at room temperature.

2.7. Adsorption isotherm utilizing radiolabelled IgG

Quartz slides cut in rectangular areas of 4 × 4 cm² were covered by a PMMA layer, by spin coating on the quartz polished surface a PMMA solution. Eight PMMA-coated quartz slides were placed in an adsorption cell, where a liquid-tight seal was made by utilizing a (polyhexafluoropropylene-co-vinylidene fluoride) O ring. The area available for adsorption was 0.29 cm². Adsorption was initiated by adding 40 μl IgG solution in the adsorption buffer of known concentration in each well. The ratio of labelled (radioiodinated) to unlabelled IgG in all the adsorption measurements was 1:150. ¹²⁵I human IgG (catalogue No 68009) of a radioactivity equal to 10 μCi was purchased from ICN, UK. The adsorption block was left in contact with the protein solutions at room temperature (~22 °C). After incubation for 2 h, an aliquot of 15 μl was taken from each well in order to measure the equilibrium concentration of the IgG and the plates were rinsed three times with 1000 μl buffer solution in order to remove non-strongly-bound IgG. The plates and the radioactive IgG aliquots were counted in a gamma-counter. Adsorbed IgG was removed from the PMMA surface by sonicating the quartz plates in 500 μl dodecyl sulphate sodium salt (SDS)/Triton-X (Sigma) for 1 h.

2.8. Atrazine detection

The gold surface of the device was activated with 50 μg ml⁻¹ protein A in PBS buffer at room temperature for 1 h. The device was then connected to the network analyser and the flow cell and 250 μg ml⁻¹ anti-atrazine IgG (ESL) was applied to the activated surface followed by buffer and water rinse. 400 ppb atrazine (ESL) in water was finally applied to the IgG-activated device.

3. Results–discussion

The propagation characteristics of the Love wave were studied experimentally for two different overlayers, an elastic (silica) and an inelastic (polymer) one. For temperature-controlled systems, amplitude and phase measurements

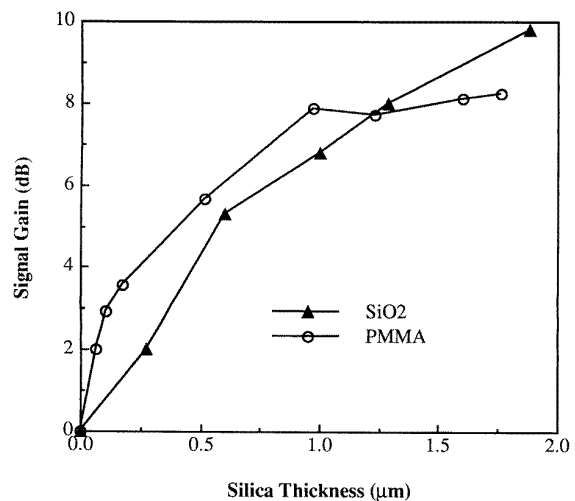


Figure 1. Signal gain of the waveguide device as silica and polymer layers of increasing thickness are deposited on the piezoelectric substrate. Signal gain is defined as the amplitude difference between the coated and uncoated devices and is always positive.

were found to provide complementary information regarding the matter/wave interaction. Amplitude changes are related to acoustic losses, normally occurring during coupling or decoupling of acoustic energy to or from the interface, respectively. Phase changes are related to velocity changes normally occurring when the properties of the propagation medium change, i.e. during mass or viscous mass deposition on the interface layer. Generally, it was found that phase is more sensitive to mass changes and should preferentially be monitored in sensing applications. However, amplitude is also a valuable measurement, since it provides important information on the acoustic energy coupling in the piezoelectric substrate in relation to the overlayer thickness.

3.1. Amplitude response during conversion of the SSBW to a guided Love wave

The mode conversion of the SSBW to a surface SH guiding Love wave, produced by depositing an overlayer on the SSBW device, is known to increase the acoustic confinement on the surface of the device in a manner related to the overlayer thickness and to result in less lossy geometries [20]. This occurs because the Love wave is trapped and guided in the overlayer and the device/overlayer interface, instead of being diffracted in the bulk of the uncoated SSBW device. Experimentally, the net result of the mode conversion and surface waveguiding can be detected by monitoring the amplitude of the SH wave for different overlayer thicknesses.

In this experiment, different thicknesses of silica and PMMA were thermally evaporated and spin coated, respectively, on the surface of SSBW devices. Experimental results are shown in figure 1. The amplitude change, given as the amplitude difference between the coated and uncoated SSBW devices, is always positive, indicating a decrease in the insertion loss of the device or, in other

words, a signal gain. According to figure 1, the signal gain increases with increasing overlayer thickness for both materials. This observation proves that the diffraction pattern of the shear wave in the bulk of the crystal changes with overlayer thickness such that more acoustic energy is located in the overlayer and that part of the substrate close to the interface when thicker layers are applied. In the case of silica and for the examined thicknesses ($<2 \mu\text{m}$), insertion loss decreases almost linearly with increasing silica thickness (rate of increase $5.2 \text{ dB } \mu\text{m}^{-1}$, $R^2 = 0.95$). For the $2 \mu\text{m}$ silica Love waveguide, the detected signal was found to be 10 dB less lossy than that of the SSBW device. In the case of PMMA, signal gain also increases with increasing the PMMA thickness until a saturation level is reached at a thickness of approximately $1 \mu\text{m}$. At that level, the acoustic wave is 8 dB less lossy than the original acoustic signal. The saturation level observed when inelastic overlayers were applied can be explained if the lossy nature of polymers is taken into account. Figure 1 suggests that for small layer thicknesses ($<1 \mu\text{m}$), the fraction of the total power confined in the PMMA to that traveling in the substrate is small and attenuation caused by polymer losses negligible. A trade-off between signal enhancement due to surface guiding and polymer losses is observed for thicknesses between 1 and $2 \mu\text{m}$, possibly suggesting that for that range an equal energy distribution occurs between PMMA and quartz substrate. Finally, as the thickness increases further ($>2 \mu\text{m}$), the fraction of the total power guided by the layer becomes appreciably larger and attenuation in the polymer becomes the predominant mechanism of acoustic loss. For a thickness of $2.5 \mu\text{m}$, the insertion loss of the coated device was found to be even greater than that of the uncoated SSBW device. It is interesting to note that the optimum overlayer thickness for signal gain does not necessarily correspond to that giving the maximum surface sensitivity. Experimental studies have shown that the optimum thickness for maximum surface sensitivity is around $6 \mu\text{m}$ [18] and $1.6 \mu\text{m}$ [17] for the silica and PMMA, respectively. Apparently, there is an overlayer-thickness trade-off between the optimum working signal and maximum surface mass sensitivity.

3.2. Effect of overlayer acoustic properties on mass sensitivity in air

The silica- and polymer-coated Love waveguide devices were utilized for detecting mass deposition on the device surface. The most straightforward application involved phase monitoring of the wave with mass deposited on the surface of the device. Highly organized layers of LB films were deposited on the surface of the device and phase measurements were used for mass calibrating each sensor. The technique of LB films is easy to apply and can be used to measure simultaneously the exact area occupied per molecule, leading to accurate calculation of the surface mass density. Results are shown in figure 2 for the $2.5 \mu\text{m}$ silica and $1.6 \mu\text{m}$ PMMA Love wave sensors. The difference between the acoustic properties of the two overlayers results in a considerable variation in the surface mass response for the two systems. The three times

higher sensitivity obtained with the PMMA ($V_{\text{PMMA}} = 1100 \text{ m s}^{-1}$) than with silica ($V_{\text{SiO}_2} = 3764 \text{ m s}^{-1}$) overlayers shows how effective the surface waveguiding is when layers with very low shear acoustic velocities are used. The sensitivity, defined as the phase change to the change in mass per unit area, was measured to be 11.08 and $31.3 \text{ deg } (\mu\text{g cm}^{-2})^{-1}$ for the silica and PMMA waveguides, respectively. The absolute sensitivity of the two systems, defined as the ratio of the phase change to the change in mass per unit area, was calculated to be 153 and $430 \text{ cm}^2 \text{ g}^{-1}$, respectively. In order to decide which of the two groups of layers, i.e. elastic or inelastic, should be used for biosensing applications, the sensitivity of the two waveguides should be compared for those overlayer thicknesses which correspond to maximum energy coupling. For the PMMA waveguide, thicknesses between 1.6 and $2 \mu\text{m}$ were found to give the best-performing devices [17]. Theoretical and experimental results [18,21] showed that 5–6 μm silica give the most sensitive waveguide structure. It was reported [18] that the absolute sensitivity of the $5 \mu\text{m}$ silica Love waveguide is $380 \text{ cm}^2 \text{ g}^{-1}$, which is lower than that of the $1.6 \mu\text{m}$ PMMA structure. Given the above results, it appears that waveguides using inelastic polymer overlayers are the most promising systems for developing sensitive biosensors. Silica waveguides not only involve more laborious procedures for depositing the overlayer, but also result in a relatively ineffective waveguide coupling since the shear acoustic velocity of the silica and quartz substrate ($V_{\text{quartz}} = 4952 \text{ m s}^{-1}$) is closely matched. In practice, PMMA layers can be easily produced by using the spin-coating technique; furthermore, the polymer can be removed by applying an appropriate organic solvent and the device re-used. PMMA couples more effectively the SSBW wave to a Love wave for much lower overlayer thicknesses since its shear acoustic velocity is significantly lower than that of quartz. It can be envisaged that further improvement of the sensitivity can be achieved by using polymers of lower shear acoustic velocity than that of PMMA.

3.3. Mass calibration in the presence of liquid—study of protein adsorption on the polymer-coated Love waveguide

The Love wave is a shear horizontal wave in polarization and can operate in the presence of liquid. In a liquid environment the acoustic wave/matter interaction will depend on the following parameters: the deposited surface mass, the viscosity of the interface and the electric and dielectric properties of the liquid. Thus, the mass sensitivity in the presence of liquid is expected to be different to that measured in air, since the nature of the wave/matter interactions in a solution is not similar to that in air. The adsorption of a protein layer on the polymer surface in presence of liquid was studied for a range of protein concentrations. To simplify the analysis, it was assumed that any phase change observed during protein adsorption was only due to interfacial mass change. Such an assumption can be only valid if the viscosity and electric properties of the interface do not change significantly during the process of protein adsorption.

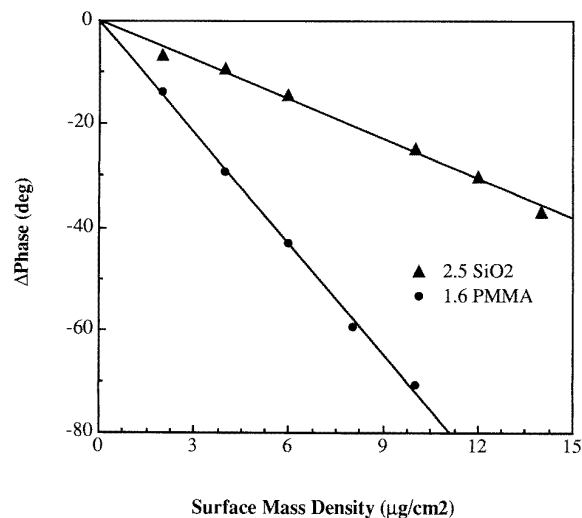


Figure 2. Phase change of the 2.5 μm SiO_2 and 1.6 μm PMMA waveguide mass sensors as a function of the deposited surface mass density in air.

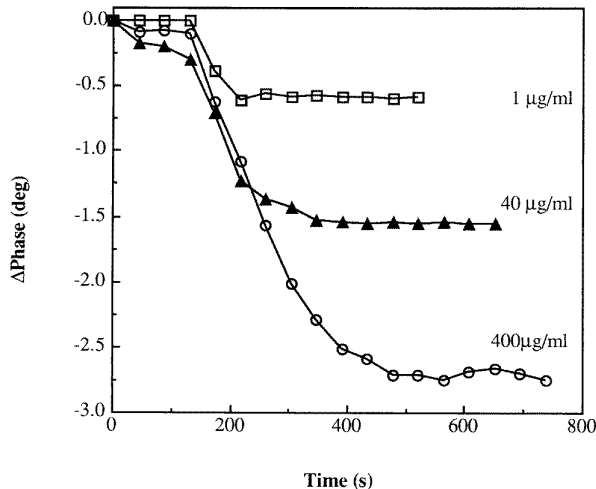


Figure 3. Phase monitoring of the adsorption of 1, 40 and 400 $\mu\text{g ml}^{-1}$ IgG on the PMMA-coated surface of the device in real time.

In the present study, protein solutions of IgG in PBS buffer within the concentration range of 1–400 $\mu\text{g ml}^{-1}$ were applied to the polymer surface of the device using a flow-through system. Figure 3 follows the kinetics of the adsorption of three different IgG concentrations, as detected in real time by monitoring the phase response of the Love wave. At the beginning of each experiment PBS buffer (pH 7.3) was pumped through until a stable phase reading was obtained, followed by injection of the corresponding IgG solution and final rinse with PBS buffer. Figure 4 gives the phase change of the Love wave as a function of the bulk concentration of the IgG solutions. Phase change was measured *in situ*, and each data point was determined at equilibrium. Qualitatively, this graph reflects the general behaviour of proteins adsorbing at solid/liquid interfaces. For low IgG concentrations a linear relationship between surface density and phase change

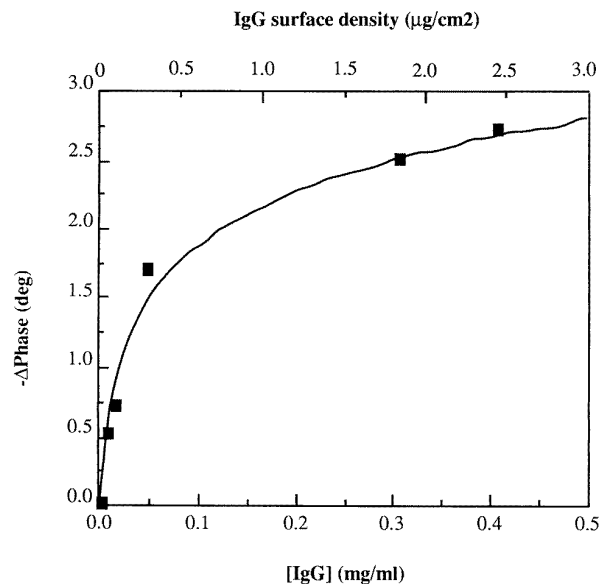


Figure 4. Phase change of the IgG adsorption on the PMMA-coated Love waveguide as a function of (i) the IgG concentration in solution (bottom axis) and (ii) the IgG surface mass density, as measured using radiolabelled IgG (top axis).

may be assumed. For higher IgG concentrations phase change seems to start approaching a saturation level. In order to calibrate the phase change to the protein surface mass density, radiolabelled IgG was used in a parallel experiment. Solutions of ^{125}I -labelled/unlabelled IgG, within the same concentration range, were applied to a well defined area of PMMA and a γ -counter was used to detect the amount of adsorbed protein as described in section 2.7. The protein surface density, expressed in micrograms per square centimetre, corresponds to the amount of IgG that remained adsorbed after washing with adsorption buffer (pH 7.3) divided by the adsorption area. Figure 4 gives the phase change observed during protein adsorption against (i) the bulk concentration of the protein solution ($[\text{IgG}]$, mg ml^{-1}) and (ii) the protein surface density ($\mu\text{g cm}^{-2}$). Comparison of figures 4 and 2 reveals that, for the same surface mass density, the phase response observed during mass deposition in liquid is considerably less than that observed in air. This difference can be attributed to a number of possible factors.

(i) Liquid loading of the device results in *viscoelastic* and *electric coupling* between the wave and the liquid sample. If part of the acoustic energy present on the waveguide surface is coupled to the viscous and electric components of the interface, it is possible that the remaining energy for detecting mass changes is lower compared to that available during mass detection in air.

(ii) The flow cell used for applying the liquid samples on the device surface may induce some additional *mechanical perturbations* which can affect the signal response.

(iii) Finally, it is worth mentioning that *structure* of the deposited mass may also affect the degree of energy coupling. Highly organized, homogeneous and dense LB

layers may result in more effective acoustic coupling than randomly adsorbed protein mass.

Further to the measurement of the adsorbed IgG surface density, the radiolabel technique can be used to provide information related to the strength of the IgG/PMMA-surface bond by trying to elute the radiolabelled protein after washing the device with different solutions and measuring the remaining radioactivity. This would be extremely important for biosensing applications since it would determine the possibility of re-using the PMMA-coated device after IgG adsorption. In order to elucidate the adsorbed protein, the PMMA-coated quartz slides were initially sonicated in adsorption buffer for 30 min at $\sim 22^\circ\text{C}$. This treatment did not reduce the amount of adsorbed protein, indicating irreversible adsorption. A reasonable decrease of the amount of adsorbed IgG was only found after sonicating the adsorption slides in 1 wt.% SDS/1 wt.% Triton X-100 detergent solution for 1 h (data not shown). SDS, being a small molecule, can reach the PMMA surface and can create electrostatic repulsion between the negatively charged SDS-IgG complexes and the SDS-PMMA surface. However, even after the above treatment the surface was not completely free of protein, indicating that a high degree of conformational denaturation of the protein has already occurred. Thus, it appears that the immobilization of IgG through physical adsorption results in irreversible protein binding and does not allow the recycling of the devices.

3.4. A three-layer Love waveguide

In order to eliminate some of the unwanted acoustic wave/liquid coupling the following three-layer system was considered. A polymer waveguiding layer was spin coated on the SSBW device and on top of it a gold layer was deposited in the area between the IDTs. A commercially available photoresist (Novolac) was used instead of PMMA since it was found to improve the adhesion of gold. The gold layer was considered in order to reduce unwanted interactions between the acoustoelectric field, induced by the piezoelectric substrate, and dielectric or conducting liquid samples. Gold has a relatively low shear acoustic velocity ($V_{\text{gold}} = 1200 \text{ m s}^{-1}$) and a high density. In order to ensure that gold will only short the electric field and not interfere with the propagation of the Love wave, a range of thicknesses between 10 and 100 nm was applied to the polymer surface and the amplitude and phase of the multilayer geometry was compared to that of the polymer-coated device. Initially, it was found that the thickness of the gold layer is inversely related to that of the polymer overlayer; as the thickness of the polymer increases thinner gold layers are required in order to avoid mode conversion of the Love waveguide. For the optimum-thickness polymer-coated device, the best-performing geometry was found to be that utilizing a 50 nm gold layer. The dramatic effect of the metal layer on the response of the waveguide was observed when water loading reduced the normally observed 40 deg phase change to 20 deg. Although there may still be some remaining acoustoelectric interaction, it is believed that its effect has

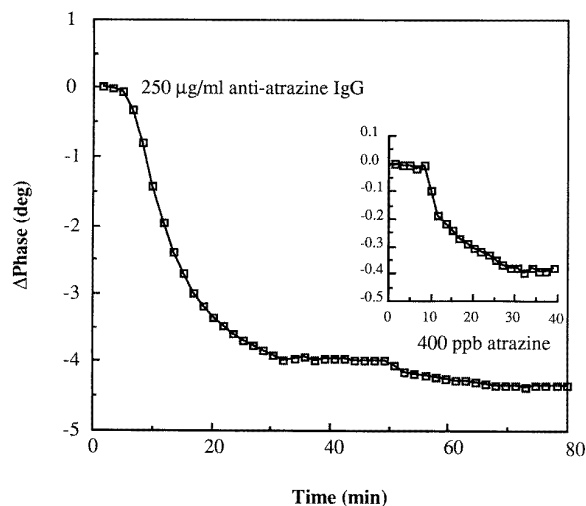


Figure 5. Phase detection of the binding of $250 \mu\text{g ml}^{-1}$ anti-atrazine IgG, followed by addition of 400 ppb atrazine. The gold-coated polymer waveguide used in this experiment was initially activated with protein A.

been considerably eliminated by using the above three-layer waveguide structure and the performance of this system is closer to that of a liquid-based mass acoustic sensor. It is worth mentioning that the gold layer is not expected to reduce the viscoelastic acoustic wave interaction.

3.5. A three-layer Love waveguide biosensor; detection of low-molecular-weight analytes

In addition to the reduction of the acoustoelectric interaction, the gold layer provides a versatile substrate for selective surface modification for biosensing applications. The potential of the novel multi-waveguide structure was initially realized during the direct detection of a low-molecular-weight analyte. Initially, gold was modified by applying a solution of $50 \mu\text{g ml}^{-1}$ protein A in PBS buffer for 1 h. Protein A is known to have a high affinity for gold and adsorb strongly on its surface. The protein-A-coated gold surface was then exposed to a solution of $250 \mu\text{g ml}^{-1}$ sheep anti-atrazine IgG in PBS buffer, which resulted in 4 deg phase drop (figure 5). Protein A is known to have five binding sites for IgG per molecule. A considerable advantage of this immobilization procedure is that IgG is bound to protein A via its Fc side, leaving its Fab fragments free for the analyte binding. Atrazine, a pesticide with a molecular weight of 215, was finally applied at a concentration of 400 ppb. Its direct binding to the immobilized anti-atrazine IgG was observed as a further 0.4 deg phase drop. Given the low molecular weight of atrazine, its direct detection at such a low concentration can be explained based on its high affinity for the IgG-modified surface and the sensor's high sensitivity to mass deposition. The detection of the same concentration of atrazine on the two-layer polymer waveguide was not possible.

4. Conclusions

The effect of the acoustic properties of the overlayer used for guiding the Love wave was studied for two different materials: an elastic (silica) and an inelastic (PMMA) one. It was shown that the low-shear-acoustic-velocity PMMA results in a much more effective coupling of the Love wave and, thus, in more sensitive waveguide sensors comparing to silica waveguides. The 1.6 μm PMMA waveguide structure was shown to have a mass sensitivity of 430 g cm^{-2} , which, to our knowledge, is the maximum ever reported for a Love wave sensor. In addition to its high sensitivity, spin-coated PMMA layers can be easily produced and, further, chemically removed for re-using the device. Calibration of the phase change with radiolabelled IgG showed that the nature of the acoustic wave/matter interaction is very different in air and in the presence of liquid. In the latter case, viscoelastic and electric interactions also appear to be important as well as wave/mass interactions. In order to eliminate the effect of the acoustoelectric coupling, a three-layer waveguide structure incorporating a gold layer on top of the polymer was used. It was found that the gold can reduce considerably the effect of the acoustoelectric interaction without affecting the Love wave. The above system was used successfully to detect the direct binding of 400 ppb atrazine on the anti-atrazine-IgG-modified surface. It is anticipated that further work on the optimization of the polymer and metal layers will lead to the development of powerful acoustic waveguide biosensors.

Acknowledgment

The author would like to acknowledge the Biotechnology and Biological Sciences Research Council (BBSRC) for supporting this work.

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