

Comparison of Poly(methylmethacrylate) and Novolak waveguide coatings for an acoustic biosensor

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In order to optimize the geometry of the acoustic waveguide biosensor, the performance of the device was assessed for two different guiding layers: a photoresist (Novolak) and a polymer Poly(methylmethacrylate) (PMMA) one. Initially, the effect of the thickness of each layer on the insertion loss of the device and frequency of the wave was monitored. The 1.5 and 1.7 μm Novolak and PMMA, respectively, coated devices were used to monitor the binding of Immunoglobulin G to the protein A activated device surface. Both devices were found to exhibit the same phase sensitivity to protein deposition. However, Novolak waveguides showed a higher stability in contact with water, making them more suitable for biosensing applications than PMMA waveguides. Finally, the reproducibility of the Novolak-coated waveguide was studied during the formation of the polymer layer, addition of buffer, and antibody binding. © 2001 American Institute of Physics.
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I. INTRODUCTION

The acoustic waveguide geometry comprises a surface acoustic wave device, which supports a shear wave and is overlaid by a dielectric layer. If the shear velocity in the overlayer is lower than that in the substrate, the wave is trapped at the substrate/overlayer interface giving rise to a guided wave known as the Love wave. The Love wave was first reported in 1911;¹ since then, the characteristics of both the wave and the waveguide have been analyzed theoretically by many investigators.²⁻⁴

The first application of the Love acoustic wave device as a biosensor was reported in 1992.⁵ This work introduced a novel waveguide geometry, which utilized a polymer overlayer. During the last few years, a number of articles have appeared in literature covering both theoretical and experimental aspects of the Love wave biosensor.⁶⁻¹⁰ In these studies, the effect of the waveguide layer on the sensitivity of the device has been reported for silica and poly(methylmethacrylate) (PMMA) films. Silica is an elastic, relatively dense material ($\rho = 2.2 \text{ g cm}^{-3}$) with a high shear acoustic velocity ($V_{\text{SiO}_2} = 3764 \text{ m s}^{-1}$).¹¹ Amorphous films of silica were formed on the whole active surface of the device by using the chemical vapor deposition and sputtering technique. Unfortunately, not only is silica evaporation quite laborious, but the acoustic coupling is relatively ineffective unless very thick silica layers are applied ($\sim 6 \mu\text{m}$).¹² This is because the acoustic velocities of silica and quartz substrate are closely matched ($V_{\text{Quartz}} = 4952 \text{ m s}^{-1}$). A significant advantage of silica layers, however, is that they exhibit low acoustic losses for frequencies in the MHz range due to the elastic and amorphous nature of the material. Polymer overlayers of (PMMA) have been applied on the whole active surface of the device by using simpler techniques such as spin coating. Thin layers ($\sim 2 \mu\text{m}$) of PMMA are sufficient to guide the

Love wave due to the low shear acoustic velocity of the polymer ($V_{\text{PMMA}} = 1100 \text{ m s}^{-1}$).^{5,13} However, in practice, the high sensitivity predicted by theory for the PMMA waveguide device has never been achieved, mainly due to high acoustic losses occurring inside the soft polymer.

In an attempt to investigate further the effect of the overlayer, two polymers of different chemical structure and possibly mechanical properties were used as the guiding layer: Novolak, a commercially available photoresist consisting of phenyl/formaldehyde groups, and PMMA. Different thicknesses of Novolak and PMMA were deposited on the device surface and the efficiency of each waveguide was compared by monitoring the frequency of the wave and insertion loss of the device. To extend this work to biosensing applications, the sensitivity of the two polymer-coated devices was investigated by monitoring the phase of the wave during the binding of IgG to surface-bound protein A. Finally, research issues such as the stability and reproducibility of the waveguide devices were also addressed.

II. EXPERIMENTAL SECTION

A. Materials

Phosphate buffered saline tablets, pH 7.4 (0.01 M phosphate, 2.7 mM potassium chloride, and 0.137 M sodium chloride) and protein A, were purchased from Sigma. Medium-molecular weight PMMA and 2-ethoxyethyl acetate were purchased from Aldrich. Novolak (or Novolac) was purchased from Shipley. Monoclonal antibodies raised against the hormone Estrone 3 glucuronide (anti-E3G IgG) were obtained from Unilever Research, Colworth, UK.

B. Device and instrumentation

Acoustic wave devices were manufactured in the Southampton Microelectronics Center. The piezoelectric crystal used was 0.5 mm thick single Y-cut (42.5°), z-propagating quartz, and supported a surface skimming bulk

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wave (SSBW). The interdigitated transducers (IDTs) comprised 10 nm chromium and 200 nm gold layer; 80 pairs of IDTs with a periodicity of $45 \mu\text{m}$ were patterned by conventional photolithographic techniques. A Hewlett–Packard 4195A network analyzer was used to perform acoustic measurements. To minimize interfering bulk wave reflections, a standard adhesion tape was applied to the lower side of the device.

C. Deposition of the polymer overlayer

Different polymer concentrations were prepared by diluting the polymer in 2-ethoxyethyl acetate. Novolak (10, 20, 30, 40, 50, 60, 70% w/w) and PMMA (5, 10, 15, 20, 22, 25% w/w) solutions were applied to the device surface and spread by using a spin coater (Specialty Coating Systems P6700) at 4000 rpm for 40 s. The solvent was evaporated by heating the polymer-coated devices in an oven at 190°C for 2 hs. The thickness of the waveguide layer was measured by using a surface profilometer (Dektak).

D. Gold deposition

A gold layer of 10 nm was deposited on top of the polymer and between the IDTs. Gold was deposited by thermal evaporation at a pressure of 10^{-6} mbar by using an Edwards (Auto 306) evaporator.

E. Protein A binding: IgG isotherm

Protein A was dissolved in phosphate buffer saline at $50 \mu\text{g/ml}$ and added to the freshly prepared gold-coated acoustic devices. Different concentrations (1–200 $\mu\text{g/ml}$) of the monoclonal antibody were added to the device surface at a flow rate of $80 \mu\text{l s}^{-1}$, followed by a buffer rinse.

III. RESULTS AND DISCUSSION

Layers of different thicknesses of Novolak and PMMA were deposited on the surface of the device and the acoustic signal was recorded before and after the deposition. Two parameters were measured: the resonant frequency and insertion loss. Figure 1 shows a typical transmission spectrum of the device, before and after coating it with polymer which in this case was $1.6 \mu\text{m}$ of Novolak. Generally, the effect of the polymer layer is (1) to decrease the insertion loss and (2) decrease the frequency of the device at which maximum power transmission is observed. The change in the frequency and insertion loss as a function of the overlayer thickness is shown in Figs. 2 and 3, respectively, for the two polymers. Frequency drop is directly related to the decrease of the velocity of the propagating wave as the latter is converted from a SSBW to a Love wave. As the thickness (h) of the polymer layer increases, the acoustic wave progressively transits from the quartz substrate into the guiding layer and the velocity changes so that: $V_{\text{Polymer}} < (V_{\text{Love}})_h < V_{\text{Quartz}}$. According to Fig. 2, the velocity of the Love wave in the PMMA and Novolak waveguides is very similar.

The positive change of the insertion loss observed in Fig. 3 shows that, in both cases, more acoustic energy is trapped close to the upper surface of the device as the thickness of

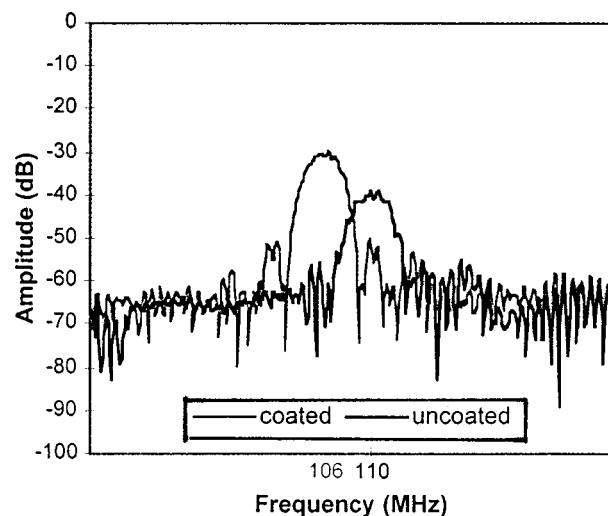


FIG. 1. Transmission spectrum of the uncoated and $1.6 \mu\text{m}$ Novolak-waveguide device is shown.

the overlayer increases. A small difference in the shape of this graph is observed for the two polymers: amplitude change increases gradually with Novolak thickness while a step change is observed with PMMA. The amplitude change in both waveguides becomes maximum around $1.5 \mu\text{m}$ indicating that at that thickness, an optimum trade off has been achieved between energy trapping and losses due to the soft nature of the polymer. In both cases, any further increase of the polymer thickness resulted in devices with an insertion loss much higher than that of the uncoated device (data not shown). These waveguides were unsuitable for sensing applications. Based on Fig. 3, devices incorporating $1.5 \mu\text{m}$ and $1.7 \mu\text{m}$ of Novolak and PMMA layers, respectively, were used for any further experiments.

The sensitivity of the polymer-coated waveguide devices was investigated by studying protein binding on the device surface. A thin layer of protein mass bound to the device surface will oscillate with the surface, which, in turn oscillates as a result of the wave propagation. In order to perform

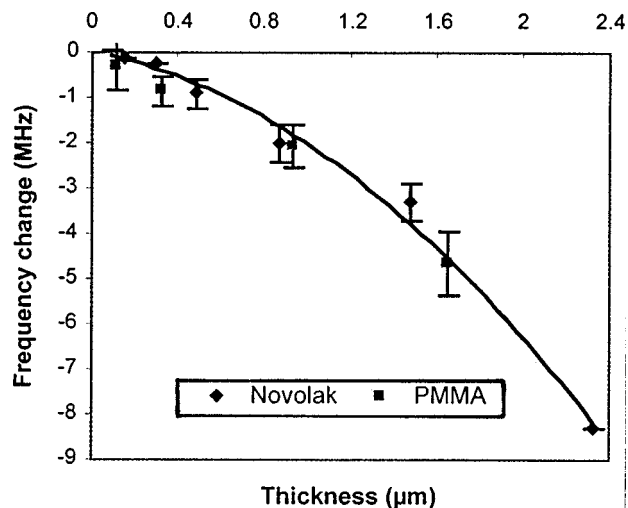


FIG. 2. Frequency change as a function of the waveguide layer thickness, when Novolak and PMMA are used as waveguide layers is shown.

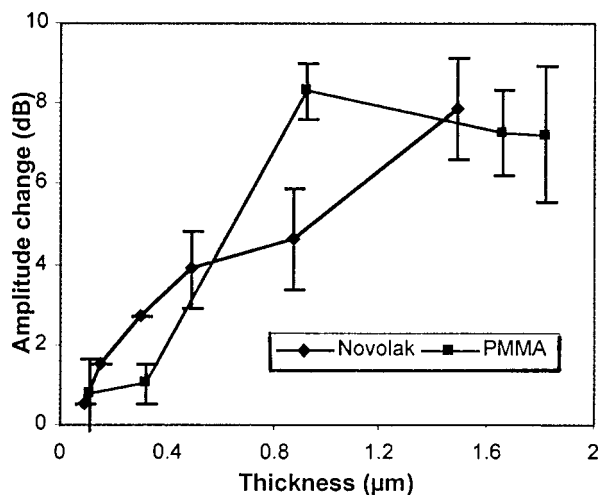


FIG. 3. Amplitude change as a function of the waveguide layer thickness, when Novolak and PMMA are used as waveguide layers is shown.

a biosensing experiment, a thin gold layer was deposited on the polymer surface between the IDTs, which was subsequently exposed to 50 $\mu\text{g/ml}$ of protein A. Different concentrations of IgG were applied on the activated gold surface and the binding of the protein was recorded by following the phase of the wave in real time. Figure 4 shows the phase change as a function of the antibody concentration for the two devices. Phase change was measured *in situ* after a reaction time of 8 min, when, in all cases, equilibrium was reached. Apparently, the binding of IgG to protein A follows the expected pattern of a Langmuirian isotherm; for low IgG concentrations ($<50 \mu\text{g ml}^{-1}$) a linear behavior between concentration and phase change can be assumed. For higher IgG concentration, phase change approaches a saturation level. Figure 4 shows that within experimental error, no difference is observed between Novolak and PMMA waveguides indicating that the sensitivity of the two devices to surface perturbations is similar.

In order to explore further whether there were any advantages in applying Novolak instead of PMMA, the stability of the two devices in the presence of a liquid was com-

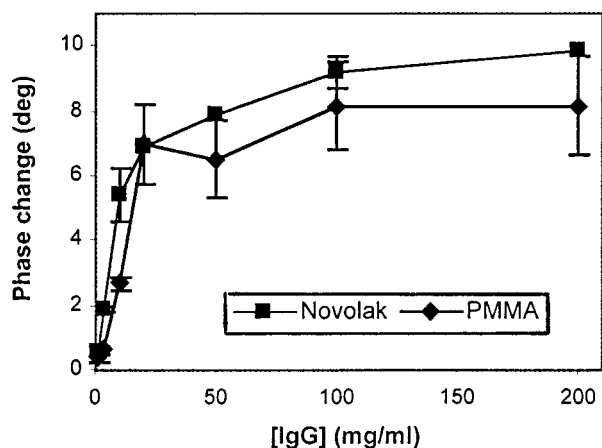


FIG. 4. Detection of IgG binding to the protein A modified surface when 1.5 μm and 1.7 μm of PMMA and Novolak, respectively, are used as a waveguide layer is shown.

pared. Results showed that PMMA-coated devices exhibited a significant signal drift when in contact with liquid while Novolak-coated devices showed a good long-term stability. This is probably due to swelling of the PMMA waveguide layer following the absorption of water. In addition, it was found that gold adheres better to Novolak than PMMA resulting in a more stable interface when the former waveguide layer is used. Finally, further work revealed that Novolak exhibited higher chemical stability than PMMA. The latter is important in those experiments where organic solvents are applied.

As a final study, the reproducibility of the Novolak-coated waveguide biosensor was investigated during the coating of the polymer layer and performance of biosensing experiment. The spin coating of Novolak on 20 devices gave a variation of 35% in the change of the insertion loss of the device. The poor reproducibility of this step is most probably due to the presence of dust particles and bubbles in the polymer solution which apparently affect the morphology and guiding properties of the layer. The variation of the phase change as a result of adding a buffer on the device surface was found to be 25%, probably due to changes in the roughness and hydrophilicity of the surface. Finally, the reproducibility of the phase change observed during the addition of antibody was found to be 15%. The latter most probably reflects changes in the surface properties of the surface, which affect protein binding.

IV. CONCLUSIONS

Waveguide devices comprising a photoresist (Novolak) and a polymer (PMMA) guiding layer were compared. The frequency response as a function of the polymer thickness suggests that the velocity of the Love wave is very similar in the Novolak- and PMMA-coated devices indicating that the two materials exhibit similar mechanical properties. As a result, no significant difference was detected in the mass sensitivity of the two biosensors during the phase detection of protein binding on the device surface. However, waveguide devices comprising Novolak as a guiding layer were found to be superior for biosensing applications due to their high chemical and long-term stability when in contact with a liquid sample.

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