

# Acoustic waves and the study of biochemical macromolecules and cells at the sensor–liquid interface

The  
Analyst  
Critical Review

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*I am skeptical of the principle of objectivity, which, in my view, is often simply the current popular viewpoint in disguise.*

**Darryl Reaney<sup>1</sup>**

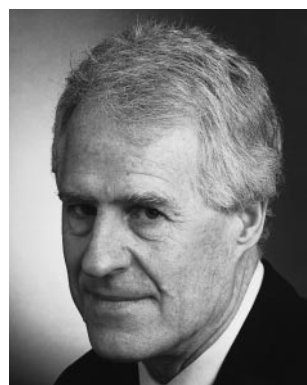
## 1 Introduction

The launching of acoustic waves in materials at ultrasonic frequencies, of the order of one to several hundred megahertz (MHz), depends upon the conversion of electromagnetic into

acoustic energy. This process may involve piezoelectric substrates or the coupling of non-piezoelectric solids with devices capable of generating acoustic fields. Piezoelectricity is associated with the reversible electric polarization produced by mechanical strain in crystals belonging to certain classes.<sup>2</sup> The Curie brothers have been universally credited with the discover-



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*Guelph. The focus of his present research is the design of biological and chemical sensors, particularly ones based on bulk wave acoustic devices. In addition, some of his research involves fuzzy logic control systems, a natural extension of sensor technology into less tangible measurement problems. Dr. Hayward is a registered professional engineer in the province of Ontario.*

ing of this phenomenon (1880). From a chemical sensor standpoint it is interesting to note that the first application of piezoelectric effects in the development of such devices was that of Marie Curie.<sup>3</sup> The Curies discovered the element radium and, in the course of their work, employed what was termed a 'quartz electric balance'. It was about 60 years after the work of the Curies that Sauerbrey<sup>4</sup> started using a thickness-shear mode (TSM) acoustic device, based on quartz plates, in the study of thin-deposited films. This research led to the famous Sauerbrey equation, which, essentially, treats any added layer on the device as being composed of quartz, that is, the new film is considered as extending the acoustic wavelength of the device. As we shall see, Sauerbrey's expression is repeatedly invoked, even in modern times, to explain frequency responses in liquid media despite the fact that the original model was specified, reasonably, for the gas phase deposition of ultra-thin films.

The first report of a TSM sensor being used as a selective analytical device concerned an application as a chromatographic detector.<sup>5</sup> With respect to liquid phase operation, before the 1970s, it was commonly believed that the device could not function in such media because of viscous damping forces.<sup>6</sup> However, in 1980, two separate groups were able to achieve operation in liquids by exposing only one face of the device to fluid.<sup>6,7</sup> The first paper to suggest that acoustic wave devices could be employed in the field of biosensor technology involved the placement of antibody protein at the interface of a surface acoustic wave (SAW) sensor.<sup>8</sup> Some 20 or so years earlier, White and Voltmer<sup>9</sup> had pioneered the marriage of piezoelectricity with microelectronics through the demonstration that photolithographic techniques could be employed to deposit interdigital substrates to excite Rayleigh waves. Despite the obvious attractive possibilities for the SAW as a biosensor, later work cast serious doubt as to whether the sensor operates in a liquid in true Rayleigh format.<sup>10</sup> Subsequent to this controversy, Thompson *et al.*<sup>11</sup> successfully detected immunochemical interactions at the liquid–solid interface of a TSM device. Since this work, a number of different devices have been employed in conjunction with a wide variety of biological moieties, and much of this is detailed in the present review.

Finally, before proceeding to review the theory and applications of acoustic wave technology in the field of biosensors, it is important to point out that not all such devices are based upon piezoelectric materials. These substrates can present a number of restrictions in terms of the attachment of biomolecules to the appropriate interface. In part, this is the reason that recent times have seen the introduction of thin rod, tube and magnetic acoustic resonator sensors where acoustic waves can be launched into non-piezoelectric materials. Although these structures have not yet seen use in biosensor development, we introduce them here because their physical structure and mode of excitation offer considerable potential for the future.

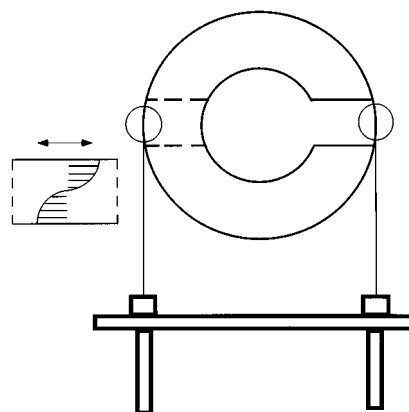
## 2 Overview of acoustic wave devices

The operation of acoustic wave devices is based on the propagation of bulk or surface launched acoustic waves through piezoelectric and other materials. Quartz is the most frequently used piezoelectric matrix because of its stability with respect to temperature. Metal transducers launch acoustic waves at ultrasonic frequencies and the type and resonant frequency of such waves are determined by the crystal orientation, the thickness of the piezoelectric material and the geometry of the transducers. The generated waves are transverse or shear waves when particle displacement is perpendicular to the direction of propagation. In the case of compressional or longitudinal waves, particle displacement is parallel to the direction of travel. While bulk waves propagate through the volume of the piezoelectric substrate, the propagation of surface waves is

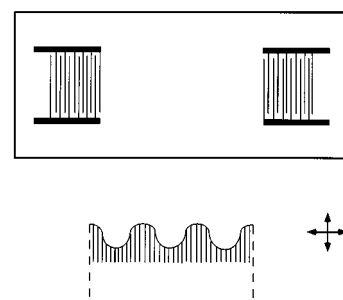
restricted to a distance of about one acoustic wavelength from the piezoelectric substrate surface. With respect to shear wave devices, depending on the polarization of acoustic waves, particle displacement can be parallel or normal to the sensing surface creating horizontal or vertical shear waves.<sup>12–15</sup>

The most frequently used acoustic wave sensors are the thickness-shear mode devices that generate bulk transverse waves with particle displacement parallel to the surface of the sensor. Such waves, on propagation into liquid, suffer attenuation in acoustic energy because of viscous forces in the liquid. However, the overall behaviour in liquids is governed not only by the viscosity of the liquid medium but also by the particular conditions present at the liquid–solid interface. The TSM device (Fig. 1) consists of an AT-cut quartz disc with metal electrodes on opposite sides to effect the application of an oscillating electric field. The applied transducer configuration categorizes the TSM as a one-port device. The thickness ( $t$ ) determines the wavelengths ( $\lambda$ ) of the fundamental ( $n = 1$ ) and harmonic ( $n = 3, 5, 7, \dots$ ) resonances through the expression  $\lambda = 2t/n$ . Consequently, the resonant frequency for the fundamental mode is restricted by the thickness of quartz wafers to the approximate range 5–20 MHz.<sup>12,13</sup>

SAW devices (Fig. 2) comprise thicker ST-cut quartz plates and interdigital metal transducers (IDTs) that generate Rayleigh waves propagating in both directions from the IDTs within the limit of approximately one acoustic wavelength from the sensing surface. The surface particles move elliptically, resulting in a wave consisting of both shear and compressional components. The latter provokes an important attenuation effect in liquids which prevents the application of SAW devices in such media. The particle displacements of the shear wave are transverse relative to the propagation direction and normal to the plane of the surface, so that the generated wave is categorized as shear vertical (SV). The most frequently applied, 'delay line', configuration of IDTs involves input and output transducers being positioned on two opposite sides of a



**Fig. 1** Top view of a TSM consisting of a quartz disc, metal electrodes and electric contacts. The smaller figure illustrates the wave motion and the direction of particle displacement.



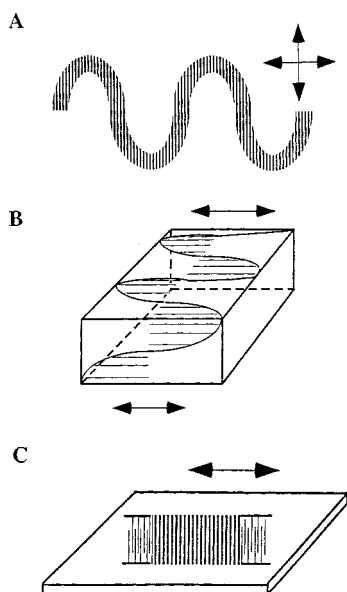
**Fig. 2** Top view of a SAW delay line consisting of a quartz plate and interdigital metal transducers. The lower figure indicates the wave motion and direction of surface particle displacement.

piezoelectric substrate. Such a structure forms a two-port device. The frequency of a SAW device is determined by the 'finger' spacing of the IDTs ranging from 30 to 500 MHz.<sup>12,15</sup>

An additional category of surface-launched acoustic wave sensors is those comprised of plate devices, where the thickness of a piezoelectric substrate is reduced to a dimension corresponding to the order of the acoustic wavelength. Although surface-excited, the generated waves travel through the bulk of the piezoelectric material. Lamb waves generated in a plate of finite thickness can be of both symmetric and antisymmetric modes when referring to the median plane of the plate. Antisymmetric Lamb waves exhibit flexural character and their velocity decreases with decreasing plate thickness. Flexural Love waves are composed of elliptical motion (as with Rayleigh waves) and, therefore, contain shear and compressional components. However, as a consequence of the minimal thickness of the plate, the wave velocity is lower than the compressional velocity of sound in liquids. Accordingly, compressional waves are not coupled in liquids, resulting in less attenuation of acoustic energy. The devices that generate such waves are termed flexural plate wave (FPW) devices [Fig. 3(A)] and can operate in liquids. FPW devices are microfabricated from very thin silicon or composite membranes with a sputtered ZnO piezoelectric layer. IDTs are positioned in a way to form a delay-line configuration. Since the piezoelectric plate is so thin, the operating frequencies of FPW devices are substantially lower than for other surface-launched acoustic wave devices (2–7 MHz).<sup>12,13,15</sup>

Another type of device in this category, shear horizontal acoustic plate (SH-APM) mode devices [Fig. 3(B)], produce waves where particle displacement is parallel to the sensing surface. Generated waves reflect between the plate surfaces which results in their superposition and the formation of a series of plate modes of different frequencies. With a configuration similar to typical SAW devices, SH-APM devices operate at frequencies in the range 25–200 MHz and are designed for tuning to a particular plate mode by adjusting the transducer bandwidth (determined by the number of finger pairs in the IDTs) and plate thickness. The device can operate in both liquid and gas media.<sup>12,15</sup>

Thick quartz plates of a different crystal orientation can produce shear horizontal surface waves (SH) parallel to the

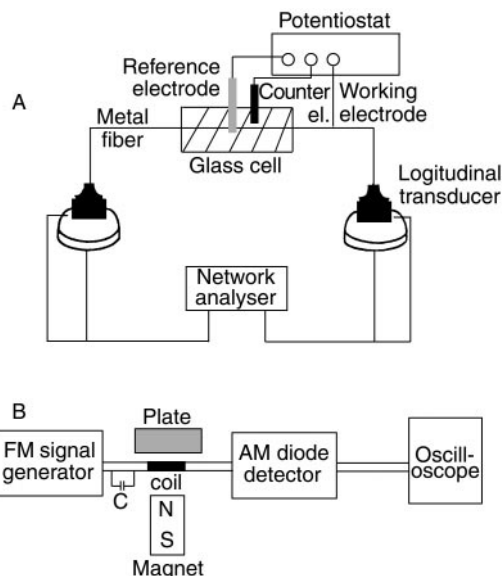


**Fig. 3** (A) FPW: schematic diagram of a wave motion and direction of particle displacement. (B) SH-APM: schematic diagram of a wave motion within the plate and direction of particle displacement for the  $n = 1$  mode. (C) STW: the structure of the device and direction of particle displacement.

surface of devices. Structures producing this kind of wave are surface transverse wave (STW) devices [Fig. 3(C)] where a metal grating is used to prevent wave diffraction, and Love-wave devices which involve a structure where thin films are applied for the purpose of guiding acoustic waves. These acoustic devices also involve interdigital transducers.<sup>12</sup>

Lately, the development of acoustic wave devices that do not require piezoelectric materials for the propagation of acoustic waves has been under way. Thin-rod acoustic wave devices (TRAW) [Fig. 4(A)] are based on the propagation of acoustic waves in a cylindrical rod, which actually consists of a fibre with a radius much smaller than the acoustic wavelength. Piezoelectric input transducers generate and receive acoustic waves in a delay line configuration with respect to the thin rod. The rod itself does not have to be made of a piezoelectric substrate, because the acoustic wave propagation is based on a purely mechanical process. Two modes of vibration in TRAW devices are apparent, *i.e.*, flexural and extensional. Whereas particle displacement for flexural waves is perpendicular to the plane of the thin rod surface, it is in the plane of the sensing surface for extensional waves, which results in an increased viscous damping on propagation into liquids. The possibility of increasing the mass sensitivity of the sensor by decreasing the fibre radius with facile operation of the device, particularly in the flexural mode, in liquid media, make this type of acoustic wave device appealing for the study of interfacial electrochemistry.<sup>16,17</sup> By introducing a tube instead of a thin rod, a sensor with a high mass sensitivity which is provided by a small thickness of the tube wall has been designed. Suitable geometry combined with only minor observed acoustic losses when the device is immersed in liquids makes it suitable for perspective application in on-line monitoring of physical and chemical changes in flow.<sup>18</sup> The most recently described device exploits a coupling mechanism between electromagnetic and acoustic waves. A magnetic-acoustic-resonator sensor (MARS), presented in Fig. 4(B), consists of a non-piezoelectric acoustic plate which is excited from distance. Generation and detection of shear acoustic waves are performed by the means of a radiofrequency signal and a strong magnetic field. It was reported that the effect of mass loading of a metallized glass plate in air, incorporated into MARS device, can be predicted from Sauerbrey's equation similarly to TSM devices.<sup>19</sup>

Table 1 depicts the comparison of some of the characteristics of the acoustic wave devices that have been described in brief above, together with the correlation of their responses to mass loading.



**Fig. 4** (A) The configuration of a TRAW system in flexural mode. (B) The configuration of a MARS device.

### 3 Acoustic waves and the liquid–solid interface

When a crystal whose lattice does not have a centre of symmetry is stressed, the asymmetric motion of charge centres produces an electric field.<sup>2</sup> Conversely, when an electric field is placed across this type of crystal, the attraction and repulsion of the charge centres generate stress in the crystal. The converse piezoelectric effect provides a convenient method for generating motion at ultrasonic frequencies.

The physical properties of various piezoelectric materials make each suitable for a particular application. For ultrasonic resonators, quartz is the material of choice. Its primary advantage is its high stiffness. Resonators made of quartz have low losses and very sharp resonances.

Quartz is a hexagonal crystal with Cartesian axes shown in Fig. 5.<sup>20</sup> The crystal may be rotated, but the *X*-axis passes through a vertex and the *Y*-axis passes at right-angles through a face. When a field is placed across the crystal parallel to the *X*-axis, the thickness in the *X* direction increases. The *X*-axis is defined as positive at the side where a negative charge is generated by compressing the crystal. If the field is aligned with the *Y*-axis, the resulting deformation is a shear along the *Z*-axis. The *Z*-axis is electrically neutral.

The quartz crystals used in sensor applications are wafers cut from a larger crystal. To achieve a shear motion, the cut must be normal to the *Y*-axis so that the field can be produced by charging electrodes placed on the two faces of the wafer. If the

cut is rotated about the *X*-axis, the temperature coefficient changes markedly. The AT cut is normal to the *Y*-axis but rotated 35.25° from the *Z*-axis. This has a zero temperature coefficient at 40 °C, making the AT cut ideal for frequency control applications. The resulting mass production has made these crystals very inexpensive.

Since quartz is anisotropic, most of its physical and electrical properties are direction dependent. The dielectric constant, elasticity and piezoelectric constants, and others, are expressed as matrices. Since the AT wafer is cut at a particular rotation and the electric field is placed across the wafer, the result is a set of scalar properties valid for the particular rotation. The field results in a shear deformation across the wafer with no thickness extension.

When used as a thickness-shear mode sensor, the crystal is operated as a resonator. Since the applied field across an AT cut wafer causes both free surfaces to move in parallel but opposite directions, both surfaces are motion antinodes. This means that the thickness of the wafer will be an odd multiple of half of the acoustic wavelength in quartz. The velocity of the shear wave is given by<sup>20</sup>

$$v = (c/\rho)^{1/2} \quad (1)$$

where  $v$  is the acoustic shear wave velocity,  $\rho$  is the density of quartz and  $c$  is the stiffness calculated for the direction of motion and the wafer cut angle. This gives a resonant frequency

$$f_0 = nv/2d_Q \quad (2)$$

where  $f_0$  is the resonant frequency in hertz,  $n$  is an odd integer and  $d_Q$  is the thickness of the wafer. The quartz resonator is a mechanically resonant system which has two forms of energy storage, elastic deformation and inertia. Energy is transferred from one to the other at a characteristic rate dependent on the mass and stiffness of the resonator. These parameters are both represented in the above equations.

This analysis works well for a crystal resonator operating in a vacuum. However, if the crystal is loaded by an additional mass attached to the surface or by immersion in a viscoelastic medium, the resonance behaviour will be affected. These loading effects provide the basis for the TSM and other resonant sensors.

The first quartz sensors were developed by Sauerbrey<sup>4</sup> to measure mass deposited on the crystal surface. The model, which is still widely used, considers the added mass to be simply an extension of the crystal thickness:

$$\Delta f = -2f_0^2 (c\rho)^{-1/2} \Delta m/A \quad (3)$$

where  $\Delta f$  is the resonant frequency shift due to the added mass,  $\Delta m$  is the added mass and  $A$  is the area of the crystal. This is valid for small amounts of added mass; however, the equation overpredicts the frequency shift when the added mass gives a frequency shift greater than about 2% of the unloaded resonant frequency. Miller and Bolef<sup>21</sup> considered the loaded crystal as a compound resonator which gave Sauerbrey's equation with higher order terms. This extends the model range to frequency shifts up to 15% of the unloaded value.

When the resonator is immersed in a fluid, energy is lost to the fluid through viscous coupling. Kanazawa and Gordon<sup>22</sup> modelled the resonant frequency shift due to viscous coupling by assuming no slip between the fluid and the crystal surface. The resulting solution of the fluid equations of motion is a transverse shear wave that propagates into the liquid. This wave decays with a characteristic length  $\delta$ :

$$\delta = (2\eta_L/\omega\rho_L)^{1/2} \quad (4)$$

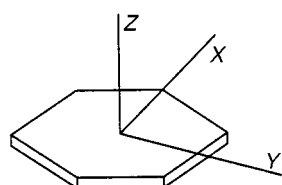
where  $\eta_L$  is the fluid viscosity and  $\rho_L$  is the fluid density. In water this length is short, of the order of 0.25  $\mu\text{m}$ . The frequency shift due to the fluid loading was also calculated:

$$\Delta f = -f_0^{3/2} (\rho_L\eta_L/\pi c\rho)^{1/2} \quad (5)$$

**Table 1** Survey of some of the properties of various acoustic wave devices<sup>12,15–18</sup>

Type of device	Particle displacement (relative to the wave propagation direction)	Typical operation frequency MHz	Mass sensitivity <sup>a,b</sup>
TSM	Transverse	5–20	$-1/(\rho t)$
SAW	Transverse and parallel component	30–500	$-(f_0 K_1)/(\rho V)$
FPW	Transverse and parallel component	2–7	$-1/(2\rho t)$
SH-APM	Transverse	25–200	$-J/(\rho t)$
STW	Transverse	200–500	$-(f_0 K_2)/(\rho V)$
TRAW	Transverse (flexural mode) and parallel (extensional mode)	0.2–2	$-1/(n\rho a)$
Tube	Parallel (extensional mode)	2	$-1/[\rho(b - a_1)(1 + a_1/b)]$

<sup>a</sup> Mass sensitivity ( $S_m$ ) is defined as  $S_m = \lim (\Delta v)/(v_0 \Delta m) \Delta m \rightarrow 0$ , where:  $\Delta m$  is added mass,  $\Delta v$  is change in phase velocity and  $v_0$  is unperturbed phase velocity. <sup>b</sup>  $\rho$ , Density of the sensor material;  $t$ , plate thickness;  $f_0$ , fundamental, unperturbed frequency of the device;  $K_1$ , constant dependent on the properties of the plate of the SAW device;  $V$ , wave velocity;  $J$ ,  $J = 1/2$  for an isotropic plate mode ( $n_1$ )  $n_1 = 0$  and  $J = 1$  for  $n_1 > 0$ ;  $K_2$ , constant dependent on the properties of the STW device;  $n$ ,  $n = 1$  for the first extensional mode and  $n = 2$  for the flexural mode;  $a$ , radius of the thin rod;  $b$  and  $a_1$ , external and internal diameter of the tube, respectively.



**Fig. 5** Cartesian axes for a quartz crystal.

When only the resonant frequency shift of a sensor operating in a fluid is measured, it is not possible to assess the individual contributions of mass and viscosity. To obtain additional information, the quartz resonator system can be modelled as an electrical equivalent circuit based on a simple analogy, the Butterworth–van Dyke model.<sup>20</sup> A simple derivation may be made by performing a force balance on a damped mass and spring system as shown in Fig. 6. The force balance is given by

$$m \frac{d^2x}{dt^2} + kx + \eta \frac{dx}{dt} = F \quad (6)$$

where  $m$  is the mass,  $k$  is a spring constant,  $\eta$  is a damping coefficient and  $F$  is an applied force. Taking the Laplace transform of eqn. (6), dividing by the velocity and transforming the equation into the frequency domain with  $s = j\omega$  gives

$$j\omega m + k/j\omega + \eta = F/V \quad (7)$$

where  $j$  is the square root of  $-1$ ,  $\omega$  is an angular frequency and  $V$  is the velocity of the mass.

$F/V$  is a driving force divided by a flow like term, and so is analogous to a resistance. This provides the motional arm of the Butterworth–van Dyke (BVD) model of a quartz resonator. The impedance represented by eqn. (7) consists of an inductor arising from the inertial term, a capacitor arising from the elastic stress term and a resistor arising from the damping. The imaginary terms represent energy storage while the real resistance term represents energy dissipation. The complete BVD model of a resonator in an electric circuit, as shown in Fig. 7, includes a parallel capacitor representing dielectric energy storage.

The previous model has been extended to include the effects of mass deposition and liquid loading.<sup>23</sup> The deposited mass adds an inductor to the model, but the viscous loading adds both a resistor and an inductor. The resistance accounts for the energy dissipated by the fluid. Some of the energy transferred to the fluid is returned through viscous coupling so that the coupled fluid acts as an attached mass. Simpson<sup>24</sup> showed that the impedance magnitude of the viscous resistance and the corresponding inductance are equal. By measuring the resistance and the resonant frequency shift, mass and viscous effects may be determined simultaneously.<sup>25</sup>

Hayward and Thompson<sup>26</sup> developed a model of a chemical sensor based on the previous work of Reed *et al.*<sup>27</sup> and Ferrante *et al.*<sup>28</sup> The sensor was a coated crystal operating in a fluid where the coating represented a thin layer of a chemically or biologically sensitive agent. These models solved nine fundamental differential equations to predict the response of the sensor. The formulation of these models introduces additional sensor response mechanisms that have not yet been exploited.

Reed *et al.*<sup>27</sup> modelled the response of a resonator to the properties of an attached layer of fluid. Good agreement with experimental data was obtained for low molecular mass liquids; however, polymers gave lower losses and resonant frequency shifts than predicted. This was attributed to the elastic behaviour of these materials, particularly at the high frequencies used with

quartz resonators. This film elasticity acts as an additional energy storage mechanism.

Ferrante *et al.*<sup>28</sup> developed a similar model, but the fluid layer was assumed to be only viscous and infinitely thick. This is the case when a resonator is immersed in a fluid medium. Changes in the losses and frequency shifts with the hydrophilic–hydrophobic nature of the surface were attributed to slip at the fluid–resonator interface. Here the fluid layer at the surface and the surface itself move at different velocities and out of phase.

Amplitude calculations from the Hayward–Thompson model<sup>26</sup> show that in water, the motion is approximately  $30 \text{ nm V}^{-1}$ . At this scale, slip at the surface is possible and will depend on the affinity between the surface and the medium. Hayward and Thompson also showed that the effect of this slip and that of viscoelasticity cannot be separated in the resonance model, however they occur together.

Recently, Bandey *et al.* developed a very versatile, general equivalent circuit model describing TSM impedance response under different types of loadings, varying from rigid solids, Newtonian and Maxwellian fluids to viscoelastic layers and even multilayers and overlayers.<sup>29</sup> According to the authors, the model permits the characterization of any combination of described physical conditions at the interface.

These models indicate that a resonator can provide detailed information regarding the interaction between a resonator, a sensing layer and a medium. These can be changed by several factors: mass deposited on the resonator surface; energy dissipation by viscous action in the medium; energy storage by elastic deformation in the sensing film or the medium; and changes in the coupling between the surface and the medium. All of these process occur together, but in many systems one will dominate the others.

## 4 Measurement techniques

The technique of choice for the monitoring acoustic wave device operation, in most studies, involves the oscillator method. Within such a configuration, the device constitutes the frequency-controlling element of a circuit. The oscillator method measures only one electrical parameter, the series resonant frequency of the resonating sensor. A typical oscillator circuit consists of two inverters connected in series producing a non-inverting amplifier. The sensor is connected from the output to the input of the amplifier to give a positive feedback.<sup>30</sup> Mechanical damping of the generated acoustic wave results in a decrease in the amplitude of the received/reflected signal. At the point where admittance magnitude becomes too attenuated, as in a liquid medium where the viscosity exceeds a certain value, the oscillator circuit stops functioning.<sup>12</sup> Alternatively, if an oscillator circuit is employed with automatic gain control, the amplitude of the output voltage of the amplifier is kept constant.<sup>11,25</sup>

In contrast to the oscillator technique, operation of acoustic devices in tandem with an impedance analyser involves the application of a sinusoidal voltage to the sensor resulting in a generated current which, by internal computing, provides admittance or impedance data.<sup>31,32</sup> The method also permits the measurement of both series and parallel resonant frequencies together with corresponding energy dissipation factors.<sup>33</sup> When the system is in series mode, the crystal oscillation is sensed by measuring the current that flows through the device, whereas when it is in parallel mode, the voltage over the crystal is detected. In both cases the decay curve is numerically fitted to the exponentially damped sinusoidal equation, yielding both resonant frequency and energy dissipation parameters.

The application of the network analysis method, provides the most complete characterization of the electrical information

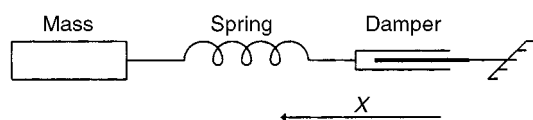


Fig. 6 Mechanical analogy of a quartz resonator.

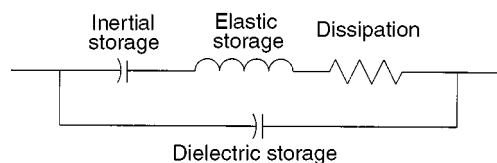


Fig. 7 Extended Butterworth–van Dyke model.

obtained when an acoustic wave device operates in liquids.<sup>34</sup> Values of the magnitude and phase of the impedance of the sensor are continuously calculated and registered. By fitting recorded data to the equivalent electrical circuit, the values of the circuit parameters can be calculated from the impedance–frequency curves. Among the quantities obtained from such an analysis are the series and parallel resonant frequencies, motional resistance and interfacial capacitance. The data obtained are related to the physical properties of the quartz, surface mass and contacting liquid, providing multiple information regarding the behaviour of the sensor in the liquid phase.<sup>23,35</sup>

The coupling of a flow injection analysis (FIA) system to an acoustic wave sensor [Fig. 8(a)]<sup>36</sup> allows the monitoring of kinetic processes involved at the sensor surface–liquid interface. The design of a flow-through cell incorporating the sensing element varies with different experimental set-ups, but the essential requirement is always the attempt to generate a continuous laminar flow of liquid with minimized mechanical and electrical influence on sensor performance. As an approach to FIA-type analysis, a typical flow-through cell incorporating a TSM sensor [Fig. 8(b)]<sup>36</sup> consists of two blocks clamped together to give a minimal volume of liquid in place over the device surface. Only one side of the sensor is exposed to the liquid, the other usually being kept under nitrogen. Generally, the sensing element is held between two O-rings, but in some designs it can be sealed or glued to the supporting holder.<sup>13</sup> The fragility of quartz wafers makes the design of such a flow-through cell a particularly difficult task in practical terms and significant further advances can be expected in the future.

## 5 Protein adsorption

Research involving the study of protein adsorption at the solid–liquid interface has become an extremely important activity. A better understanding of such phenomena is essential for any investigation of crucial processes such as blood coagulation or solid-phase immunoassays, because the adsorption of proteins on solid surfaces appears to be the initial step in both events. The great number of related studies, with very often contra-

dictory findings, makes this field even more scientifically intriguing.

The complex structure of protein molecules and the existence of the considerable structural variety among the protein population, together with the essentially irreversible nature of certain adsorption processes, make the study of surface phenomena particularly complex and the description of appropriate kinetics very difficult to unravel. While traditional experimental techniques, predominantly involving radiolabelling protocols, provide important information concerning the amount of adsorbed protein, the general lack of adequate on-line techniques to study the kinetics of the process reliably has been widely recognized. Acoustic wave devices are among only a few of the available methods that can reach this goal, without the necessity for any modification of the protein molecule under study.<sup>13,37</sup>

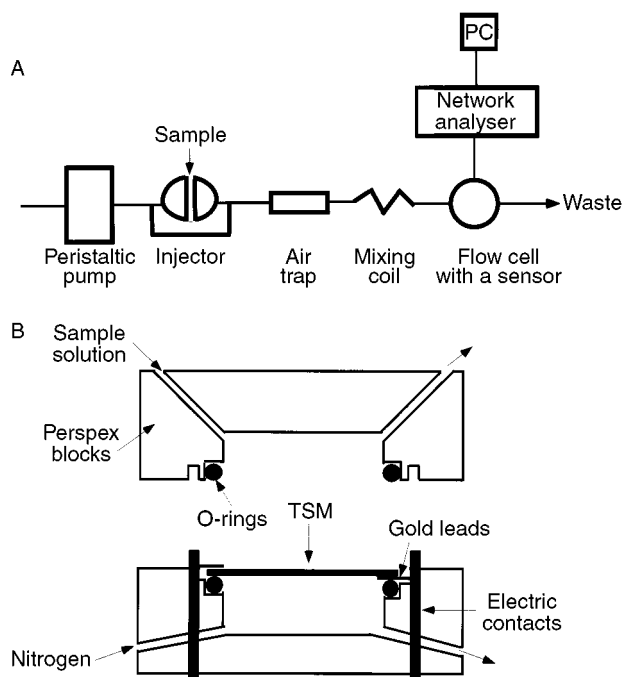
A number of important studies dealing with basic protein adsorption to unmodified and chemically modified electrodes of TSM sensors have been published. Often, in many cases, the point has been made that frequency responses could not be correlated with the adsorbed mass of protein predicted by Sauerbrey's equation.<sup>4</sup> These findings indicate that for TSM devices operating in liquids, the mass response concept cannot be invoked to predict the amount of adsorbed protein. The change in frequency is probably governed not only by mass loading but also by boundary conditions at the sensor–liquid interface, such as interfacial free energy, liquid structure and coating film properties. The formation of a viscoelastic, highly hydrated protein layer at the interface produces changes in frequency response through viscous losses together with alteration of energy and dissipation processes.

TSM devices coated with polysulfone were used to determine adsorption isotherms for albumin on a polymer surface.<sup>38</sup> Mass-based frequency responses were corrected by the introduction of a protein bulk concentration-dependent term to obtain a linear relationship. Higher values for the adsorption isotherms, obtained through the increased frequency shift, compared with predicted values, were attributed to the effect of hydration of adsorbed protein molecules.

The adsorption of mellitin and  $\beta$ -globulin to phospholipid has been studied by the attachment of a lipid monolayer to the device surface by a Langmuir film balance.<sup>39</sup> Frequency responses were measured both on-line from the solution and in air after drying, with the adsorbed mass of protein being evaluated from Sauerbrey's equation. The discrepancy between the amount of adsorbed  $\beta$ -globulin calculated on the basis of the two different types of measurement was attributed to the incapacity of the large protein to penetrate into the lipid layer. As in the previous study, water hydration was assumed to be responsible for the increased frequency response.

Acoustic network analysis was applied to characterize TSM device response for the adsorption of  $\alpha$ -chymotrypsinogen on unmodified gold electrode surfaces.<sup>40</sup> In addition to the series resonant frequency, other quantities such as parallel resonant frequency, motional resistance and static capacitance were also monitored. The shift in parallel resonant frequency, which is influenced by the composition of the charged interface, implied that the protein adsorbs initially in its native state, then undergoes later conformational changes that modify its surface charge characteristics. These findings were supported by the analysis of static capacitance response, which is primarily associated with the capacity of the electrical double layer at the charged interface.

The importance of the size, shape and hydrophobicity of adsorbed protein has been associated with the enhancement of frequency response (in solution compared with air) for the adsorption of ferritin on the gold electrodes of piezoelectric crystals.<sup>41</sup> On the basis of the amount of protein, calculated from Sauerbrey's equation for the frequency measurement in air, the authors concluded that the protein adsorbed on the



**Fig. 8** (A) A single line manifold configuration for the FIA–TSM network analyser system. (B) Diagram of a flow-through cell incorporating a TSM acoustic wave device.

electrode constituted less than monolayer coverage. The information extracted through protein film characterization by X-ray photoelectron spectroscopy, atomic force microscopy (AFM) and surface plasma resonance (SPR) spectroscopy supported the above finding.

The analysis of the energy dissipation factor, rather than frequency response alone, was performed in a study where an enhanced sensor response was obtained in the case of the adsorption of viscoelastic, non-rigid layers of different biomolecules.<sup>42</sup> The experimental results showed that the adsorption of smaller proteins (myoglobin, haemoglobin, albumin) results in less dissipation shifts than for larger proteins such as ferritin or fibrinogen, after adsorption on hydrophilic gold electrodes or devices modified with a hydrophobic self-assembled thiol monolayer. The surveyed energy dissipation was defined by the co-existing action of three phenomena superimposed on the viscoelastic porous biofilm during the oscillation: the strain of the film, trapped liquid and the load from the bulk liquid. The authors used the same technique to study the adsorption of two forms of hemoglobin (met-Hb and HbCO) to the previously mentioned hydrophobic gold surface.<sup>43</sup> For both forms of protein, the results suggested the existence of two states of adsorption process exhibiting different viscoelastic properties. In the first phase the molecules are said to be more rigidly bound, forming a partially denatured protein layer, whereas in second phase an additional, more loosely bound layer of molecules is created, which is supposed to retain a native-like state.

The adsorption kinetics of fibrinogen on the non-electrode surface of acoustic plate mode (APM) devices, sputtered with gold and modified with two differently terminated self-assembled monolayers (SAMs), has been examined.<sup>44</sup> It was observed that while no protein adsorption occurred on hydrophilic hexa(ethylene glycol)-terminated SAM, large amounts of fibrinogen were adsorbed on hydrophobic methyl-terminated SAM which could be subsequently completely removed by surfactant. On the basis of these results, a two-state kinetic model was assumed to describe the adsorption process, where fibrinogen initially adsorbs on the surface in a native state and, subsequently, unfolds.

The experimental results of another study indicated that the adsorption of fibrinogen on the gold electrodes of TSM devices modified with hydrophobic SAM resulted in an increased biosensor response compared with the case for a relatively hydrophilic gold surface.<sup>45</sup> The same study presented evidence that the tertiary structure of avidin might have been compromised by adsorption on hydrophilic surfaces.

The flow-through adsorption and characterization of the proteins human serum albumin,  $\alpha$ -chymotrypsinogen A, cytochrome *c*, fibrinogen, haemoglobin, immunoglobulin G and apo-transferrin to the two siloxane surfaces polymercaptopropylmethylsiloxane and octaphenylcyclotetrasiloxane and clean gold electrodes were monitored by acoustic network analysis.<sup>46</sup> The nature of the responses for these unmodified and siloxane-modified TSM devices was discussed in terms of the various possibilities for interfacial molecular interactions, such as sulfur-containing moieties with gold, and side-chains with the phenyl rings present in octaphenylcyclotetrasiloxane. The overall direction of the responses, reduction in series resonance frequency and increase in motional resistance suggested the introduction of a viscoelastic protein film at the interface or perturbation of acoustic coupling to the liquid.

TSM device measurements and data obtained by double-layer capacitance were compared to study the kinetics of the adsorption of albumin on gold.<sup>47</sup> It was concluded that sensor oscillations modified the adsorption kinetics by demanding an additional time constant to characterize the process. The authors considered that the biomolecular layer formed a stronger interaction with a non-oscillating than with an oscillating electrode and that, consequently, information concerning ad-

sorption mechanisms obtained by TSM devices might be misleading.

Adsorption and desorption of albumin and a thiol derivative on the gold electrode surfaces of TSMs have been monitored on-line in order to show the reversible adsorption of the former and irreversible adsorption of the latter.<sup>48</sup> This very surprising result was used to support the findings obtained by application of an electrochemical 'non-linear' impedance method, where the measurement of non-linear electrochemical properties provided qualitative information on the adsorption state of the protein through the evaluation of the differential capacitance and conductance. The assumed physisorption of albumin resulted in loosely bound molecules and, consequently, a high voltage dependence of capacitance and conductance, while the same parameters in the case of the chemisorbed thiol derivative, tightly bound to surfaces, responded poorly to a polarity change associated with applied potential.

In another study, the adsorption of albumin was monitored on the platinum electrodes of TSMs.<sup>49</sup> The authors used Sauerbrey's equation to estimate the mass loading of the protein, and suggested the existence of a monolayer of randomly oriented molecules. In contrast to a previous study, the adsorption of albumin was shown to be irreversible, based on experiments involving cyclic voltammetry. However, *in situ* stepping of the TSM electrode potential in the negative direction induced the minor desorption of albumin, which was attributed to an increase in electrostatic repulsion or decrease in electrostatic attraction within the protein molecule population, which is less tightly bound to the surface.

The elutability of the milk proteins  $\beta$ -casein and  $\beta$ -lactoglobulin from clean gold and hydrophobic gold surfaces was studied by TSM devices.<sup>50</sup> The experiments showed that proteins are harder to remove with surfactant from gold than from hydrophobic gold surfaces, possibly owing to the formation of covalent thiolate bonds between protein molecules and the gold surface. The enhanced frequency shifts that were observed in this study were attributed to changes in protein hydrodynamic layer thickness.

An interesting attempt was made to develop an acoustic biosensor based on a thin (10  $\mu\text{m}$ ) film of a piezoelectric polymer, poly(vinylidene fluoride) (PVDF), which is sometimes also applied as a material for affinity membranes.<sup>51</sup> An equation for mass sensitivity similar to that of Sauerbrey was derived and protein (IgG and BSA) adsorption was monitored in real time. Instability and numerous interferences were reported, with an enhanced Sauerbrey-like response being explained by involving the possibility of invoking multilayer protein adsorption and viscosity effects.

Considerable research has been performed with respect to multilayer protein architecture, where TSM devices have been employed for monitoring the assemblage. Multilayers have been obtained by alternate adsorption of species including either opposite charges or specificity. Charged protein layers (cytochrome *c*, myoglobin, lysozyme, histone f3, haemoglobin, glucoamylase and glucose oxidase) were linked to positively charged polyethylenimine or negatively charged poly(styrene sulfonate) through alternate electrostatic adsorption.<sup>52</sup> In this study, the assembly of up to 24 molecular layers of entire film thickness of 500  $\text{\AA}$ , incorporating adsorbed myoglobin and lysozyme, was deposited on the silver electrodes of 9 MHz piezoelectric crystals.

The development of bioaffinity sensors, which are based on highly selective and sensitive binding interactions between a ligand and a biomolecule-receptor, has been the subject of many studies which will be discussed here and in the following sections of this review. The application of the capability of biological macromolecules for recognition of complementary substrate molecules and subsequent specific binding to them is the subject of a major research effort in the field of biosensor technology. However, many such systems have limited prac-

tical application because of factors generally connected with chemical and thermal instability.

TSM devices with gold electrodes, modified with desthiobiotinylated albumin, were used to confirm the higher affinity for complementary coupling of avidin to biotin in solution rather than to the immobilized desthiobiotin.<sup>53</sup> The high affinity and selectivity of the avidin–biotin interaction were applied for monitoring the *in situ* deposition of up to 20 protein layers on gold electrodes of 6 MHz piezoelectric crystals, involving streptavidin and biotinylated albumin.<sup>54</sup> Frequency responses were measured in air, for dry protein assemblies as well, and were found to be in the accordance with Sauerbrey's equation. The enhancement of frequency responses *in situ* was ascribed to the high hydration of protein layers. Considering the presumption that the sensitivity of TSM devices should decrease with increased viscoelastic coupling and after showing undiminished sensitivity of the device during the build-up of protein layers, the authors concluded that protein films behave as a rigid solid. In another study, 9 MHz TSM sensors with gold electrodes were applied for monitoring the formation of avidin–biotin conjugate multiple layer structures incorporating dextran, insulin and albumin.<sup>55</sup> Although the build-up of multilayers was detected for more than a dozen avidin–biotin complexes, it was clearly observed that the resulting frequency shifts gradually diminished, which was explained by an increase in the thickness of the film which surpassed the decay length of shear wave motion.

The specific binding of biomolecules to lipid receptors incorporated in supported membranes deposited on electrodes of TSM devices has been the subject of a number of studies. In some of this work, gold electrodes of 5 MHz TSM devices were functionalized with octanethiol and ganglioside-containing phospholipid vesicles were fused on the hydrophobic SAM.<sup>56,57</sup> Impedance spectroscopy was used to confirm the complete immobilization of the bilayer lipid membrane. The specific binding of various proteins, a lectin (peanut agglutinin)<sup>56</sup> and bacterial toxins (tetanus, cholera and pertussis),<sup>57</sup> to ganglioside receptors was detected by monitoring the series resonant frequency of TSM devices. On the basis of the experimental results, the authors concluded that the frequency shifts resulting from binding processes are specific parameters for particular ligand–receptor complexes. Discrepancies between measured frequency responses and those predicted by Sauerbrey's equation for a complete protein monolayer were attributed to factors affecting the lipid–protein interface such as viscoelasticity, the electrochemical double layer and surface free energy.

In another study, the binding affinity of a lectin for lactosylceramide was compared with the case where the glycolipid receptor monolayer was homogeneously mixed with matrix phospholipid to the situation when it was phase-separated from a phospholipid monolayer, by means of a 27 MHz TSM horizontally attached to monolayers.<sup>58</sup> On the basis of binding amounts calculated from frequency decreases, the authors concluded that the lectin shows higher affinity for the clustered glycolipid.

TSM devices modified with photoactive materials have also been the subject of study. TSM devices (9 MHz) with gold electrodes modified with nitrospiropyran–pyridine photoisomerizable-mixed monolayers were applied to monitor the photostimulated association and dissociation of cytochrome *c* to and from a photoswitchable monolayer.<sup>59</sup> Photoisomerization of the nitrospiropyran sites resulted in the protonated nitromerocyanine state which exerted electrical repulsive interactions with the positively charged cytochrome *c* associated with the pyridine sites. Consequently, the haemoprotein dissociated from the photoactive interface. It is important to mention that illumination cycles were actually performed outside the cell.

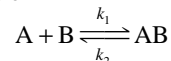
As can be seen, in many studies the attempt has been made to associate experimental frequency responses with Sauerbrey's

equation. It appears that the prevailing explanation for the enhanced frequency response of TSM sensors in a liquid medium involves the role of loosely bound water molecules. It is likely that this type of association with proteins might increase their motional freedom and, therefore, energy losses through conformational changes in the structure. However, all the efforts made to emphasize the contribution of water molecules to the effective mass load appear to be completely inappropriate for a system where the protein film is surrounded by bulk water. By reducing the function of a TSM device to a microbalance, much readily available information on biomolecules at the TSM–liquid interface remains unappreciated. In reality, the complex response of a TSM sensor provides valuable qualitative data about the nature of the adsorbed protein layer and the functional consequences of the adsorption process.

## 6 Detection of interfacial immunochemical interactions

Among the most important biological systems composed of molecules with specific binding properties are antibodies–immunogens (antigens), enzymes–substrates, lectins–oligosaccharides, receptors–various molecules (hormones, neurotransmitters, cytokines), avidin–biotin, protein A–antibodies or antibody–antigen complexes, DNA–DNA or RNA, transport proteins–various molecules and ions. In addition, the design of synthetic cognitive elements with particular binding specificities is the subject of much current research. Some of above-mentioned molecular recognition processes have already been discussed in the previous section.

A molecular interaction for an affinity sensor can be described by the equation



where A is the molecule, B a substance with binding properties towards A, AB the complex between them,  $k_1$  rate of association and  $k_2$  rate of dissociation. The binding constant ( $K$ ) represents the affinity between the two elements:

$$K = [AB]/([A][B]) = k_2/k_1$$

An antigen can induce a polyclonal response, *i.e.*, the production of a large number of antibodies with different binding constants.<sup>13,60–62</sup>

Compared with other applications of acoustic wave devices as biosensors, far more work has been carried out with respect to the development of immunosensors. In this section we shall discuss studies where various immunochemical reactions have been performed at the acoustic sensor–liquid interface.

The earliest attempt to develop an immunosensor based on a TSM device was in 1972 when 5 MHz piezoelectric crystals were used to detect the formation of a BSA–anti-BSA sera complex by the 'dip and dry' method.<sup>63</sup> Such a procedure consisted of dipping piezoelectric crystals, pre-coated with antibody/antigen, into a solution of antigen/antibody protein with subsequent measurement of the frequency of the dried sensor for comparison to the frequency obtained before immunoreaction. This type of approach has been adopted in a surprising number of later studies. For example, in a recent investigation, different human herpes viruses were detected with a reported excellent correlation between frequency change and the number of viral cells.<sup>64</sup> Synthetic peptides have been used to generate herpes virus-specific monoclonal antibodies, which were, in turn, immobilized *via* protein A on modified gold electrodes of TSMs. The 'dip and dry' method was also used for the characterization of an atrazine immunosensor based



on protein A-modified TSM with atrazine antibody immobilised on the device surface<sup>65</sup> and for the detection of a microbe (*Vibrio cholerae*) by a TSM immunosensor.<sup>66</sup> An assay with excellent precision for the detection of atrazine has been reported, in contrast with the observations of many researchers who have experienced great difficulty in obtaining reproducible frequency measurements in air after the devices have been submitted to any significant manipulation and (bio)chemical transformation. Experience shows that conclusions based on any relatively small, off-line, frequency changes in air related to the mass sensitivity of TSMs can only be regarded as, at best, highly speculative. Another system for the detection of the same herbicide was proposed later when the specific reaction between atrazine immobilized on modified TSMs and its monoclonal antibody was monitored in real time.<sup>67</sup>

In contrast to the above, the complexity of the physics of propagation of acoustic shear waves through the TSM–bulk liquid interface has been recognized for some time. Silanized TSM devices and those modified with polyacrylamide gel were used to monitor the formation of the complex between the immobilized IgG antigen and the IgG from the solution in real time.<sup>11</sup> In this study it was clearly pointed out, for the first time, that TSM systems with viscoelastic proteinaceous layers at the interface do not respond to absolute amount of added material associated with Sauerbrey-type mass frequency physics, but rather to perturbations in interfacial conditions. Similar immunoreactions were monitored on the surface of TSMs precoated with protein A, which specifically binds human IgG.<sup>68</sup> In another study, silanized TSMs were used to monitor the preferential binding affinity of IgG subclasses to immobilized protein A.<sup>69</sup> In both studies, unsuccessful attempts were made to correlate the amount of theoretically bound immunoreactant with the assumed mass sensitivity of the device. In a later study, where the interaction between anti-HSA and HSA on the surface of TSMs was observed, the enhanced frequency response was positively established by radiolabelling and attributed to interfacial factors.<sup>70</sup> Recently, the binding of HSA to complementary monoclonal and polyclonal antibody precoated on TSMs was examined and compared.<sup>71</sup> Assuming that the change in resonant frequency varies linearly with the mass of adsorbed protein, the authors were able to estimate association constants and rate constants for the immunoreactions.

The detection of HIV antibodies by means of synthetic HIV peptide (the epitope of the protein) immobilized on 20 MHz TSM was performed with a selectivity comparable to that of a licensed ELISA-based test.<sup>72</sup> The authors claimed that by application of a differential FIA method, which eliminated the damping effect, the validity of Sauerbrey's mass–frequency dependence was assured. Other authors applied a dual modulation method that employed a sealed crystal providing frequency difference relative to the working crystal to overcome damping disturbances.<sup>73</sup> A specific reaction between an enterotoxin and its antibody immobilized on modified electrodes of TSMs was monitored by this procedure.

The enhancement of the frequency signal has been recognized in many immunoreaction-related studies, and various attempts to elucidate the observed phenomenon have been made. Many of the efforts have concentrated on the distinguishing of effective mass load and the presence of a viscoelastic biomolecular layer on the surface of the device. The oscillator frequency and the electroacoustic admittance were alternately measured during the selective reaction between a peroxidase and the corresponding antibody immobilized on a 27 MHz transducer, in order to compare the on-line change of parameters characterizing TSM equivalent circuit with the oscillator frequency which was supposed to depict only a mass variation.<sup>74</sup> The adsorption of the viscoelastic protein layer resulted in an increase in motional resistance and an alteration in rheological properties that produced an overestimation of the

mass given by the oscillator frequency. However, the lack of the motional resistance increase during the immunoreaction itself was explained by the inadequacy of the performed immunoassay. In another study, where the acoustic network analyser method was used to monitor immunochemical interactions, the role of boundary viscoelastic properties of the surface of a TSM was examined through a shear acoustic impedance signal which was connected to rigid mass and interfacial liquid variation.<sup>75</sup> Two types of immunoreactions were discussed. In the case of selective binding of erythrocytes to IgM, changes of boundary liquid viscosity dominated, whereas in the case of the formation of an IgG–anti-IgG complex, mass deposition appeared to be the controlling process.

The simultaneous analysis of both frequency and dissipation factor was performed during HSA–anti-HSA immunoreaction on the surface of hydrophobic gold electrodes of TSMs.<sup>76</sup> The enhanced frequency response and the increase in dissipation were attributed to the contribution made by the trapped interprotein capillary-like water to bare protein mass or to the non-specific binding in the second phase of the interaction. An entirely different experimental procedure was applied when the covalent coupling of IgG antibody fragments to linkers embedded in a phospholipid monolayer matrix and subsequent binding of the antigen were monitored at the air–water interface by means of a TSM device in the contact with a bioactive layer.<sup>77</sup> The authors observed that a substantially larger surface density of bound biomolecules was determined on the basis of measured frequency response than by radioimmunoassay. Similarly to the study described earlier, the results were explained by hydration and/or slippage in the protein layer.

As can be gleaned from the above analysis of different studies, the proteins that take part in immunoreactions have been immobilized on acoustic wave devices following different immobilization methods. While it is possible simply to adsorb the protein on the gold electrodes of TSMs through hydrophobic and sulfur–gold interactions,<sup>66,70–72,74</sup> in other investigations gold TSM electrodes were modified with polyethylenimine *via* glutaraldehyde,<sup>64,65,73</sup> aminosilane *via* glutaraldehyde,<sup>11,64,65,67,69,75</sup> polyacrylamide *via* glutaraldehyde,<sup>11</sup> hydrophobic thiol SAMs<sup>76</sup> or by protein A<sup>68</sup> in order to attach further the active biolayer by hydrophobic or covalent bonds. In some of these studies, various immobilization procedures were evaluated.<sup>11,64,65</sup> Recently, different immobilization methods, *i.e.*, adsorption on gold, avidin–biotin binding and two types of bindings on thiol SAMs [dithiobis(succinimidyl) propionate] and dextran-modified], were compared regarding BSA–anti-BSA and IgG–anti-IgG immunoreactions.<sup>78</sup> Various specific interactions on TSM surfaces have been monitored in real time, which raises the interesting point that none of the suggested immobilization protocols was recommended as possessing any clear advantage over any other protocol. A similar conclusion might be derived from a study where specific interaction between IgG and anti-IgG was observed on the surfaces of unmodified TSMs and those modified following three different protocols: protein A binding, thiolation of gold electrodes and thiolation of anti-IgG.<sup>79</sup> The estimation of adsorbed amounts of proteins was performed by the 'dip and dry' method, although the monitoring of the immunoreactions in real time was also presented, in order to evaluate orientational aspects and the immunological activity of immobilized proteins. A similar immunochemical interaction was observed in a related study where alternating polyelectrolyte films and anti-IgG multilayer films were used for the detection of IgG.<sup>80</sup>

Many other experimental procedures for immunoassays on TSM surfaces comprise various techniques for modification of gold electrodes. SAMs of a thiol-containing synthetic peptide constituting an epitope of the foot-and-mouth disease virus were covalently attached to gold electrodes and applied to the detection of different concentrations of the specific antibody in

the solution.<sup>32</sup> It was further reported that *Chlamydia trachomatis* from urine samples might be detected on the surface of gold TSM electrodes primarily modified by a cystamine monolayer and linked to the corresponding antibody.<sup>81</sup> In another investigation, by use of the Langmuir–Blodgett technique, fluorescein lipids embedded in a phospholipid matrix were deposited on gold TSM electrodes and the binding of antifluorescein antibody to fluorescein-conjugated lipid was monitored.<sup>82</sup> Also, there have been attempts to modify gold electrodes of TSM devices by anodic polymerization of *m*-phenylenediamine in order to prepare them for the immobilization of bioactive molecules.<sup>83</sup> The selective interaction of a microbe (*S. paratyphi* A) against its antibody, cross-linked within the electropolymerized film, was monitored. Somewhat earlier, the immobilization of antibodies on TSM surfaces modified by ethylenediamine plasma-polymerized films was examined and evaluated.<sup>84</sup> The sensor was calibrated, in liquid, for the specific interaction between HSA and its antibody.

The limited mass sensitivity of commercial TSM devices, which is of primary concern for many investigators, is imposed by manipulating difficulties with extremely thin quartz discs operating at higher resonant frequencies. Consequently, there have been a number of attempts to overcome this problem. A unique approach, described as an amplified-mass immunosorbent assay, was used for the detection of an enzyme (adenosine 5'-phosphosulfate reductase).<sup>85</sup> Gold electrodes of TSMs were modified with the corresponding antibody and after the formation of the immuno-complex, which did not result in a significant frequency decrease in the applied range of concentrations, another antibody–enzyme conjugate reagent was employed to catalytically amplify the mass response. A histochemical staining agent, that was added to the solution, was enzymatically converted into an insoluble precipitate on the TSM surface, inducing a decrease in frequency with a magnitude corresponding to the level of enzyme bound to the antibody-modified TSM. The intensification of the signal by the enzymatically enhanced formation of the deposits, for another immunoreaction on the surface of TSM, was obtained using the amplified mass immunosorbent assay for detection of antibodies against the recombinant African swine fever virus attachment protein.<sup>86</sup> Liposomes have been used as a different kind of signal-intensifying reagents for monitoring an immunoreaction on the surface of a TSM device in a type of competitive assay.<sup>87</sup> The binding of antibody-bearing liposomes to the corresponding antigen (phenyloxazolone–BSA conjugate) immobilized on gold TSM electrodes has been examined in real time. The specific interaction was inhibited by the presence of the soluble hapten in a concentration-dependent manner.

Different immunochemical binding processes have been monitored on the surfaces of other types of acoustic wave device. A surface transverse wave (STW) device operating at 250 MHz, silanized and modified with avidin–biotinylated atrazine complex, was used for the detection of atrazine.<sup>88</sup> Applying a type of a competitive assay, very low concentrations of the herbicide (0.06 ppb) were detected.

The possibility of improving the sensitivity of Love wave devices by optimizing the thickness of the waveguiding layer has made them attractive for immunosensing applications. Low (ppb), levels of anti-IgG were detected using a Love wave sensor operating at 110 MHz, which was silanized and modified with IgG.<sup>89</sup> It was reported that a Love wave device showed an improved performance in liquids when a thin gold film was deposited on top of the polymer waveguide layer to eliminate unwanted acoustoelectric coupling.<sup>90</sup> In a later study, such a system was fully applied as an immunosensor.<sup>91</sup> A biotinylated, supported phospholipid bilayer that bound streptavidin and biotinylated goat IgG was used to detect anti-goat IgG. An excellent suppression of non-specific binding, provided by streptavidin attachment to the phospholipid layer, was achieved. In a recent study, the viscoelastic behaviour of protein films,

composed of IgG antibodies deposited on a shear horizontal surface acoustic wave device, was examined.<sup>92</sup> The attenuation of Love waves in a film composed of up to 20 layers was discussed. The authors concluded that for the one- or two-layer films normally used in acoustic wave immunosensing, a pure mass effect is dominant, whereas for thick films, saturation of the sensor response was observed.

Acoustic plate mode (APM) devices have also been applied to the detection of low levels of IgG in a static experimental cell.<sup>93</sup> The IgG antigen–antibody system was examined in another study involving an APM device.<sup>94</sup> A detailed investigation of the effect of viscoelasticity of the films of different reactants used to modify sensor surfaces prior to the immobilization of antibodies was performed. The authors concluded that the optimization of the device response would require minimizing viscoelastic effects of the coatings.

A careful examination of the studies described above suggests that despite the existence of a significant amount of work devoted to the development of immunosensors based on acoustic wave devices, their full development is still problematic. Having in mind that antigenic sites are often conformation dependent and that hydrophobic, hydrophilic and ionic interactions are all involved in the binding process, it is understandable that the immobilization and adsorption of proteins on the surfaces compromises their activity in affinity based reactions, thus making the design of immunosensors particularly troublesome. Another generally acknowledged source of difficulty is misleading results related to non-specific adsorption of the species on the sensing surface. It appears that a more extensive analysis of these obvious difficulties is often conveniently neglected during the presentation of experimental results.

## 7 Nucleic acids and DNA/RNA–protein/peptide interactions. Drug discovery

Recently, in an issue of *Time* magazine that was entirely devoted to the development of genetic engineering, the 21st century was proclaimed as the century of biotechnology.<sup>95</sup> Indeed, having in mind the accelerated work and intensified efforts with respect to deciphering the human genome, it is realistic to assume that the coming decades will bring us perhaps the most exciting turning points in the history of medicine, through the full implementation of genetic profiling and genetically customized drugs. Much of the general availability of the above methods will depend on the further development of gene probe technology. Currently, radioactive, enzymatic or luminescence labelling are applied to obtain detectable signals during the hybridization of nucleic acids and for the determination of specific DNA sequences.<sup>96,97</sup> Constant efforts have been made to develop simple, label-free methods to monitor not only hybridization processes, but also nucleic acid–protein interactions and the binding of small molecules and drugs. The compatibility of most biosensor technologies with microelectronics makes genosensors promising tools for fast and continuous screening of the above-mentioned processes. In this respect, research into genosensors based on acoustic wave devices presents exciting possibilities for the future.

Most of the relevant work involves the monitoring of the hybridization of single-stranded nucleic acid molecules, immobilized on TSM surfaces by various techniques, to their complementary strands present in solution. In one of the earlier attempts, the hybridization of synthetic RNAs was identified on the surfaces of a polymer (styrene–acrylic acid copolymer)-coated 9 MHz PQC's using the often-mentioned 'dip and dry' method.<sup>98</sup> Ebersole *et al.* used the procedure for amplifying TSM signals<sup>85</sup> to demonstrate the potential of TSMs modified with avidin and streptavidin for performing hybridization

assays with biotinylated oligonucleotides.<sup>99</sup> The application of TSM surface modification by the formation of avidin–biotinylated DNA complexes was exploited in the number of hybridization studies.<sup>100–103</sup> Multilayer biotinylated DNA films formed on 9 MHz TSM surfaces by the successive deposition of avidin and poly(styrene sulfonate) showed increased hybridization capacity compared with single-layer films.<sup>100</sup> Another approach for building multilayer structures including DNA molecules on the surfaces of TSMs consisted of units built of double-stranded biotinylated DNA sandwiched between streptavidin layers.<sup>101</sup> A biotinylated lipid matrix was primarily deposited on TSM electrodes. Neutravidin-modified TSM surfaces were also used to study the hybridization of complementary, non-complementary oligonucleotides and targets with a mutated base with a biotinylated 25-mer oligonucleotide probe.<sup>102</sup> It was shown that distinctively different signals were obtained for the complementary and non-complementary cases and for different types and locations of induced mismatches. In a further study with 27 MHz TSM, involving an avidin–biotin nucleotide probe immobilization method, the comparison of the results obtained with those acquired by SPR (commercial system, BIAcore) was performed.<sup>103</sup> The monitored hybridization process included alterations in the reacting oligonucleotide molecules, such as integration of different mismatched bases and changes in DNA chain length.

Other methods to immobilize DNA probes on the gold electrodes of TSMs, have included thiol-derivatized nucleic acid molecules at the 5'-phosphate end.<sup>104–106</sup> It was also shown, using the genoprobe obtained through the immobilization of a thiol-derivatized peptide nucleic acid on gold TSM electrodes, that it was possible to differentiate single-base mismatches during the hybridization event.<sup>107</sup> In some studies, silver TSM electrodes were applied for the immobilization of TSMs through modification with thioglycolic acid<sup>108</sup> or with a didodecyldithionooxamide–BSA complex.<sup>109</sup>

Other approaches to immobilize nucleic acids on TSMs for monitoring hybridization processes have been exploited. A DNA probe, where a nucleic acid was covalently immobilized on gold TSM electrodes hydroxylated with (3-glycidoxypropyl)trimethoxysilane, was reported earlier.<sup>110</sup> In a unique attempt at adsorption and electropolymeric (polyphenol) entrapment for immobilizing a four-layer DNA dendrimer on gold TSM electrodes, trioxsalen (4,5',8-trimethylpsoralen) was used to cross-link covalently dendrimer layers.<sup>111</sup> The branched DNA structure consisting of multiple single-stranded arms showed significantly improved hybridization capacity compared with that for conventional DNA probes.

In several earlier studies, the hybridization of nucleic acids adsorbed on PdO sputtered on gold TSM electrodes was investigated.<sup>112–117</sup> Applying radiochemical labelling with <sup>32</sup>P, the authors showed that the added mass resulting from the formation of a DNA hybridization complex corresponded to a considerably larger change of frequency than that predicted by Sauerbrey's equation.<sup>113</sup> The observation of an enhanced frequency response was interpreted in terms of the perturbation of acoustic energy transmission by changes in interfacial properties upon hybridization. By means of acoustic network analysis, DNA hybridization kinetics at the TSM–solution interface were examined in detail.<sup>114</sup> The kinetics of hybridization of RNA homopolymer was also discussed and a hybridization mechanism was proposed after the examination of time profiles for the parameters measured by the network analyser such as series and parallel resonant frequency, motional resistance or maximum phase angle.<sup>117</sup>

The monitoring of the binding of proteins to nucleic acids immobilized on TSM surfaces has been the subject of a number of studies. The kinetics for sequence-specific binding of NFI (nuclear factor I) to DNA, including its recognition site, were monitored and compared with non-specific binding of histone f3.<sup>118</sup> For another specific protein–DNA interaction, the authors

compared the kinetic parameters calculated on the basis of data obtained by TSM measurements with those found by gel mobility shift assays and demonstrated a good agreement between the two techniques.<sup>119</sup> A recent study involved the detection of the interactions between two Tat-derived peptides and HIV-1 *trans*-activation (TAR) RNA immobilized on TSM surfaces.<sup>120</sup> The frequency responses related to two different binding processes showed opposite directions, reflecting a structural distinction between the two Tat–TAR complexes at the TSM interface.

Small molecule binding to nucleic acids is very important for research efforts related to drug discovery. However, only a few studies with acoustic wave sensors have dealt with the monitoring of such processes. These mainly involve the kinetics of the binding of chemotherapeutic agents to DNA. In a study of the DNA–platinum-based drug interaction mechanism, both series frequency and motional resistance signals obtained from acoustic network analysis were monitored.<sup>121</sup> A kinetic analysis showed the detection of DNA complexation with hydrolysis products of two platinum isomers (*cis* and *trans*). In another study, the alkylation of DNA by different antitumour agents was monitored by TSM, through the change in viscosity of DNA solution induced by the alkylation process.<sup>122</sup> The described method, unique because of the omission of a DNA immobilization step, could not, however, provide a more detailed kinetic analysis. Recently, a ruthenium complex binding to DNA immobilized on gold TSM electrodes through a cationic (methylated thiopyridine) monolayer was studied using cyclic voltammetry and an acoustic wave sensor.<sup>123</sup> The binding properties of an antibiotic (doxorubicin) to DNA immobilized on TSMs were also studied.<sup>124</sup> This investigation showed that, despite the differences in applied DNA immobilization procedures and sample injection methods, the affinity constants for ligand–DNA interactions determined by the acoustic wave sensor and by a commercial SPR device (BIAcore) were comparable.

Regarding other types of acoustic wave sensor, work connected with the development of a gene probe has been performed using acoustic plate mode (APM) devices. In an earlier study, where a probe nucleic acid sequence was covalently attached to the sensing surface through an aminosilane film, the hybridization reaction was monitored.<sup>125</sup> Depending on the concentration of the target nucleic acid, both elastic stiffening of the interface, due to the possibility of target DNA cross-linking the surface, and mass loading were observed. Later, the same authors demonstrated the sensing properties of an improved APM device design for the selective detection of chemically denatured DNA by means of polymerase chain reaction amplification.<sup>126</sup>

Finally, considering the fact that acoustic sensor technology might be conveniently incorporated into the design of high-density arrays of genoprobes, widely known as gene chips, it can be expected that work in this field will intensify.

## 8 Cell adhesion and cell function

The monitoring of cell adhesion, spreading and proliferation on solid surfaces is crucial for a better understanding and evaluation of the capability of biomaterials for assimilation within natural tissue and the activation of tissue repair mechanisms. However, the investigation of these phenomena by means of acoustic wave devices has been the subject of only a few studies, in spite of the obvious feasibility and the attractiveness that the method provides for investigating similar processes.

In some earlier studies, the attachment of platelets,<sup>127</sup> osteoblasts (bone-forming cells)<sup>128</sup> and epithelial (monkey kidney) cells<sup>129</sup> to gold electrodes of piezoelectric crystals was

monitored. In each study the amount of the adhered cells derived from a mass-based response could not be correlated with experimentally determined values, although different explanations were offered. Whereas in the former study<sup>127</sup> it was supposed that the TSM sensor did not detect the total mass of whole bodies of adherent platelets, but only the weight of their focal contact region, the latter studies<sup>128,129</sup> assumed that viscoelastic damping throughout the viscous layer of adhered cells was responsible for the decrease of the theoretically expected response. However, in the study with osteoblasts,<sup>128</sup> it was shown that a linear correlation between frequency change and the surface coverage could be established and possibly applied to monitor cell growth. In the same study, a previous model<sup>22</sup> for a purely viscous film was used to determine the apparent viscosity of osteoblasts. Similarly, the viscosities of epithelial (canine kidney) cell monolayers cultured on gold electrodes of TSM were determined by impedance analysis.<sup>130</sup> The authors used an equivalent circuit model<sup>23</sup> to quantify the viscous damping. The low values obtained for apparent viscosities were attributed to the existence of additional space between the cells immobilized through their focal contact regions and the TSM surface, where the shear wave might be considerably damped before contacting the cellular monolayers. Data obtained from simultaneously measured changes in frequency and energy dissipation during the adhesion of small colonies of epithelial (monkey kidney and hamster ovarian) cells to TSM surfaces modified with hydrophobic and hydrophilic polystyrene surfaces were used to evaluate the potential application of TSM for studying cell-surface interactions.<sup>131</sup> The authors concluded that the TSM response provides a 'fingerprint' of the cell adhesion process reflecting different cell adsorption kinetics for various cell types and various surfaces.

Experimental results suggesting that protein adsorption is the initial event during blood interaction with surfaces goes back to earlier studies.<sup>132</sup> Further, it was shown that proteins such as fibrinogen, fibronectin or von Willebrand factor strongly promote platelet adhesion and spreading, whereas the same process is inhibited by pre-adsorbed albumin.<sup>133</sup> Unfortunately, there are few TSM studies dealing with the problem of cell adhesion on a pre-adsorbed protein layer or any biospecific layer. The strong adhesion of bovine epithelial cells on TSM surfaces pre-coated with fibronectin was observed and confirmed using other techniques such as electron microscopy, interference reflection microscopy and fluorescence microscopy.<sup>134</sup> In another study it was shown that TSM devices with gold electrodes modified with collagen respond substantially more to the adhesion of platelets than corresponding surfaces modified with albumin.<sup>135</sup> The smaller frequency response compared with a theoretically expected mass based response derived from the amount of platelets counted by radiolabelling was not a surprising finding considering the high water content of platelets. The sensor response was attributed to viscoelastic properties and morphology of the adhered platelet layer rather than to their mass. Finally, it was found that various tumour cells adhered and spread on galactose-bearing lipid film immobilized on the surface of TSM in serum-containing medium, but not in serum-free medium.<sup>136</sup> The results suggested that serum components pre-adsorb on synthetic glycolipid films and further influence cell spreading.

The necessity for the evaluation of the haemocompatibility of biomaterials and a better understanding of the processes controlling thrombus formation on solid surfaces in contact with blood suggests the importance of the described TSM studies and further investigation of relevant phenomena. It would be interesting to monitor the processes related to 'multilayer protein passivation' which has frequently been suggested for attaining long-term antithrombogenicity of implanted biomedical devices.<sup>137-139</sup> Further studies involving monitoring of interactions of blood clotting factors with cell membranes

would be extremely helpful for extending our knowledge of processes involved in the blood coagulation system and, consequently, the short-term blood compatibility of biomaterials.

## 9 Other applications

In several studies, the dependence of the TSM frequency response on the interfacial viscosity was used to monitor various biochemical processes. The changing viscosity of the pre-adsorbed fibrin clot, as it dissolved after the addition of plasminogen activator, was monitored by a TSM device.<sup>140</sup> The clot dissolution time was correlated with the amounts of two types of plasminogen activators in the examined samples. Whereas in the case of streptokinase the observed standard error was comparable to that exhibited in rapid fibrin plate assay, in the case of tissue plasminogen activator a much higher standard error was calculated. The discrepancy was attributed to diffusion effects and to acoustic compression wave resonances. In another study, the rheological behaviour of a blood drop deposited on the surface of silver TSM electrodes was investigated.<sup>141</sup> Equivalent circuit parameters of the TSM were monitored with an impedance analyser. The authors examined processes such as blood clotting and urokinase activated fibrinolysis and concluded that the frequency response was dominated by a viscosity change only at the initial stage, followed subsequently by a mass effect and surface stress.

A TSM sensor combined with a spectrum analyser was used to monitor the viscosity decrease during the depolymerization of a muscle protein, actomyosin, induced by the addition of adenosine 5'-triphosphate in its solution.<sup>142</sup> On the basis of the experimental results, the authors suggested the further development of a sensor for monitoring the freshness of meat. In another study, the same authors applied the TSM device to monitor the viscosity change of the solution due to an immunoreaction in association with the specific agglutination of streptolysin-bearing latex particles induced by the introduction of lysin.<sup>143</sup>

In an early investigation, the resonance frequency and resistance of a palladium-plated TSM sensor were monitored with an impedance analyser during the gelation of limulus amoebocyte lysate induced by *E. coli* produced endotoxin.<sup>144</sup> The monitoring of increased viscosity by a TSM sensor has been claimed to be superior in performance to a conventional optical method used for the determination of endotoxin. The growth of urease-producing bacteria, through detection of the dissolved ammonia in the culture medium using TSM devices, has also been reported.<sup>145</sup> Ammonia, produced by the hydrolysis of urea, diffused across a gas-permeable membrane into an internal electrolyte solution, where conductivity alteration caused a change in the resonant frequency of the TSM sensor which was actually not in contact with the solution. The growth characteristics of *Proteus vulgaris* were investigated and the method apparently exhibited satisfactory accuracy and precision.

Attempts have been made to develop a bioaffinity sensor for monosaccharides (glucose and ribose) based on a 6 MHz thiol-modified TSM devices coated with a synthetic receptor resorcinol-dodecanal cyclotetramer.<sup>146</sup> The authors reported that no meaningful signal was obtained owing to the binding of low molecular mass substrates to the sensitive layer composed of a low surface concentration of receptor molecules. However, the binding of a disaccharide (sucrose) to a fully hydrated (thermally treated) Langmuir phospholipid film transferred onto the electrodes of 5 MHz TSM was possible, although not *in situ*.<sup>147</sup> On the basis of frequency measurement of the dried

substrate, the authors concluded that the amount of bound sucrose increases linearly with its concentration in the solution. The results were explained by the formation of a mingled layer with inseparably incorporated sucrose molecules within a hydrated phospholipid film.

The adsorption behaviour of phospholipid containing liposomes on synthetic phospholipid polymers and poly[hydroxyethyl methacrylate] (HEMA) was monitored and compared, using 5 MHz TSM devices with gold electrodes spin-coated with the above mentioned polymers.<sup>148</sup> On the basis of larger frequency responses, the authors concluded that liposomes can penetrate a hydrated poly(HEMA), and, thus, probably change their structure. The unchanged structure of liposomes adsorbed on phospholipids was, however, confirmed by AFM. It was emphasized that such findings might enhance the application of otherwise unstable liposomes, immobilized on phospholipid polymers, as model cell membranes for studying different interactions and processes.

In another study, gold plated TSM devices with C- and N-terminated L-alanine immobilized on the surface were used to monitor artificial boundary lipid-containing liposome-induced release of membrane proteins and the subsequent reconstitution.<sup>149</sup> The authors suggested that different shapes of time-frequency responses obtained during the binding of the proteoliposome to two differently terminated L-alanine forms might make TSM devices a useful instrument for distinguishing conformational changes in amino acids.

TSM devices were coated with cholestyramine resin and exposed to solutions of various bile acid salts (cholate, glycocholate, taurocholate) in a batch-operating one-side liquid cell.<sup>150</sup> The authors reported apparently good sensitivity of the sensor obtained in such a way and suggested a multi-step regeneration protocol permitting the resin-based sensor to be rinsed numerous times over extended period. Such a sensor has been suggested as an important tool in the study of bile salts binding non-absorbable resins as a therapy for the elevated serum cholesterol.

An interesting study of the inhibition of catalytic activity of a haem peptide, microperoxidase from cytochrome *c* by binding of strong ligands, was performed on the gold electrodes of 5 MHz TSM devices modified with a mercaptosilane and fabricated as a dual-response biosensor, giving simultaneous electrochemical and piezoelectric responses.<sup>151</sup> The inhibition of the catalytic activity upon binding of ligands (imidazole, histamine and histidine) was observed through electrochemical response as a decrease in the cathodic current response to H<sub>2</sub>O<sub>2</sub>. The mass increase due to the binding of the same ligands was detected through the decrease in frequency response. Such a configuration apparently permitted both qualitative and quantitative analysis of interfering ligands present in the solution. It is interesting that the authors claimed, although supporting data were not shown, that by the mass-related decrease in frequency response it was possible to distinguish the binding of histamine ( $M_r = 111.15$ ) from the binding of histidine ( $M_r = 155.16$ ) to the peptide.

Here we shall mention some attempts to develop enzyme based biosensors using acoustic wave devices. It was reported that glucose could be detected continuously by using the enzyme-catalysed formation of precipitates of an oxidised dye on the TSM surface.<sup>152</sup> Similarly to previous studies,<sup>85,86</sup> an enzyme-related reaction resulting in insoluble species was used to intensify the impedance response. In another study, the rate and extent of the inhibitory effect of organophosphorus and carbamate pesticides on the activity of acetylcholinesterase immobilised on the surface of TSMs were followed in real time by measuring the frequency changes associated with catalytically induced deposition of a pigment.<sup>153</sup> However, the application of these methods may be problematic because of the simultaneous non-specific adsorption of other components present in the system.

## 10 Concluding remarks and future perspectives

The literature associated with the operation of the TSM in liquid media offers a classic example of the nature of scientific dogma. When it comes to the interpretation of frequency responses for a wide variety of chemical systems instigated at the TSM device-liquid interface, the wavelength extension model has almost invariably been invoked. (Often, because the oscillator experimental configuration is employed, the frequency response is not the true series resonance frequency.) Although facile explanations based on a mass response model are convenient, particularly for situations where it is important to correlate other processes with deposited (or lost) amounts, it is unreasonable to ignore the fact that in liquids acoustic energy is propagated into the surrounding medium to decay lengths of the order of micrometres. Moreover, chemistry conducted at the TSM surface is occurring at precisely the location where acoustic coupling phenomena occur. Accordingly, the perturbation of the transmission of acoustic energy by biochemical pairs combined at the device-liquid interface provides an exciting biosensor mechanism. The reason for this lies not only in the fact that interfacial biochemical pairs often involve changes in physical properties connected to tertiary structural and counterion alterations, but also to the possibility to obtain multi-dimensional information from the TSM. For example, the latter yields mass, charge and energy dissipation responses through consideration of inductance, parallel resonance frequency and motional resistance changes, respectively. Electrochemical and optical-based biosensors do not provide this range of versatility. Finally, it is worth noting that the sensitivity of acoustic wave sensors has generally been considered to be inferior to that of the above-mentioned devices. In reality, because of the sensing mechanisms outlined previously, the various acoustic wave structures offer unique sensitivity to interfacial biochemical phenomena. A brief summary of some of the features provided by acoustic wave sensor technology follows:

1. Basic and equivalent circuit parameters offer the potential to study the tertiary structure, charge, viscoelastic properties, *etc.*, of biochemical macromolecules and cells attached to a device surface immersed in water.
2. Acoustic wave sensors yield responses to various properties of a wide variety of biological moieties such as proteins, nucleic acids and cells.
3. Signalling is direct in that no labels such as radiochemical or fluorescent tagging agents are required.
4. Most acoustic wave devices are functionally compatible with FIA technology.
5. The behaviour of devices in liquids is compatible with a number of different protocols available for attaching biochemical moieties to the sensor surface.
6. It is clearly possible to work with immobilized biochemical monolayers rather than the situation for other sensors where adjacent 'amplifying' layers such as dextran are required.
7. The detection of the binding of relatively small molecules to various receptor sites imposed on the device surface appears to be feasible, especially if such a binding event results in a change of receptor tertiary structure.
8. It is apparent that new acoustic wave structures such as the tuneable MARS device may offer not only enhanced sensitivity, but also new dimensions for the study of biochemical macromolecules in micro-assay format.

We now turn to a brief look at what is on the horizon in the development of acoustic wave biosensor technology. One research activity that is common to all biosensors is the immobilization of various biological species on the device surface. With respect to acoustic wave devices this may, for example, involve gold surfaces such as those in place on TSM structures, or silicon in other devices. A key problem for all

sensors has been the stability of biochemical macromolecules attached to device surfaces, from the perspective of storage, heat and light effects. If a device is to be employed over relatively long periods of time, without calibration, denaturation of proteins, for example, cannot be tolerated. In this regard, we are beginning to see exciting new methods for the enhancement of macromolecular stability through the use of sol-gel and polymer-based systems.

In terms of acoustic wave devices, we are likely to see considerably more research being directed at an understanding of the interactions between acoustic energy and the properties of the liquid-solid interface. This is likely to be reflected in attempts to dissect out from sensor responses, mass, charge, viscoelastic and acoustic coupling processes, particularly with respect to frequency and equivalent circuit parameters. An exciting possible result of such understanding will be the generation of new ways of studying biochemical phenomena such as tertiary structural changes and counter-ion release. In this respect, it is interesting that the link between acoustic wave responses and biochemical structure may require a new detailed understanding of the theory of acoustic wave propagation into liquids. This is so because explicit correlation with other techniques may be unavailable because of the dearth of optical and other methods for the detection of subtle biochemical structural alterations.

The comment given above leads one to conclude naturally that we are likely to see future significant advances in the development of true hybrid sensors, including those involving acoustic wave devices. This type of structure will incorporate a substrate together with its immobilized biochemical apparatus being interrogated by completely different physical techniques. Possibilities include opto-acoustic devices and the use of materials that are both pyroelectric and piezoelectric. Such combinations in tandem with chemometric procedures may lead to enhanced analyses in terms of the information generated by sensor response.

Finally, as we mentioned above, we will see the introduction of new acoustic wave sensors in the near future. Such structures will involve better sensitivity, facile attachment of biochemical macromolecules and cells, use of non-piezoelectric materials, the generation of several levels of chemical information from physical parameters and commercial presentation of equipment incorporating a sensor into sophisticated FIA set-ups.

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## 12 References

- D. Reaney, *After Death*, Morrow, New York, 1991.
- W. G. Cady, *Piezoelectricity. An Introduction to the Theory and Applications of Electromechanical Phenomena in Crystals*, Dover, New York, 1964.
- M. Curie, *Radioactive Substances*, (translation of the Thesis presented to the Faculty of Science, Paris), Philosophical Library, New York, 1961.
- G. Sauerbrey, *Z. Phys.*, 1959, **155**, 206.
- W. H. King, *Anal. Chem.*, 1964, **36**, 1735.
- T. Nomura and A. Minemura, *Nippon Kagaku Kaishi*, 1980, 1621.
- P. L. Konash and G. J. Bastiaans, *Anal. Chem.*, 1980, **52**, 1929.
- J. E. Roederer and G. J. Bastiaans, *Anal. Chem.*, 1983, **55**, 2333.
- R. M. White and F. W. Voltmer, *Appl. Phys. Lett.*, 1965, **7**, 314.
- G. S. Calabrese, H. Wohltjen and M. K. Roy, *Anal. Chem.*, 1987, **59**, 833.
- M. Thompson, G. K. Dhaliwal, C. L. Arthur and G. S. Calabrese, *IEEE Trans. Ultrason. Ferroelec. Freq. Contr.*, 1987, **UF34**, 127.
- M. Thompson and D. C. Stone, *Surface-Launched Acoustic Wave Sensors*, John Wiley and Sons, New York, 1997, pp. 34–52.
- J. Rickert, G. L. Hayward, B. A. Cavic, M. Thompson and W. Göpel, in *Sensors Update: Sensor Technology—Applications—Markets*, ed. H. Baltes, W. Göpel and J. Hesse, Wiley-VCH, Weinheim, 1999, vol. 5, pp. 105–139.
- J. C. Andle and J. F. Vetelino, *Sens. Actuators A*, 1994, **44**, 167.
- J. W. Grate, S. J. Martin and R. M. White, *Anal. Chem.*, 1993, **65**, 940A.
- P. C. H. Li, D. C. Stone and M. Thompson, *Anal. Chem.*, 1993, **65**, 2177.
- P. C. H. Li and M. Thompson, *Anal. Chem.*, 1996, **68**, 2590.
- P. C. H. Li and M. Thompson, *Anal. Chim. Acta*, 1996, **336**, 13.
- A. C. Stevenson and C. R. Lowe, *Sens. Actuators A*, 1999, **72**, 32.
- V. I. Bottom, *Introduction to Quartz Crystal Unit Design*, Van Nostrand Reinhold, New York, 1982.
- J. G. Miller and D. I. Bolef, *J. Appl. Phys.*, 1968, **39**, 4589.
- K. K. Kanazawa and J. G. Gordon, *Anal. Chim. Acta*, 1985, **175**, 99.
- S. J. Martin, V. E. Granstaff and G. C. Frye, *Anal. Chem.*, 1991, **63**, 2272.
- R. L. Simpson, PhD Thesis, University of Washington, 1985.
- G. L. Hayward and G. Z. Chu, *Anal. Chim. Acta*, 1994, **288**, 179.
- G. L. Hayward and M. Thompson, *J. Appl. Phys.*, 1998, **83**, 2194.
- C. E. Reed, K. K. Kanazawa and J. H. Kaufman, *J. Appl. Phys.*, 1990, **68**, 1993.
- F. Ferrante, A. L. Kipling and M. Thompson, *J. Appl. Phys.*, 1994, **76**, 3448.
- H. L. Bandey, S. J. Martin, R. W. Cernosek and A. R. Hillman, *Anal. Chem.*, 1999, **71**, 2205.
- S. Bruckenstein and M. Shay, *Electrochim. Acta*, 1985, **30**, 1295.
- H. Muramatsu, E. Tamiya and I. Karube, *Anal. Chem.*, 1988, **60**, 2142.
- J. Rickert, T. Weiss, W. Kraas, G. Jung and W. Göpel, *Biosens. Bioelectron.*, 1996, **11**, 591.
- M. Rodahl, J. Höök and B. Kasemo, *Anal. Chem.*, 1996, **68**, 2219.
- A. L. Kipling and M. Thompson, *Anal. Chem.*, 1990, **62**, 1514.
- M. Yang and M. Thompson, *Anal. Chem.*, 1993, **65**, 1158.
- M. Yang and M. Thompson, *Anal. Chim. Acta*, 1992, **269**, 167.
- J. J. Ramsden, *Q. Rev. Biophys.*, 1994, **27**, 41.
- M. Laatikainen and M. Lindstrom, *J. Colloid Interface Sci.*, 1988, **125**, 610.
- Y. Ebara and Y. Okahata, *Langmuir*, 1993, **9**, 574.
- M. Yang, F. L. Chung and M. Thompson, *Anal. Chem.*, 1993, **65**, 3713.
- F. Caruso, D. N. Furlong and P. Kingshott, *J. Colloid Interface Sci.*, 1997, **186**, 129.
- M. Rodahl, F. Hook, C. Fredriksson, C. Keller, A. Krozer, P. Brzezinski, M. Voinova and B. Kasemo, *Faraday Discuss. Chem. Soc.*, 1998, **107**, 229.
- F. Hook, M. Rodahl, B. Kasemo and P. Brzezinski, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 12271.
- R. R. Seigel, P. Harder, R. Dahint, M. Grunze, F. Josse, M. Mrksich and G. M. Whitesides, *Anal. Chem.*, 1997, **69**, 3321.
- B. A. Cavic, F. L. Chu, M. Furtado, S. Ghafouri, G. L. Hayward, D. P. Mack, M. E. McGovern, H. Su and M. Thompson, *Faraday Discuss. Chem. Soc.*, 1997, **107**, 159.
- B. A. Cavic and M. Thompson, *Analyst*, 1998, **123**, 2191.
- F. Lacour, R. Torresi, C. Gabrielli and A. Caprani, *J. Electrochem. Soc.*, 1992, **139**, 1619.
- S. Nakata, N. Kido, M. Hayashi, M. Hara, H. Sasabe, T. Sugawara and T. Matsuda, *Biophys. Chem.*, 1996, **62**, 63.
- B. Guo, J. Anzai and T. Osa, *Chem. Pharm. Bull.*, 1996, **44**, 800.
- B. S. Murray and L. Cros, *Colloids Surf. B: Biointerfaces*, 1998, **10**, 227.
- P. W. Walton, M. R. O'Flaherty, M. E. Butler and P. Compton, *Biosens. Bioelectron.*, 1993, **8**, 401.
- Y. Lvov, K. Ariga, I. Ichinose and T. Kunitake, *J. Am. Chem. Soc.*, 1995, **117**, 6117.
- M. Masson, K. Yun, T. Haruyama, E. Kobatake and M. Aizawa, *Anal. Chem.*, 1995, **67**, 2212.
- J. Rickert, A. Brecht and W. Göpel, *Anal. Chem.*, 1997, **69**, 1441.
- S. Ghafouri and M. Thompson, *Langmuir*, 1999, **15**, 564.
- A. Janshoff, C. Steinem, M. Sieber and H.-J. Galla, *Eur. Biophys. J.*, 1996, **25**, 105.
- A. Janshoff, C. Steinem, M. Sieber, A. Bayà, M. A. Schmidt and H.-J. Galla, *Eur. Biophys. J.*, 1997, **26**, 261.
- M. Hashizume, T. Sato and Y. Okahata, *Chem. Lett.*, 1998, 399.

- 59 I. Willner, A. Doron and E. Katz, *J. Phys. Org. Chem.*, 1998, **11**, 546.
- 60 L. J. Kricka, in *Chemical Sensors*, ed. T. E. Edmonds, Blackie, London, 1988, pp. 3–14.
- 61 L. Bui, R. De Bono, V. Ghaemmaghami, K. M. R. Kallury, P. Li, N. McKeown, D. Stone, H. Su, L. Tessier, S. Vigmond and M. Thompson, in *Advances in Biosensors*, ed. A. P. F. Turner, JAI Press, Greenwich, CT, 1992, vol. 2, pp. 181–213.
- 62 D. Leech, *Chem. Soc. Rev.*, 1994, **23**, 205.
- 63 A. Shons, F. Dorman and J. Najarian, *J. Biomed. Mater. Res.*, 1972, **6**, 565.
- 64 B. König and M. Grätzel, *Anal. Chem.*, 1994, **66**, 341.
- 65 G. G. Guilbault, B. Hock and R. Schmid, *Biosens. Bioelectron.*, 1992, **7**, 411.
- 66 R. M. Carter, J. J. Mekalanos, M. B. Jacobs, G. J. Lubrano and G. G. Guilbault, *J. Immunol. Methods*, 1995, **187**, 121.
- 67 C. Steegborn and P. Skládal, *Biosens. Bioelectron.*, 1997, **12**, 19.
- 68 K. A. Davis and T. R. Leary, *Anal. Chem.*, 1989, **61**, 1227.
- 69 H. Muramatsu, J. M. Dicks, E. Tamiya and I. Karube, *Anal. Chem.*, 1987, **59**, 2760.
- 70 M. Muratsugu, J. Ohta, Y. Miya, T. Hosokawa, S. Kurosawa, N. Kamo and H. Ikeda, *Anal. Chem.*, 1993, **65**, 2933.
- 71 G. Sakai, T. Saiki, T. Uda, N. Miura and N. Yamazoe, *Sens. Actuators B*, 1997, **42**, 89.
- 72 C. Kösslinger, S. Drost, F. Aberl and H. Wolf, *Fresenius' J. Anal. Chem.*, 1994, **349**, 349.
- 73 C. Zhang, G. Feng and Z. Gao, *Biosens. Bioelectron.*, 1997, **12**, 1219.
- 74 K. Bizet, C. Gabrielli, H. Perrot and J. Therasse, *Biosens. Bioelectron.*, 1998, **13**, 259.
- 75 L. Tessier, N. Schmitt, H. Watier, V. Brumas and F. Patat, *Anal. Chim. Acta*, 1997, **347**, 207.
- 76 F. Höök, M. Rodhal, P. Brzezinski and B. Kasemo, *Langmuir*, 1998, **14**, 729.
- 77 I. Vikholm and W. M. Albers, *Langmuir*, 1998, **14**, 3865.
- 78 S. Storri, T. Santoni, M. Minunni and M. Mascini, *Biosens. Bioelectron.*, 1998, **13**, 347.
- 79 F. Caruso, E. Rodda and D. N. Furlong, *J. Colloid Interface Sci.*, 1996, **178**, 104.
- 80 F. Caruso, K. Niikura, D. N. Furlong and Y. Okahata, *Langmuir*, 1997, **13**, 3427.
- 81 I. Ben-Dov, I. Willner and E. Zisman, *Anal. Chem.*, 1997, **69**, 3506.
- 82 H. Ebato, C. A. Gentry, J. N. Herron, W. Müller, Y. Okahata, H. Ringsdorf and P. A. Suci, *Anal. Chem.*, 1994, **66**, 1683.
- 83 S. Si, F. Ren, W. Cheng and S. Yao, *Fresenius' J. Anal. Chem.*, 1997, **357**, 1101.
- 84 K. Nakanishi, H. Muguruma and I. Karube, *Anal. Chem.*, 1996, **68**, 1695.
- 85 R. C. Ebersole and M. D. Ward, *J. Am. Chem. Soc.*, 1988, **110**, 8623.
- 86 J. M. Abad, F. Pariente, L. Hernandez and E. Lorenzo, *Anal. Chim. Acta*, 1998, **368**, 183.
- 87 K. Yun, E. Kobatake, T. Haruyama, M.-L. Laukkanen, K. Keinänen and M. Aizawa, *Anal. Chem.*, 1998, **70**, 260.
- 88 M. Tom-Moy, R. L. Bae, D. Spira-Solomon and T. P. Doherty, *Anal. Chem.*, 1995, **67**, 1510.
- 89 G. L. Harding, J. Du, P. R. Dencher, D. Barnett and E. Howe, *Sens. Actuators A*, 1997, **61**, 279.
- 90 E. Gizeli, *Smart Mater. Struct.*, 1997, **6**, 700.
- 91 E. Gizeli, M. Liley, C. R. Lowe and H. Vogel, *Anal. Chem.*, 1997, **69**, 4808.
- 92 M. Weiss, W. Welsch, M. Schickfus and S. Hunklinger, *Anal. Chem.*, 1998, **70**, 2881.
- 93 J. C. Andle, J. T. Weaver, D. J. McAllister, F. Josse and J. F. Vetelino, *Sens. Actuators B*, 1993, **13**, 437.
- 94 J. Renken, R. Dahint, M. Grunze and F. Josse, *Anal. Chem.*, 1996, **68**, 176.
- 95 *Time*, 1999, **153** (1), 26.
- 96 R. L. P. Adams, J. T. Knowler and D. P. Leader, *The Biochemistry of the Nucleic Acids*, Chapman and Hal, London, 11th edn., 1992, pp. 593–656.
- 97 M. Yang, M. E. McGovern and M. Thompson, *Anal. Chim. Acta*, 1997, **346**, 259.
- 98 N. C. Fawcett, J. A. Evans, L. Chien and N. Flowers, *Anal. Lett.*, 1988, **21**, 1099.
- 99 R. C. Ebersole, J. A. Miller, J. R. Moran and M. D. Ward, *J. Am. Chem. Soc.*, 1990, **112**, 3239.
- 100 F. Caruso, E. Rodda, D. N. Furlong, K. Niikura and Y. Okahata, *Anal. Chem.*, 1997, **69**, 2043.
- 101 K. Ijiro, H. Ringsdorf, E. Birch-Hirschfeld, S. Hoffman, Y. Schilken and M. Strube, *Langmuir*, 1998, **14**, 2796.
- 102 L. M. Furtado and M. Thompson, *Analyst*, 1998, **123**, 1937.
- 103 Y. Okahata, M. Kawase, K. Niikura, F. Ohtake, H. Furusawa and Y. Ebara, *Anal. Chem.*, 1998, **70**, 1288.
- 104 Y. Okahata, Y. Matsunabu, K. Ijiro, M. Mukae, A. Murakami and K. Makino, *J. Am. Chem. Soc.*, 1992, **114**, 8299.
- 105 K. Ito, K. Hashimoto and Y. Ishimori, *Anal. Chim. Acta*, 1996, **327**, 29.
- 106 F. Caruso, E. Rodda, D. N. Furlong and V. Haring, *Sens. Actuators B*, 1997, **41**, 189.
- 107 J. Wang, P. E. Nielsen, M. Jiang, X. Cai, J. R. Fernandes, D. H. Grant, N. Ozsoz, A. Beglieter and M. Mowat, *Anal. Chem.*, 1997, **69**, 5200.
- 108 H. Zhang, H. Tan, R. Wang, W. Wei and S. Yao, *Anal. Chim. Acta*, 1998, **374**, 31.
- 109 H. Zhang, R. Wang, H. Tan, L. Nie and S. Yao, *Talanta*, 1998, **46**, 171.
- 110 S. Yamaguchi, T. Shimomura, T. Tatsuma and N. Oyama, *Anal. Chem.*, 1993, **65**, 1925.
- 111 J. Wang, M. Jiang, T. W. Nielsen and R. C. Getts, *J. Am. Chem. Soc.*, 1998, **120**, 8281.
- 112 H. Su, M. Yang, K. M. R. Kallury and M. Thompson, *Analyst*, 1993, **118**, 309.
- 113 H. Su, K. M. R. Kallury, M. Thompson and A. Roach, *Anal. Chem.*, 1994, **66**, 769.
- 114 H. Su and M. Thompson, *Biosens. Bioelectron.*, 1995, **10**, 329.
- 115 H. Su, S. Chong and M. Thompson, *Langmuir*, 1996, **12**, 2247.
- 116 H. Su and M. Thompson, *Can. J. Chem.*, 1996, **74**, 344.
- 117 H. Su, S. Chong and M. Thompson, *Biosens. Bioelectron.*, 1997, **12**, 161.
- 118 K. Niikura, K. Nagata and Y. Okahata, *Chem. Lett.*, 1996, 863.
- 119 Y. Okahata, K. Niikura, Y. Sugiura, M. Sawada and T. Morii, *Biochemistry*, 1998, **37**, 5666.
- 120 L. M. Furtado, H. Su, M. Thompson, D. P. Mack and G. L. Hayward, *Anal. Chem.*, 1999, **71**, 1167.
- 121 H. Su, P. Williams and M. Thompson, *Anal. Chem.*, 1995, **67**, 1010.
- 122 Z.-H. Lin, G.-L. Shen, Y. Lin and R.-Q. Yu, *Fresenius' J. Anal. Chem.*, 1997, **357**, 921.
- 123 M. Aslanoglu, A. Houlton and B. R. Horrocks, *Analyst*, 1998, **123**, 753.
- 124 M. Yang, H. C. M. Yau and H. L. Chan, *Langmuir*, 1998, **14**, 6121.
- 125 J. C. Andle, J. F. Vetelino, M. W. Lade and D. J. McAllister, *Sens. Actuators B*, 1992, **8**, 191.
- 126 J. C. Andle, J. T. Weaver, J. F. Vetelino and D. J. McAllister, *Sens. Actuators B*, 1995, **24–25**, 129.
- 127 T. Matsuda, A. Kishida, H. Ebato and Y. Okahata, *ASAIJ*, 1992, **38**, M171.
- 128 J. Redepenning, T. K. Schlesinger, E. J. Mechalke, D. A. Puleo and R. Bizios, *Anal. Chem.*, 1993, **65**, 3378.
- 129 D. M. Gryte, M. D. Ward and W. S. Hu, *Biotechnol. Prog.*, 1993, **9**, 105.
- 130 A. Janshoff, J. Wegener, M. Sieber and H.-J. Galla, *Eur. Biophys. J.*, 1996, **25**, 93.
- 131 C. Fredriksson, S. Kihlman, M. Rodahl and B. Kasemo, *Langmuir*, 1998, **14**, 248.
- 132 J. L. Brash and D. J. Lyman, *J. Biomed. Mater. Res.*, 1969, **3**, 175.
- 133 A. T. Poot, J. P. Bengeling, J. P. Casenave, A. Banthes and W. G. van Aken, *Biomaterials*, 1988, **9**, 126.
- 134 H. Ebato, Y. Okahata and T. Matsuda, *Kobunshi Ronbunshu*, 1993, **50**, 463.
- 135 M. Muratsugu, A. D. Romaschin and M. Thompson, *Anal. Chim. Acta*, 1997, **342**, 23.
- 136 T. Sato, M. Endo and Y. Okahata, *J. Biomater. Sci., Polym. Ed.*, 1995, **7**, 587.
- 137 L. Vroman, *Science*, 1974, **184**, 585.
- 138 J. L. Brash, in *Blood Compatible Materials and Devices*, ed. C. P. Sharma and M. Szycher, Technomic Press, Lancaster, 1991, pp. 3–24.
- 139 V. I. Sevastianov, in *High Performance Biomaterials*, ed. M. Szycher, Technomic Press, Lancaster, 1991, pp. 313–341.
- 140 G. L. Hayward, R. L. Dutton, Z. Zhang, J. M. Scharer and M. Moo Young, *Anal. Commun.*, 1998, **35**, 25.
- 141 S. H. Si, T. A. Zhou, D. Z. Liu, L. H. Nie and S. Z. Yao, *Anal. Lett.*, 1994, **27**, 2027.
- 142 S. Kurosawa, E. Nemoto, M. Muratsugu, M. Yoshimoto, Y. Mori and N. Kamo, *Anal. Chim. Acta*, 1994, **289**, 307.

- 143 M. Muratsugu, S. Kurosawa and N. Kamo, *Anal. Chem.*, 1992, **64**, 2483.
- 144 H. Muramatsu, E. Tamiya, M. Suzuki and I. Karube, *Anal. Chim. Acta*, 1988, **215**, 91.
- 145 H. Tan, L. Deng, L. Nie and S. Yao, *Analyst*, 1997, **122**, 179.
- 146 J. Falter, T. Medina and H.-L. Schmidt, *Sens. Actuators*, 1994, **18–19**, 694.
- 147 T. Hasegawa, J. Nishijo and J. Umemura, *J. Phys. Chem. B*, 1998, **102**, 8498.
- 148 Y. Iwasaki, S. Tanaka, M. Hara, K. Ishihara and N. Nakabayashi, *J. Colloid Interface Sci.*, 1997, **192**, 432.
- 149 M. Nakamura, K. Tsujii and J. Sunamoto, *J. Med. Biol. Eng. Comput.*, 1998, **36**, 645.
- 150 J. J. Chance and W. C. Purdy, *Anal. Chem.*, 1996, **68**, 3104.
- 151 T. Tatsuma and D. A. Buttry, *Anal. Chem.*, 1997, **69**, 887.
- 152 S. M. Reddy, J. P. Jones, T. J. Lewis and P. M. Vadgama, *Anal. Chim. Acta*, 1998, **363**, 203.
- 153 J. M. Abad, F. Pariente, L. Hernández, J. D. Abrûna and E. Lorenzo, *Anal. Chem.*, 1998, **70**, 2848.

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