

Piezoelectric pH Sensors: AT-Cut Quartz Resonators with Amphoteric Polymer Films

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Piezoelectric AT-cut quartz resonators immersed in aqueous media, coated with cross-linked films of the random copolymer -{[CH₂CH(CO₂H)]_e-[CH₂- $C(CH_3)(CO_2CH_3)]_{b}$ -[CH₂C(CH₃)(CO₂CH₂CH₂- NMe_2]_{c}_n (1), exhibit large frequency changes} when the pH is changed in the vicinity of the isoelectric point of the polymer film. The frequency changes are attributed to changes in the viscoelastic properties of the films that occur during phase transitions between the isoelectric form and the cationic polymer $(1-NMe_2H^+)$ present at low pH or the anionic polymer $(1-CO_2)$ present at high pH. These phase transitions are accompanied by dramatic changes in acoustic energy attenuation, film thickness changes, and film surface energy, as indicated by acoustic impedance analysis, phase measurement interferometric microscopy, and contact angle measurements. The results are consistent with pH-dependent segregation of the isolectric and ionic phases within the bulk and between the bulk and the surface. The unique pH-sensing capabilities of the coated resonators, combined with their robustness, ease of fabrication, and low cost, provide a convenient approach for the measurement of "threshold" pH changes. Real-time measurements of enzymatic activity and microbe metabolism are demonstrated as examples of potential applications of these sensors.

INTRODUCTION

The ability of piezoelectric acoustic wave devices to respond to small changes in mass at their surfaces while immersed in liquids has led to numerous fundamental investigations of

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interfacial phenomena with these devices.¹ The technological significance of these devices is also becoming increasingly appreciated in that sensors can be readily designed by appropriate modification of the transducer surfaces with a chemically active film. For example, different acoustic wave sensors (shear,² surface acoustic,³ flexure,⁴ and shear-horizontal acoustic plate mode⁵ devices) have been reported for the detection of biologically significant analytes,⁶ metal ions,⁵ and nerve toxin analogs.⁷ These devices generally rely on interfacial chemical events that result in mass changes, which alter the frequency of the acoustic wave that propagates through the transducer and the chemically active film.

The most extensively studied transducer in this regard has been the shear mode AT-cut quartz resonator, commonly referred to as the quartz crystal microbalance (QCM), which comprises an AT-cut crystal sandwiched between two metal excitation electrodes that generate a standing shear wave

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across the thickness of the quartz crystal. The shear acoustic wave experiences an antinode at the surface of the quartz crystal and therefore can propagate into a film immobilized on the surface of the crystal. The thickness (or mass per unit area) of the film and the nature of the shear wave propagation in the film determine the frequency response. The mass change is generally determined from the frequency response according to the Sauerbrey relationship⁸ (eq 1), where Δf is

$$\Delta f = -2f_0^2 \Delta m / A(\rho_0 \mu_0)^{1/2} \tag{1}$$

the measured frequency shift, f_0 the initial (resonant) frequency of the quartz crystal, Δm the mass change, A the piezoelectrically active area defined by the two excitation electrodes, ρ_q the density of quartz (2.648 g/cm³), and μ_q the shear modulus $(2.947 \times 10^{11} \text{ dyn cm}^{-2} \text{ for AT-cut quartz})$.

Sensor design based solely on mass changes, however, can be somewhat limiting if the analytes have low molecular mass. For example, the mass increase associated with binding of a protein to an active surface of a shear mode AT-cut quartz crystal may not be sufficient to achieve a frequency response of practical significance.^{6d} While other piezoelectric transducers may provide somewhat greater sensitivity to mass changes, detection of analytes with low molecular mass remains difficult, practicularly at low coverages. Other considerations include the rigidity of the bound analyte; for example, cell layers that bind to the surface of a QCM give frequency shifts that are much smaller than predicted by eq 1, suggesting that these films behave more like fluids than rigid masses when attached to the resonator surface.⁹ Under these conditions, the acoustic wave propagation and the resonant frequency of piezoelectric resonators can be sensitive to the viscosity and density of materials in contact with the resonator.¹⁰ In the case of thin films immobilized on the quartz resonator, it is becoming increasingly apparent that the viscoelastic properties of the films can play a large role in the frequency response of piezoelectric transducers.^{11,12} The suggestion that thin films whose viscoelastic properties change dramatically upon interaction with an analyte could serve as a basis for the design of piezoelectric sensors prompted us to examine this approach for the detection of low molecular weight analytes. We have been particularly interested in developing piezoelectric sensors for pH measurements that are economical, robust to hostile environments, and amenable to miniaturization and small sample volumes. The need for a pH measurement system with these qualities is embodied in recent reports describing a light-addressable pH sensor which can be utilized for small-volume microbiological and cytotoxicity applications^{13,14} and optical fibers modified with pH indicator dyes.¹⁵ We describe herein a unique, highly sensitive, piezoelectric pH sensor based on composite resonators fabricated from AT-cut quartz resonators coated with cross-linked films of the amphoteric polymer 1. The phase behavior and viscoelasticity of these composite resonators are strongly dependent upon pH, and this behavior is



manifested as dramatic changes in the resonant frequency and electrical characteristics of the composite resonator during phase transitions between ionic and isoelectric forms of 1. The sensors are capable of detecting "threshold" pH changes in which significantly large frequency changes are observed over very small pH changes. These studies also provide insight into the unique fundamental properties of this polymer and illustrate the sensitivity of the QCM to factors other than mass changes. We also provide some illustrative examples of applications of these sensors which demonstrate their utility, namely, measurement of the rates of an enzymatic reaction and microbial metabolism. While our studies have employed a QCM as the piezoelectric element, the concepts can be transferred readily to other piezoelectric transducers capable of operating in liquids.

EXPERIMENTAL SECTION

Apparatus. The pH sensor comprises a 1-in. diameter 5-MHz AT-cut quartz crystal coated with a cross-linked polymer film of 1 and an oscillator circuit designed to drive the crystal at its resonant frequency as described recently.16 Gold electrodes (2000 Å thick) on titanium underlayers (500 Å) were deposited on both sides of the crystal by electron beam evaporation. The quartz crystal is inserted into the feedback loop of a broad-band rf amplifier via the two gold electrodes, and an alternating potential field across the crystal induces oscillation in the shear mode (displacement parallel to the electrodes) at the series resonant frequency. The quartz crystal was mounted between two 9-mm O-rings confined by standard 18/9 glass O-ring fittings, such that the upper glass joint comprised the sample chamber. The gold electrode facing the solution is at hard ground. The output of the oscillator circuit is connected to a Hewlett-Packard 5384A frequency counter, which is interfaced to a Macintosh personal computer for data collecton. The sensitivity of a 5-MHz QCM as given by eq 1 is $0.057 \text{ Hz cm}^2 \text{ ng}^{-1}$. An asymmetric electrode format was employed in which the side having the larger gold pad is coated with the polymer film and faces the solution (A =0.35 cm² polymer side, $A_{\text{pieso}} = 0.18 \text{ cm}^2$ on opposite side).

Impedance analysis was performed with a Hewlett-Packard 4194A impedance/gain-phase analyzer capable of performing measurements over a frequency range of 100 Hz-40 MHz in the impedance mode. Data collection was accomplished via an HPIB interface with a Macintosh personal computer. Programs were configured to measure impedance (Z), susceptance (B), conductance (G), and phase angle (θ) over the entire frequency range at an appropriate resolution or to measure key parameters related to the properties of the composite resonator (that is, the quartz crystal and polymer film). These parameters included the maximum conductance (G_{max}) and the frequency of that value $(f_{G_{max}})$, minimum and maximum impedance (Z_{min}, Z_{max}) and the frequencies of those values $(f_{Z_{\min}}, f_{Z_{\max}})$, the frequencies of the half-power points (f_1, f_2) , minimum and maximum susceptance (B_{\min}, B_{\max}) and the frequencies of those values $(f_{B_{\min}}, f_{B_{\max}})$. Equivalent circuit values based on the Butterworth-van Dyke model¹⁷ were determined using the internal simulation program

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of the HP4194A. Quality factors were calculated using the relationship $Q = f_{G_{max}}/(f_2 - f_1)$.

Measurements of \overline{pH} were made simultaneously with frequency measurements or network analysis with a Beckman Model $\Phi 32$ pH meter. The analog output of the pH meter was connected to an IO/Tech analog-digital converter which was interfaced to the HPIB bus, enabling automatic measurement of pH.

Film thicknesses in aqueous solutions were measured directly with a phase measurement interferometric microscope (Zygo, Inc.). Cross-linked films of 1 were coated on evaporated gold films on quartz substrates according to the procedure described below and were immersed under a thin film (ca. 1 mm) of water contained over the sample with a glass cover slip. The $\mathbf{p}\mathbf{H}$ of the solution was changed by replacing the water between the sample and the cover slip with water adjusted to the desired pH value. This replacement was performed with a syringe so that the sample compartment and optical path length was not disturbed. A reference height difference for calibration was provided by the edge of the gold films, whose thickness was established from the frequency shift of the quartz crystal microbalance during electron beam evaporation. Sessile contact angle measurements were performed with a Rame-Hart contact angle goniometer using a $10-\mu L$ water droplet.

Polymer Preparation and Film Immobilization. The polymer 1 (with a = b = c = 1) was synthesized according to a previously reported procedure.¹⁸ Under a nitrogen atmosphere, an emulsifier solution consisting of 1000 mL of distilled water, 10 g of Triton QS-30 surfactant (Rohm & Haas), and 10 g of (N,N-dimethylamino)ethanol was heated to 60 °C. Then a mixture containing 0.58 mol of methyl acrylate, 0.58 mol of methyl methacrylate, and 0.58 mol of (dimethylamino)ethyl methacrylate was added at 4 mL/min. When the solution became saturated (saturation is evident when the solution begins to exhibit translucency), addition of an initiator solution containing 5 g of ammonium persulfate in 250 mL of distilled water was started at 0.75 mL/min while the monomer addition was continued simultaneously. Addition of the initiator results in immediate polymerization, as evidenced by a temperature increase. After all the monomer was added, the mixture was stirred for an additional 15 min and then poured into 2-L polyethylene beaker. Acetone was added until the product coagulated. The product was then collected by filtration and washed with water to remove remaining emulsifier and other impurities. The polymer was transferred to a flask equipped with a high-shear blade stirrer, ethanol added, and the mixture heated to 80 °C. After the polymer had dissolved, a solution of 32.65 g of KOH in 100 mL of distilled water was added via an addition funnel to selectively convert the methyl acrylate to the potassium salt of acrylic acid. The addition rate should be controlled so that polymer does not precipitate during this step. After the addition was complete, the mixture was stirred for an additional 30 min at 80 °C. The product either can be retained in the ethanol solution in this basic form or can be neutralized by addition of an amount of acid equivalent to the amount of base added during the hydrolysis step. The product was isolated by isoelectric precipitation by transferring the ethanol solution of the polymer to a large excess of distilled water and then adding acid to precipitate the isoelectric polymer. The polymer was isolated by centrifugation, washed with water, and then redissolved in water at a pH either above or below its isoelectric point. It is important to store the polymer as a solution because redissolution of the dried polymer is difficult.

Films of 1 were cross-linked with a multifunctional aziridine (Xama-7; Virginia Chemical Co.) according to the procedure depicted in Scheme I. Xama-7 is the pentaerythritol-tris(β -aziridinyl)propionate 3 which is prepared by the reaction of ethyleneimine with 2, the ester of pentaerythritol and acrylic acid. Cross-linked films of 1 were prepared by spin coating a basic solution (NH₄OH) containing 5.44% 1 and a required amount of 3, followed by heating for 5 min at 100 °C. It was found that generally a 50:1 molar ratio of 1:3 gave the best results. The heating step eliminates NH₃, thereby acidifying the coating opening reaction between the aziridine groups and a small number of the CO₂H groups of 1. The film thickness was controlled by

(18) Foss, R. P. U. S. Patent 4,749,762, 1988.

Scheme I



spin-coating rotational speeds, which ranged from 1000 to 4000 rpm. Film thicknesses were determined independently with a Sloan Dektak IIA stylus profilometer.

Enzyme Activity Measurements. The response to pH changes induced by urease-catalyzed hydrolysis of urea was measured in 2 mL of a buffer solution in which the resonator coated with cross-linked 1 was immersed. The buffer solution was prepared from 1.48 mL of 1.0 mM NaOH, 8 mL of 0.2 mM EDTA, and 100 mL of deionized water adjusted to a pH of 5.5. Determinations of the response dependence upon urease concentration were performed by adding known amounts of a urease solution (1 mg of urease in 100 mL of deionized water) to the buffer solution containing 0.25 M urea while the resonator was immersed in the solution. Conversely, the response dependence upon urease solution to the buffer solution containing 0.1 μ g/mL urease.

Microbe Metabolism. The aqueous growth medium (pH = 7.4) for microbe metabolism measurements contained 1.0% (w/ v) protease peptone No. 3 (Difco), 0.1% beef extract (Bacto), 0.002% Bromo Cresol Purple, 0.5% NaCl, and 0.1% 1-CO2-. The base medium was supplemented with carbohydrates (1% w/v)where required and filtered through a $0.2 \mu m$ Corning sterilization filter. Response of the piezoelectric sensor to bacterial metabolism was investigated using reference strains obtained from the American Type Culture Collection (ATCC). Escherichia coli (ATCC 25922) was first grown overnight in a 3% tyrpticase soy broth (TSB) medium at 37 °C to a cell density of $\sim 10^9$ cells/mL. Just before use, the cells were diluted to minimal media composed of 1 part in 20 in Difco Bacto Purple broth (BPB) containing no carbohydrates. The cell density was determined microscopically in a hemocytometer using a light microscope. Portions of the diluted culture are then used as a starting inoculum for the piezoelectric sensor experiments. Cell numbers determined on aliquots removed from the QCM growth chamber were obtained by first arresting the cell growth of the aliquot by addition of a 0.1% sodium azide solution. The samples were vortexed and counted visually in a Petroff Hauser counting chamber. In some cases the cell concentrations were counted automatically using an Olympus Q2 image analyzer equipped with a Compaq 386/25 computer.

RESULTS AND DISCUSSION

We recently reported an approach to pH sensing employing AT-cut quartz resonators and an acrylate-based polymer derivatized with NMe₂ and CO₂H functionalities (1) whose solubility was dependent upon the pH of the medium (Scheme II).¹⁹ As the pH of the medium approached the isoelectric point of the polymer, deposition of the isoelectric insoluble polymer onto the surface of the resonator resulted in a corresponding shift in the resonant frequency of the resonator.

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Scheme II



This approach was employed successfully for the measurement of microbe metabolism.

The polymer 1 can be prepared readily from acrylate monomers so that the (dimethylamino)ethyl methacrylate, methyl methacrylate, and acrylic acid groups are present in controlled amounts (see Experimental Section). The experiments described herein were all performed with the polymer formulation a = b = c = 1. Light scattering studies suggested that $pI \approx 6.1$ for this composition; at this value the optical density of aqueous solutions of this polymer was maximum due to scattering by the insoluble polymer. At pH values below or above the pI, however, the polymer was very soluble owing to its high degree of ionicity under these conditions. For pH > pI values, the polymer can be considered as consisting of neutral NMe2 and anionic CO2- groups, whereas for pH < pI the polymer can be considered as consisting of neutral CO₂H and cationic NMe₂H⁺ groups. Accordingly, the ionic forms of the polymer are herein referred to as 1-CO₂-(pH > pI) and 1-NMe₂H⁺ (pH < pI). Light scattering and buffering capacity studies of non-cross-linked 1 indicated that the width of the isoelectric region was approximately 1 pH unit. In this region the polymer's buffering capacity is reduced, presumably owing to its decreased solubility and lower activity of the acid and base centers resulting from hydrogen bonding or electrostatic bonding of zwitterionic pairs. The buffering capacity studies actually suggested that $pI \approx 5.5$; this value may be more accurate because the light scattering studies are susceptible to errors associated with the size of the polymer particles and their agglomeration. The pI and the width of the isoelectric region can be adjusted synthetically by altering the molecular weight and/or the ratio of the monomers. For example, the width of the isoelectric region would be decreased by increasing polymer uniformity (i.e., minimizing the variability of a, b, and c among different chains).

The utility of the aforementioned sensing approach that relies on polymer precipitation is limited because it requires the presence of the pH-sensitive polymer in the medium. We therefore pursued a strategy employing polymer films immobilized on the AT-cut quartz resonator; wherein mass and viscoelastic properties were sensitive to pH changes. We surmised that the mass and viscoelastic properties of polymer films of 1 would depend upon pH for several reasons. At pH values below and above the isoelectric point the polymer film is positively and negatively charged, respectively. Under these conditions it was expected that the film would swell with solvent and electrolyte to minimize the repulsion between the charged centers. At the isoelectric point, however, the polymer can be considered as either neutral or zwitterionic. Hydrogen bonding between functional groups, the absence of charge repulsion, and hydrophobic effects were expected to reduce the swelling of the polymer. Such dimensional changes would presumably be accompanied by large changes in viscoelasticity and surface energy (i.e., hydrophobicity)²⁰ of the polymer that could be manifested in large QCM frequency shifts. Changes in mass due to exchange of electrolyte ions and solvent between the film and solution are also to be expected. Indeed, impedance analysis indicated that precipitated films of un-cross-linked 1 on an AT-cut quartz resonator exhibited large bandwidths in the conductance spectrum and a corresponding decrease in the quality factor, suggesting energy dissipation associated with viscoelastic behavior.

Preparation of Cross-Linked Polymer Films. Composite resonators comprising the AT-cut quartz crystals with immobilized films of 1 were prepared by a procedure in which an esterified pentaerythritol reagent containing reactive aziridine groups served to cross-link 1 by an acid-assisted ring-opening reaction between the aziridine groups and a small number of CO₂H functionalities of 1. Films were prepared by spin coating a mixture of 1 and the cross-linking agent 3 onto a 1-in. AT-cut quartz crystal from a solution basified with NH₄OH, the basic medium supressing the cross-linking step. Cross-linking in the film was then induced by gentle heating at 100 °C for ~ 10 min, which eliminated NH₃ and decreased the basicity of the film. We have found that, for our purposes, films with a 50:1 ratio of 1:3 gave optimum combination of negligible solubility and large frequency responses to pH changes. Smaller amounts of cross-linking agent resulted in films that were either soluble or very tacky, with significant instability in the composite resonator. Conversely, large amounts of cross-linking agent resulted in films that are highly cross-linked and rigid and are not responsive to pH changes.

Frequency Response to pH. Composite resonators with the coated side of the crystal immersed in aqueous solution exhibited stable oscillation provided the film thickness did not exceed 1 μ m. Films with thicknesses exceeding this value resulted in severe acoustic losses, as indicated by the low quality factors of the resonator. These losses likely result from viscous damping associated with coupling between polymer chains and between polymer chains and solvent. Stable oscillation in air was achieved when these films were dry, corroborating the participation of solvent in the energy loss mechanism.

The series resonant frequency (f_s) , measured with the composite resonator in the feedback loop of a broad-band rf amplifier, and the frequency of maximum conductance $(f_{G_{max}})$ measured by impedance analysis, were found to depend significantly upon the pH of the solution in contact with the resonator (we will assume throughout that the difference between f_s and $f_{G_{max}}$ is negligible²¹). When the pH of an aqueous solution, previously acidified with H₃PO₄, was gradually increased from pH = 3 the onset of an abrupt frequency decrease at pH ≈ 4.6 was observed (Figure 1). The magnitude of the frequency shift increased with polymer film thickness, giving exceptionally large shifts approaching -6000

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⁽²¹⁾ The values of f_s and $f_{G_{max}}$ are rigorously different for the quartz resonator owing to the dielectric contribution of the quartz crystal, which effectively adds a capacitance in parallel to the series "motional branch" of the oscillator, in accord with the Butterwork-van Dyke electrical equivalent representation. In the feedback mode, f_s is actually measured since this mode requires oscillation at $\theta = 0^\circ$, where the reactance of the series branch is zero and the actual conductance is less than G_{max} . Impedance analysis, however, gives G_{max} and $f_{G_{max}}$ explicitly, the latter occurring at a slightly lower frequency that f_s . In practice, the difference between the two is negligible, and factors that result in a shift in f_s will give a corresponding shift in $f_{G_{max}}$.



Figure 1. (a) Comparison of $f_{Q_{max}}$ at different pH values for a 5-MHz AT-cut quartz resonator coated with cross-linked polymer films of 1 with the buffering capacity (\blacklozenge) of a solution of non-cross-linked 1. (b) Comparison of $f_{Q_{max}}$ at different pH values for a 5-MHz AT-cut quartz resonator coated with a cross-linked polymer film of 1 with the optical density (\blacklozenge) of a solution of non-cross-linked 1. The pH was adjusted by addition of NaOH to an aqueous solution previously acidified with H₃PO₄: thickness, 0.4 (**III**) and 0.8 μ m (**II**). Below: Schematic representation of the dimensional changes in the film and segregated domain structure when both ionic and isoelectric phases are present. No spatial ordering of the domains is implied by this representation. Key for the lower portion of the figure that identifies these phases: 1-NMe₂H⁺ (**III**); 1(isoelectric) (**II**); 1-CO₂⁻ (**ZB**).

Hz for an 0.8- μ m-thick polymer film. The frequency exhibited a slight maximum at pH = 5.5, followed by an abrupt *increase* when the pH \approx 6.1. The width and pH range of this isoelectric region was similar to that indicated by the buffering capacity studies of soluble 1. These data strongly suggested that the observed frequency changes were related to the changes accompanying the transitions between the ionic and isoelectric forms of 1. The small maximum in the center of the isoelectric region at pH = 5.5 was more apparent for the thinner films, which also exhibited a broader isoelectric region based on the frequency changes.

Under ideal rigid-layer conditions the frequency changes of the composite resonator would correspond only to mass changes in the polymer film that result from exchange of electrolyte required to maintain electroneutrality in the film. Electrolyte ion pairs and solvent exchange, which would contribute to the degree of swelling of the polymer, are also likely. For example, the egress of $H_2PO_4^-$ or HPO_4^{2-} counterions from the film (depending on the actual pH; $pK_a^1 =$ 2.12; $pK_a^2 = 7.21$) can provide electroneutrality during the transition from 1-NMe₂H⁺ to isoelectric 1 upon increasing Scheme III



the pH, resulting in a mass decrease and a corresponding frequency increase (Scheme III). When pH > pI, electroneutrality could be provided by insertion of Na⁺, which would result in a frequency decrease. This mechanism, however, is contrary to observation. It is also feasible that upon entering the isoelectric region from the low-pH side electroneutrality is preserved by loss of H⁺ with concomitant insertion of Na⁺ and retention of phosphate ions, which would give a net decrease in frequency. Leaving the isoelectric region on the high-pH side the frequency increase may then correspond to loss of anions from the film with retention of the Na⁺ ions. While this latter scheme corresponds qualitatively to the observed behavior, the frequency of the composite resonator on opposite sides of the isoelectric region, however, were identical. In addition, the frequency changes for the 0.4- and 0.8-µm-thick films correspond to mass changes of approximately 20 and 90 μ g/cm², respectively. These mass changes are not proportional to film thickness and are comparable the total areal mass $(\Delta m/A)$ of these films after spin coating (estimated as 40 and 80 μ g/cm² based on a film density of 1 g/cm^3). These observations strongly suggest that the frequency changes are due to factors other than mass changes.

Phase measurement interferometric microscopy (PMIM)²² provides further evidence that factors other than mass changes contribute to the observed frequency decreases. PMIM relies on the measurement of a change in the phase angle of a He-Ne laser reflected from the surface of the polymer film immersed under a thin film (in this case 1 mm) of the aqueous solution. The change in phase angle with respect to a reference beam is used to calculate the actual height changes, provided the refractive indices of the solution and the film are known and constant. PMIM clearly revealed a rather dramatic decrease in the thickness of the cross-linked polymer film of 1 upon formation of the isoelectric phase. Typically, the film thickness at the center of the isoelectric region at pH = 5.5was half that of the thickness at the pH extremes. Topographical maps of the film obtained with PMIM over large areas (200 μ m \times 200 μ m) indicated that the thickness change was uniform across the entire film. The thickness change is fairly abrupt and mirrors the observed frequency dependence upon pH. The significant film compaction argues against an increase in film mass as the source of the frequency decrease during the transition to the isoelectric phase, strongly suggesting an important role for changes in the viscoelastic properties of the film.

In order to determine whether stress effects were responsible for the observed frequency response, we employed the double-resonator technique, in which AT-cut and BT-cut quartz crystals are used as piezoelectric substrates simultaneously or under identical conditions.²³ The stress coefficients for these crystals are nearly equal in magnitude but are opposite in sign ($K^{AT} = 2.75 \times 10^{-12}$ cm² dyn⁻¹; $K^{BT} = -2.65$

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Figure 2. (a) The contact angle, θ , of the polymer films measured with water drops of different pH values. (b) The bandwidth $f_2 - f_1$ at different pH values for a 5-MHz AT-cut quartz resonator coated with a cross-linked polymer film of 1: thickness, 0.4 (**II**) and 0.8 μ m (**II**). (c) The equivalent resistance, *R*, for a 5-MHz AT-cut quartz resonator coated with a cross-linked polymer film of 1: thickness 0.4 (**II**) and 0.8 μ m (**II**). (c) The (**II**).

× 10⁻¹² cm² dyn⁻¹), enabling isolation of the stress contributions from other contributions such as mass changes. The stress can be directly measured using eq 2, where t_q^{AT} and t_q^{BT} are the thicknesses of the AT-cut and BT-cut quartz crystals, respectively. However, these experiments indicated identical frequency response for AT- and BT-cut crystals in the entire pH range, indicating that $\Delta S = 0$.

$$\Delta S = (K^{\text{AT}} - K^{\text{BT}})^{-1} [t_{q}^{\text{AT}} \Delta f^{\text{AT}} / f_{s}^{\text{AT}} - t_{q}^{\text{BT}} \Delta f^{\text{BT}} / f_{s}^{\text{BT}}] \quad (2)$$

Sessile contact angle measurement using water drops of different pH demonstrated that the polymer is very hydrophilic at the pH extrema where the polymer is ionic (Figure 2a). As the pH of the drop was increased from pH = 3, the contact angle sequentially increased from $\theta = 35^{\circ}$ to a maximum of 62° at pH \approx 5, decreased to 40° at the isoelectric point, increased again to 65° at pH \approx 6.5, and then decreased to 35° at pH > 8.

Impedance Analysis. The shear vibration of the quartz crystal results in mechanical interactions between the crystal and the polymer film on its surface. The mechanical properties of the film therefore influence the mechanical properties of the resonator, which because of piezoelectric coupling results in correponding changes in the electrical Scheme IV



properties of the QCM. These changes can be examined readily by impedance analysis methods in which current across the quartz crystal is measured at constant voltage over a specified range of frequencies. The electrical characteristics of an unperturbed QCM are generally analyzed in terms of the Butterworth-Van Dyke equivalent circuit (Scheme IV), which comprises a LCR series branch corresponding to the mechanical motion of the crystal and a parallel capacitance associated with the static dielectric properties of the quartz crystal. The electrical properties of this circuit result in a maximum current at the resonant frequency where the capacitive and inductive reactances ($X_{\rm C}$ and $X_{\rm L}$, respectively) cancel and the circuit becomes purely resistive in the series branch. When operating the crystal is operating in a feedback loop of an rf amplifier, the series resonant frequency is measured directly because the feedback loop operates at the highest value of the conductance at zero phase angle, which occurs when $X_{\rm L} = X_{\rm C}$. Impedance analysis, however, provides other parameters that can be used to evaluate the changes in mechanical properties occurring in the polymer film. The Butterworth-Van Dyke equivalent circuit values obtained with impedance analysis represent the mechanical properties of the composite resonator, in that L corresponds to the inertial mass, C the energy stored during oscillation, and R the energy dissipation during oscillation.²⁴ The latter term is especially significant as it reflects the energy attenuation of the acoustic wave by the film.

In principle, the Butterworth–Van Dyke equivalent circuit is applicable only when the QCM does not experience losses due to viscous coupling resulting from contact with a fluid. It has been noted, however, that *near the resonant frequency*, the electrical characteristics can be used to evaluate this perturbed QCM with reasonable accuracy, thus enabling measurement of the energy loss and mass loading associated with viscous coupling.^{10c} Several reports have used this model to interpret QCM data.²⁵ Particularly useful is the electrical conductance (G) spectrum, which reaches a maximum at $f_{G_{max}}$, where the real part of the electrical admittance reaches a maximum value.

The bandwidth, $f_2 - f_1$, where f_1 and f_2 are the frequencies at $G_{max}/2$ (-3 dB below G_{max}), is proportional to the damping of the acoustic wave energy in the composite resonator. Impedance analysis of a 5-MHz uncoated AT-cut quartz crystal revealed that $f_2 - f_1$ typically has values of ~100 Hz in air and ~3000 Hz in water. Significant increases in $f_2 - f_1$ are observed when the crystal is coated with the crosslinked films of 1, typically with values of 3000 Hz in air and values in water that depended on the solution pH (vide infra). The values of $f_2 - f_1$ increase with polymer film thickness. This indicates appreciable attenuation of the acoustic energy due to the damping of the shear wave by viscous coupling to the polymer. Notably, the bandwidth decreases with in-

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Figure 3. Equivalent resistance, *R*, vs $\Delta f_{Q_{max}}$ for 5-MHz AT-cut quartz resonators coated with cross-linked polymer films of 1: (a) thickness 0.4 μ m, (b) thickness 0.8 μ m.

creasing amount of cross-linking agent during film preparation, suggesting that increased rigidity provided by greater number of cross-links reduces the viscous damping, in accord with expectations.

Impedance analysis reveals that $f_2 - f_1$ exhibits a "doublepeak" dependence upon pH, with two maxima at pH values of 5.0 and 6.4 (Figure 2b). These maxima occur at pH values corresponding to $\Delta f_{max}/2$ (Figure 1). The value of $f_2 - f_1$ in the center of the isoelectric region at pH = 5.5 approached the values observed at the pH extrema. The equivalent resistance, R, which is related to the bandwidth because both are a measure of the acoustic energy loss in the coated resonator, exhibits the same dependency (Figure 2c). Remarkably, this double-peak behavior mimics the contact angle dependence upon pH, indicating that the energy dissipation and contact angles are manifestations of the same phase transition behavior.

The correspondence between R and $\Delta f_{G_{max}}$ is depicted in Figure 3 for polymer films of two different thicknesses. As the pH is increased from an initial value of pH = 3, $\Delta f_{G_{max}}$ decreases with a concomitant increase in R, with R reaching a maximum near $\Delta f_{\rm max}/2$. Further pH increases into the isoelectric region result in a decrease in R while $\Delta f_{G_{max}}$ still decreases. The slight increase in $\Delta f_{G_{max}}$ in the center of the isoelectric region is evident here for the thin film by the shortlived shift to less negative $\Delta f_{G_{max}}$ values. The data then essentially retrace themselves with R now increasing with more positive values of $\Delta f_{G_{max}}$ to $\Delta f_{G_{max}}/2$, followed by a decrease in R until pH = 9, where the value of $(\Delta f_{G_{\text{max}}}, R)$ is identical to the value obtained at pH = 3. We note that these data are functionally equivalent to traces of $\Delta \alpha / k$ vs $\Delta v / v_0$ reported recently to describe the glassy transitions in polymer films immobilized on surface acoustic wave devices, where $\Delta \alpha / k$ is the attenuation per wavenumber and $\Delta v/v_0$ is the fractional velocity change.^{1a}

The frequency changes, network analysis data, and physical characterization clearly indicate that the chemical, physical, and rheological properties of 1 depend upon pH in a rather complicated manner. Based on our previous experiments with soluble 1, it is reasonable to suggest that for $pH \ll pI$ and $pH \gg pI$ the polymer is highly ionized. Under these conditions the tendency of the film to minimize Coulombic repulsion by solvent incorporation can result in swelling that leads to the observed dimensional changes. Alternatively, the expulsion of water and accompanying deswelling upon transformation to the isoelectric form of the polymer may be driven by entropic effects associated with the release of bound water molecules from the increasingly hydrophobic polymer, as has been postulated for temperature- and pH-sensitive hydrogels.²⁶

The contact angle behavior corroborates complicated phase transitions in the polymer film. The low contact angles observed at the pH extremes were expected, based on the ionicity of the polymer in these regions, similar to contact angle behavior at low pH reported for polyethylene modified with surface carboxylic acid functionality.²⁷ The increase in contact angle to the maximum values at $\Delta f_{\text{max}}/2$ is consistent with decreasing ionicity or the emergence of hydrophobic methyl methacrylate regions on the surface. Upon approaching the center of the isoelectric region from these maxima resegregation of the hydrophobic and hydrophilic regions of the polymer is suggested by the decrease in contact angle, with the hydrophobic withdrawing to the interior of the polymer film. The similar pH dependence of the energy dissipation strongly suggests that these two phenomena are related to the same phase transitions involving the formation of ionic and isoelectric phases throughout the pH range. At the pH extremes and at the isoelectric point, a single phase exists and energy dissipation will depend upon viscous losses associated with interfacial friction between polymer chains, and between polymer chains and solvent. In contrast, at pH values approaching the isoelectric region, the polymer will consist of isoelectric and ionic phases whose relative amounts will depend upon pH. If Δf_{max} is assumed to be proportional to the fraction of the ionic and isoelectric phases, the observation of maximum energy attenuation when $\Delta f =$ $\Delta f_{\rm max}/2$ suggests that the maximum energy attenuation occurs when these phases are present in equal amounts. It is reasonable to suggest that this energy dissipation is a consequence of scattering of the acoustic wave off phase domains formed from segregation of ionic and isoelectric regions (or the interfacial regions between the phase domains). as scattering would be most significant when the isoelectric and ionic phases are present in equal amounts. The viscous loss is apparently much more significant between the isoelectric and ionic phases than between polymer chains and solvent in the single-phase regions. Further examination of the data suggests that the energy attenuation is greater for the isoelectric phase than for the ionic phase. We emphasize that the postulate of phase domains is speculative, as we have no independent corroboration of their existence. Measurements of thin cross-linked films of 1 with PMIM did not indicate any evidence of phase segregation, which would have been observable because of the difference in experimentally measured refractive indices of the ionic ($\eta_D = 1.37$) and isoelectric forms ($\eta_D = 1.42$). However, the limited lateral

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resolution of PMIM would prevent detection of domains smaller than ${\sim}2~\mu\text{m}.$

The pH-induced dimensional changes occurring in the polymer suggest changes in the viscoelasticity and density of the polymer film; these changes are expected to result in frequency shifts because of changes in the propagation characteristics of the shear acoustic wave in the polymer film. The double-resonator experiments indicated that there are negligible contributions from stress changes in the polymer film during the phase transitions; that is, contributions from changes in the film modulus are negligible. This behavior is not entirely unexpected because the elasticity of the film is associated with the restoring force of the chains after application of a shear stress. This property is affected primarily by the polymer network structure (i.e., cross-linking density and polymer chain configuration), which does not necessarily change during phase transitions.²⁸ Indeed, it has been shown that the shear modulus of gels does not change substantially during a phase transition, when normalized for the polymer network concentration.²⁹ The frequency changes clearly are not consistent with mass changes alone, as it is unlikely that film mass would double during deswelling or that the mass of the film would be identical at both pH extremes.

If changes in the polymer film modulus are ignored, the changes in the properties of the composite resonator may best be understood by considering contributions from changes in the viscosity of the polymer film. This can be accomplished by treating the film as a viscous Newtonian fluid, in which the shear wave amplitude decreases with distance from the resonator surface as an exponentially damped cosine function given by eq 3, where $V_x(z,t)$ is the instantaneous velocity of

$$V_{x}(z,t) = A e^{-kz} \cos(kz - \omega t)$$
(3)

$$\Delta f = -f_0^{3/2} (\rho_{\rm L} \eta_{\rm L} / \pi \rho_{\rm q} \mu_{\rm q})^{1/2} \tag{4}$$

$$\delta = (\eta_{\rm L}/\pi f_{\rm s}\rho_{\rm L})^{1/2} \tag{5}$$

the shear wave parallel to the surface of the quartz resonator at a distance z from the resonator surface at time t, A is the maximum amplitude of the shear wave, and ω the resonant frequency.^{10ab} The solution of this equation indicates that increases in the viscosity and density give corresponding decreases in the resonant frequency according to eq 4, where $\eta_{\rm L}$ and $\rho_{\rm L}$ are the absolute viscosity and density of the liquid, respectively. This dependence stems from the increase in the decay length of the shear wave (δ) with increasing $\eta_{\rm L}$ and decreasing $\rho_{\rm L}$ (eq 5), the decay length representing the distance from the resonator surface over which the acoustic wave amplitude decreases to 1/e of its original value. Compaction of the film during transformation to the isoelectric phase results in a higher viscosity film confined closer to the resonator surface where the amplitude of the shear wave is greater, the higher viscosity increasing the amplitude and decay length of the shear wave (Figure 4). Under these conditions, the shear wave will also extend further into the solution above the polymer film as well. The enhanced coupling of the acoustic wave with the film during compaction thereby increases the effective mass detected by the propagating shear wave. The decay length of the shear wave in water for a 5-MHz QCM is $\sim 0.25 \,\mu m$. The R values for the polymer film at pH > pI and pH < pI are not much greater that those for a bare resonator (ca. 700 Ω), suggesting similar damping characteristics and decay lengths for these two cases.



Figure 4. Schematic representation of the velocity profile of a shear wave, and the effect on shear wave propagation of compaction and increasing viscosity of the polymer film resulting from the phase transition between the ionic and isoelectric forms. The thick line represents the decay of the acoustic wave in water at room temperature, where the decay length for a 5-MHz shear wave is $\delta = 250$ nm. The dashed lines represent the thickness of the ionic phase ($d_{\text{ionic}} = 0.8 \,\mu\text{m}$) and shear wave propagation in this phase. The thin solid lines represent the thickness of the higher viscosity, compacted isoelectric phase (diso = 0.4 μ m). Compaction of the film during transformation to the isoelectric phase results in a higher viscosity film confined closer to the resonator surface where the amplitude of the shear wave is greater, the higher viscosity increasing the amplitude and decay length of the shear wave. Under these conditions, the shear wave will also extend further into the solution above the polymer film. The enhanced coupling of the acoustic wave with the film during compaction thereby increases the effective mass detected by the propagating shear wave.

Therefore, the thicknesses of the films $(0.4-0.8 \ \mu m)$ and the dimensional changes (ca. $0.2-0.4 \ \mu m$) are of the same magnitude as the shear wave decay length. It is not surprising that substantial frequency decreases would be observed upon compaction under these conditions. We therefore attribute the frequency decrease in the isoelectric region to viscosity effects and corresponding changes in the shear wave propagation during the dimensional changes of polymer film.

Interfacial viscosities and the corresponding surface energies at the polymer film-water interface may also play a role in the observed behavior, particularly if the films are rigid enough so that the shear wave has significant amplitude at this interface. An increase in the surface energy of the polymer film interface is equivalent to an increase in the interfacial viscosity, which would favor the "no-slip" condition that is generally assumed for shear mode AT-cut resonators in liquid media. When slip occurs, the mass of the liquid layer above the interface will effectively "decouple" from the shear motion, resulting in an apparent mass decrease and a corresponding frequency increase. The data, however, cannot be interpreted on the basis of changes in the slip condition because Δf does not correspond to the contact angle behavior. Indeed, the resonator frequency is highest at the pH extremes where the contact angle is largest and the no-slip condition is expected to be most applicable. We conclude that, while interfacial viscosity effects may be present, their contribution is not sufficient to dominate the frequency of the composite resonator.

Piezoelectric Measurement of Enzymatic Reactions Rates. In order to demonstrate the possible utility of crosslinked films of 1 in sensing applications, we have examined

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Figure 5. (upper) Frequency response of a 5-MHz AT-cut quartz resonator coated with a cross-linked polymer film of 1 to the ureasecatalyzed hydrolysis of urea at three different urease concentrations: 0.83 (■), 0.43 (□), and 0.22 µg/mL (◆). Conditions: phosphate buffer solution containing 0.015 M NaOH, 0.016 M EDTA, and 0.25 M urea adjusted to a pH of 5.5 with H₃PO₄. Inset: Dependence of τ , the time of minimum frequency on urease concentration. (lower) Frequency response of a 5-MHz AT-cut quartz resonator coated with a crosslinked polymer film of 1 to the urease-catalyzed hydrolysis of urea at three different urea concentrations: $3.4 \times 10^{-3} \,\mathrm{M}(\diamondsuit)$, $6.8 \times 10^{-3} \,\mathrm{mM}$ (□), 13.6 × 10⁻³ mM (■). Conditions: phosphate buffer solution containing 0.015 M NaOH, 0.016 M EDTA, and 0.1 µg/mL urease adjusted to a pH of 5.5 with H₃PO₄. Inset: Dependence of τ , the time of minimum frequency on urea concentration.

the frequency response of the composite resonator to the urease-catalyzed hydrolysis of urea. This reaction results in the formation of NH₃ with a corresponding increase in the pH of the medium. Accordingly, when urea is added to a phosphate buffer solution (initial pH = 4.0) containing urease a monotonic frequency decrease is observed after a short time, followed by a monotonic increase until the original frequency is attained (Figure 5). The rates of frequency change in both branches are essentially identical. The time at which the frequency reaches the minimum shifts to longer values with decreasing urease concentration. These data are consistent with the pH-dependent frequency of the composite resonator: urease-catalyzed hydrolysis increases the pH of the medium, resulting in conversion of the 1-NMe₂H⁺ polymer film to its isoelectric form where the frequency decreases. Further urease-catalyzed increases in pH result in conversion from the isoelectric form to the $1-CO_2^-$ film with a concomitant increase in frequency. The relative rates for different urease concentrations can be discerned from the inverse of the time of the frequency minimum, τ . The experiments were performed at large urea concentrations to ensure a saturated concentration of the enzyme-substrate complex and an enzymatically catalyzed reaction occurring at the maximum velocity, V_{max} . Under these conditions $\tau \propto V_{max}$, as indicated by the linear dependence of τ on [urease]. Similarly, experiments in which [urea] is varied at constant urease concentration (but not at saturation of the enzyme-substrate complex) indicate that τ increases with urea concentration.

Piezoelectric Measurement of Microbe Metabolic and Growth Rates. The growth of microorganisms and cells alters the composition of their growth medium as nutrients are consumed and converted into metabolic end products. During this process, the pH of a medium is altered by conversion of carbohydrates into smaller molecules, such as lactic acid, succinic acid, and acetic acid, or through the conversion of amino acids to ammonia and bicarbonate. The production of metabolic acids has long been recognized as a useful and broadly applicable indicator of cell metabolism and growth. For example, acid production has been used to monitor changes in cellular metabolism due to environmental influences, toxins, and chemicals.^{13,14} We previously reported that the metabolic and growth rates of Escherichia coli could be determined by measuring the frequency changes due to pH-induced deposition of soluble 1 resulting from E. coli metabolism of carbohydrates.¹⁹ Our results and previous work³⁰ indicate that E. coli readily metabolizes carbohydrate with a concomitant decrease in the pH of its medium; at 37 °C the population doubling rate is ~ 1800 s.

The pH-dependent frequency of AT-cut quartz resonators coated with cross-linked polymer films of 1 provides a unique and convenient approach for evaluating metabolism and growth rates. Cell metabolism rates were measured with the coated side of the composite resonator submerged in a growth medium (pH = 7.5; 37 °C) containing a carbohydrate nutrient. The medium was then inoculated with $E. \ coli$ and covered with type A immersion oil to exclude air. Under these conditions the bacteria metabolize carbohydrates to organic acids and carbon dioxide, thus lowering the pH of the medium. After an induction time required for the medium to reach the pH of the upper end of the isoelectric region of the polymer film, the decreasing pH results in a gradual decrease in frequency due to formation of the isoelectric phase (Figure 6). This is followed by a gradual increase in the frequency to the initial frequency as the pH of the medium decreases through the isoelectric region. The time of the frequency minimum is inversely proportional to the metabolic and growth rate. The inverse of this time, τ , therefore corresponds to the rate. The value of τ varies for different carbohydrate nutrients. Smaller values of τ are accompanied by a broader minimum and a slower approach to the minimum frequency, behavior which is internally consistent with slower metabolic rates. The observed nutrient profiles were consistent with the known metabolic requirements of this strain with lactose > mannitol > arabinose. Experiments performed in carbohydrate-deficient media did not afford a significant response.

CONCLUSIONS

The resonant frequency of composite resonators fabricated from AT-cut quartz crystals and cross-linked films of the amphoteric polymer 1 is very sensitive to the pH of the medium in which the resonator is immersed. While the frequency changes are not linear across the entire pH range,

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Figure 6. (upper) Frequency response of a 5-MHz AT-cut quartz resonator coated with a cross-linked polymer film of 1 to *E. coll* in different carbohydrates: lactose (---), mannitol (-), arabinose (- - -), no carbohydrate (----); polymer film thickness 0.8 μ m; cell concentration 10⁷ cells/mL; 1% carbohydrate nutrient in Bacto beef broth with beef extract. (lower) Relative frequency responses of the sensor in different carbohydrate nutrients based on τ , the inverse of the time for the response curve to reach the minimum value of f_s .

they are very large within two very narrow ranges, exceeding 5000 Hz for a change of 0.1 pH unit. Assuming a reasonable signal-to-noise requirement of 2.5 and a minimum detectable frequency change of 1 Hz, this sensor is capable of detecting

pH changes of 0.001 unit in this region. This property suggests that pH sensors based on this design are particularly well suited for detecting "threshold" pH changes that may be especially useful in process control such as fermentation, enzyme-based sensors, or microbiological applications. These films are very robust, exhibiting reproducible pH response to at least 25 cycles through the isoelectric region. The ability to shift the isoelectric region, and accordingly the threshold pH, by chemically altering the acid/base ratio in the polymer enables the sensor to be tailored for a particular application and pH range. Moreover, these studies illustrate that the resonant frequency of composite resonators comprising ATcut quartz crystals and swellable polymer films can be very sensitive to effects other than mass changes. In these cases caution must be exercized when the QCM response is interpreted so that viscoelastic effects are taken into account.

The unique properties of these polymer films suggest that modification of the polymer with specific receptors such as antibodies can provide composite resonators capable of specific binding to antigens. Phase transitions and corresponding changes in the viscoelastic properties of the polymer upon binding can afford frequency responses that are substantially greater than those expected from mass changes alone. In addition, the behavior described here for AT-cut quartz resonators is transferable to other piezoelectric transducers, such as shear horizontal plate mode³¹ and flexure mode devices.³² These strategies are likely to lead to piezoelectric sensors that are economical, robust, and widely applicable toward various applications.

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