

# Viscoelastic Characterization of High Concentration Antibody Formulations Using Quartz Crystal Microbalance with Dissipation Monitoring

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**ABSTRACT:** With increasing protein concentrations, therapeutic protein formulations are increasingly demonstrating significant deviations from ideal dilute solution behavior due to protein–protein interactions. These interactions lead to unique biophysical challenges in the administration of biopharmaceuticals including high apparent viscosity and viscoelasticity as well as challenges in maintaining the physical stability of proteins in solution. Here, we describe a straightforward analytical method to calculate the complex modulus and viscosity of high concentration protein solutions from measurements made using quartz crystal microbalance with dissipation monitoring (QCM-D). Further, this methodology was used to investigate the dependence of the storage and loss moduli ( $G'$  and  $G''$ , respectively) of a humanized monoclonal antibody solution on solution pH. Unlike recent reports, the effect of protein deposition onto the surface of the quartz sensor crystal was measured and explicitly accounted for during analysis when determining the solution's complex modulus. It was found that the ratio  $G''/G'$  was significantly greater than unity for all solutions investigated, but demonstrated a distinct maximum at pH 5.5 indicating that the solution exhibited the greatest liquid-like behavior at this pH. In addition, measurements were made at higher frequencies, which were found to be more sensitive to the changes in pH than those made at lower frequencies. It was also found that the viscoelastic ratio was relatively insensitive to the frequency of measurement at lower pH, but showed greater dependence on frequency as pH increased. The characterization of the rheological properties of high concentration antibody solutions provides insight into protein–protein interactions, and the methodology presented here demonstrates a straightforward way to determine the viscoelastic properties using ultrasonic rheology without the drawbacks of numerical fitting. © 2008 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci

**Keywords:** high concentration antibody formulations; ultrasonic rheology; quartz crystal microbalance with dissipation monitoring; QCM-D; storage modulus; loss modulus; tan delta; viscoelastic properties; protein adsorption; adsorbed protein films

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## INTRODUCTION

High concentration protein solutions are increasingly being used for delivery of drugs in the biopharmaceutical field. They typically contain protein concentrations in excess of 100 mg/mL<sup>1,2</sup>

and may be as high as 200 mg/mL or more. While it is anticipated that proteins can be formulated within this range, high concentrations of proteins are associated with unique challenges including an increased tendency to aggregate or form gels as well as increased difficulty in administering these solutions through standard subcutaneous syringe and needle configurations. These solutions are regularly characterized in terms of viscosity and can exhibit a dramatic increase in apparent viscosity with increasing concentration that exceeds the predicted behavior for a non-interacting colloidal system.<sup>1,3–8</sup> These solution characteristics are generally ascribed to the nonideality of the aqueous solutions and an increase in interactions between protein molecules. In high concentration protein solutions, these interactions can be assessed through the viscoelastic properties of the solution.<sup>9–11</sup>

Because of excluded volume effects as well as multiple protein–protein interactions, high concentration protein solutions do not behave as Newtonian fluids and exhibit viscoelastic character that depends strongly on the frequency of measurement. Recently, Kalonia and coworkers<sup>9,12,13</sup> have applied a custom-built ultrasonic shear rheometer (or piezoelectric quartz crystal microbalance, QCM) operating in the megahertz frequency regime to characterize the viscoelastic properties of high concentration antibody formulations. They have proposed the use of the solution storage modulus,  $G'$ , that is observed in this frequency regime as a parameter to characterize protein–protein interactions and as a possible predictor of protein physical stability (i.e., likelihood of aggregation) in protein formulation development.<sup>14</sup>

The complex modulus,  $G^*$ , is a quantity that is frequently used to characterize the rheological properties of viscoelastic materials under the application of oscillatory strain. The real component, the storage modulus ( $G$ ), is a measure of the energy stored within the material per oscillatory cycle while the imaginary component, the loss modulus ( $G''$ ), is a measure of the energy lost per cycle. Both of these parameters are governed by the molecular interactions within the material (e.g., through conformational rearrangements, frictional losses, etc.). However, these parameters are dependent on frequency, and their values capture the molecular processes that occur on time scales similar to the applied oscillatory strain. Alternatively, the complex viscosity can be used in a similar fashion and it is noted that the

complex modulus is proportional to the complex viscosity,  $\eta^*$ , through a constant involving the angular frequency,  $\omega = 2\pi f$ .

Saluja et al.,<sup>14</sup> argue that interactions between protein molecules in solution at high concentrations affect diffusion, conformational rearrangements, and segmental motion in the molecules, which have been shown to occur on timescales commensurate with the operating frequency of the QCM. Their work has shown a qualitative correlation between ultrasonic rheological measurements of high concentration solutions and traditional measures of protein–protein interactions made at low concentrations, such as the self diffusion coefficient and the second virial coefficient obtained from light scattering techniques.

As an alternative to the electrical impedance analysis methodology utilized by Kalonia and coworkers, we have employed the commercially available quartz crystal microbalance with dissipation monitoring (QCM-D) technique for rheological characterization of high concentration monoclonal antibody solutions. By employing a transient measurement principle, QCM-D enables rapid analysis of several higher order overtones of the fundamental resonant frequency and provides data at multiple frequencies in real-time. In this work, we present a straightforward method to calculate the complex modulus and viscosity of high concentration protein solutions from QCM-D measurements at multiple frequencies in the megahertz regime. In addition, this methodology was used to investigate the effect of differing solution pH values on the mechanical properties of high concentration solutions of a recombinant humanized monoclonal antibody. Through direct measurements of the deposited protein film, we explicitly account for the effect of any protein adsorption onto the sensor crystal surface, a process that to our knowledge has not been previously addressed for these types of measurements. The methodology does not require the use of iterative fitting of the measured parameters to viscoelastic models, which can be sensitive to the initialization of the fitted parameters. Finally, our results are discussed in the context of the viscoelastic ratio,  $G''/G'$ , and compared to results in the literature.

## QCM Background

The oscillatory motion of a resonating quartz crystal can be used to apply an oscillating strain to

a sample. The resonant frequency of a quartz crystal is determined by its mass and is significantly affected by the addition of mechanical loads. Kanazawa and Gordon<sup>15</sup> have shown that the change in the resonant frequency of a quartz disc when one side is loaded by a Newtonian liquid from the resonance frequency in vacuum is related to the viscosity and density of the liquid:

$$\Delta f_n = -n^{1/2} f_0^{3/2} \left( \frac{\rho_{\text{liq}} \eta_{\text{liq}}}{\pi \rho_q \mu_q} \right)^{1/2} \quad (1)$$

where  $\Delta f_n$  is the change in the resonant frequency at the  $n$ th harmonic,  $f_0$  is the fundamental resonant frequency of the unloaded crystal,  $\rho_q$  is the density of quartz,  $\mu_q$  is the piezoelectrically stiffened elastic modulus of quartz ( $2.947 \times 10^{10}$  Pa), and  $\rho_{\text{liq}}$  and  $\eta_{\text{liq}}$  represent the density and viscosity of the liquid, respectively.

The adsorption of material onto the surface of the quartz also alters its resonant frequency as the effective mass of the crystal increases. Sauerbrey<sup>16</sup> showed that the change in resonant frequency is linearly proportional to the areal mass density of the deposited material,  $\Delta m$ , provided that it is deposited as a thin, homogeneous film:

$$\Delta f_n = -2n f_0^2 \sqrt{\frac{\rho_q}{\mu_q}} \Delta m \quad (2)$$

Note that the Sauerbrey equation is only accurate for rigid, elastic films exhibiting no frictional energy losses. A convenient measure of the energy losses in the system is the dissipation factor, which is zero for Sauerbrey films and can be calculated by observing the decay in amplitude of a freely oscillating quartz crystal. A complete description of the dissipation factor measurement and QCM-D is provided elsewhere.<sup>17,18</sup> For purely viscous, Newtonian fluids, the change in dissipation factor relative to vacuum is given by:

$$\Delta D_n = 2n^{-1/2} \sqrt{\frac{f_0 \rho_{\text{liq}} \eta_{\text{liq}}}{\pi \rho_q \mu_q}} \quad (3)$$

For cases other than Newtonian fluids and rigid films (i.e., viscoelastic materials), several models are available that relate the mechanical properties of a sample to the measured response of the quartz resonator.<sup>19–23</sup> Depending on the physical configuration of the load, the complexity of the models varies considerably. The more general

ones require numerical fitting of the measured responses at multiple resonances to completely specify the rheological properties of multi-layered loads.

Because the electrical and mechanical properties are linked in a piezoelectric material, the electrical properties of quartz (as opposed to mechanical resonance) can be used to determine the viscoelastic properties (i.e.,  $G'$  and  $G''$ ) of the load. For this type of analysis, the electrical admittance of the quartz before and after loading the sample is measured as a function of frequency. The quartz and sample are then modeled as an equivalent electrical circuit where the numerical values associated with each circuit element (e.g., the resistance for resistors, capacitance for capacitors, and inductance for inductors) represent the values of the physical properties of the system (e.g., quartz density, sample moduli, etc.). For the case of a homogeneous sample uniformly in contact with one surface of the quartz crystal, Johansson and coworkers<sup>24,25</sup> have obtained the following expression to conveniently and concisely describe the relationship between the electrical measurements and the mechanical properties of the system:

$$\frac{\Delta \tilde{f}_n}{f_0} = \frac{1}{\pi Z_q} \frac{-1 + i}{\sqrt{2}} \sqrt{2\pi n f_0 \rho_{\text{liq}} (\eta' - i\eta'')} \quad (4)$$

In the above expression,  $Z_q$  is the acoustic impedance of quartz,  $\sqrt{\rho_q \mu_q}$ , and  $\eta'$  and  $\eta''$  represent the dynamic and out-of-phase viscosity of the sample, respectively.  $\Delta \tilde{f}_n$  is a complex quantity in which the real part,  $\Delta f_n$ , represents the change upon sample loading of the frequency at which electrical conductance is maximal; and the imaginary part,  $\Delta \Gamma_n$ , represents the bandwidth or half-maximal-half-width of the conductance versus frequency sweep. Thus, measurements of the electrical properties of the system can be used to determine the viscoelastic properties of the sample. Though the two are interchangeable through a constant, we have elected to report the viscoelastic properties in terms of complex modulus (i.e.,  $G^* = G' + iG''$ ) rather than complex viscosity (i.e.,  $\eta^* = \eta' - i\eta'' = iG^*/\omega$ ) for consistency.

Since QCM-D measures the changes in resonant frequency and dissipation factor rather than perform conductance-frequency sweeps, we used Eq. (4) to obtain the following simple analytical expressions to obtain  $G'$  and  $G''$  from QCM-D

measurements:

$$G' = \frac{\pi^2 \rho_q \mu_q}{f_0^2 \rho_{\text{liq}}} \left( \frac{n^2 f_0^2}{4} \Delta D_n^2 - \Delta f_n^2 \right) \quad (5a)$$

$$G'' = -\frac{\pi^2 n \rho_q \mu_q}{f_0 \rho_{\text{liq}}} \Delta f_n \Delta D_n \quad (5b)$$

We derived the above equations by noting that both  $\Gamma$  and  $D$  are measures of the energy losses in the system and are related through the following expression:<sup>24</sup>  $D = 2\Gamma/f_0$ . Note that both  $G'$  and  $G''$  are functions of frequency. Assuming that the load is small, it is possible to calculate the complex modulus of homogeneous viscoelastic solutions using the above equations by measuring the changes in the resonant frequency and dissipation factor when a sample is applied to an unloaded resonator.

## MATERIALS AND METHODS

A typical recombinant humanized monoclonal IgG<sub>2</sub> with  $\kappa$  light chains (mAb 1) cloned, expressed, and purified at Amgen, Inc. (Thousand Oaks, CA) was used for this study. A Chinese hamster ovary (CHO) cell line was used for expression of the antibody, and the subsequent purification was conducted by a series of anion, cation, and affinity chromatography steps. The antibody was formulated at 70 mg/mL in a 10 mM sodium acetate buffer solution at pH 5.2 containing 9% (w/v) sucrose. This formulation was frozen and stored at  $-20^\circ\text{C}$  until its use in this study. While the study was ongoing, this stock solution was stored at  $2-8^\circ\text{C}$ .

The stock solution was buffer exchanged into formulations with differing pH containing no sucrose using Amicon Ultra-15 centrifugal diafiltration units with 10 kDa MWCO membranes. Generally, 1 mL of the stock solution was diluted with 15 mL of the desired final buffer and concentrated back to 1 mL. This process was repeated twice to ensure complete exchange of the buffer and removal of sucrose. Sodium acetate was used as the buffering agent for solutions between pH 4.0–5.5 and MES was the buffering species for solutions of pH 6.2. Final protein concentration was measured by absorbance at 280 nm and adjusted to a target concentration of 70 mg/mL.

## Substrate Preparation

Gold-coated quartz sensor crystals with a 5 MHz fundamental resonance as well as those possessing a fundamental frequency of 10 MHz were purchased from Q-sense, Inc. (Glen Burnie, MD) and were stored in a solution of 0.1 M sodium dodecyl sulfate (SDS) when not in use. Prior to each run, the crystals were rinsed extensively with MilliQ 18.2 M $\Omega$  cm water. This was followed by a rinse with 200 proof ethanol before the crystals were dried under a stream of nitrogen. Organic contaminants were removed by exposing the crystal surface to UV-ozone for 15 min in a BioForce Nanosciences Procleaner<sup>TM</sup> UV/Ozone Cleaner (Ames, IA). Following UV-ozone treatment, the crystals were rinsed a final time with ethanol and dried under nitrogen before being mounted into the E4 flow modules, which facilitated the exchange of solutions above the surface of the quartz sensor crystal.

## QCM-D Measurements

QCM-D measurements were performed using a Q-sense E4 measurement platform fitted with QFM 401 flow modules. Qsoft version 1.4.4.130 software was used to record both the changes in resonant frequency ( $\Delta f_n$ ) and dissipation factor ( $\Delta D_n$ ) of the quartz sensor as a function of time at several crystal resonances. Resonant frequency and dissipation factor shifts for the third, fifth, seventh, ninth, and eleventh harmonics (i.e.,  $n = 3, 5, 7, 9,$  and  $11$ ) were collected for each sample at  $25 \pm 0.1^\circ\text{C}$ .

In general, after a stable baseline was established in water, approximately 350  $\mu\text{L}$  of the protein sample was introduced into the flow module at a flow rate of 50  $\mu\text{L}/\text{min}$  and allowed to remain in contact with the surface of the quartz until there was no further change in  $\Delta f_n$  and  $\Delta D_n$  with time ( $\sim 30$  min). This period of time allowed equilibrium to be established between protein in solution and protein adsorbed onto the surface of the sensor crystal. In order to enable the separation of the QCM-D responses due to the adsorbed protein film from that of the viscoelastic protein solution, the flow chamber was then flushed with water and the resultant changes in  $\Delta f_n$  and  $\Delta D_n$  were monitored.

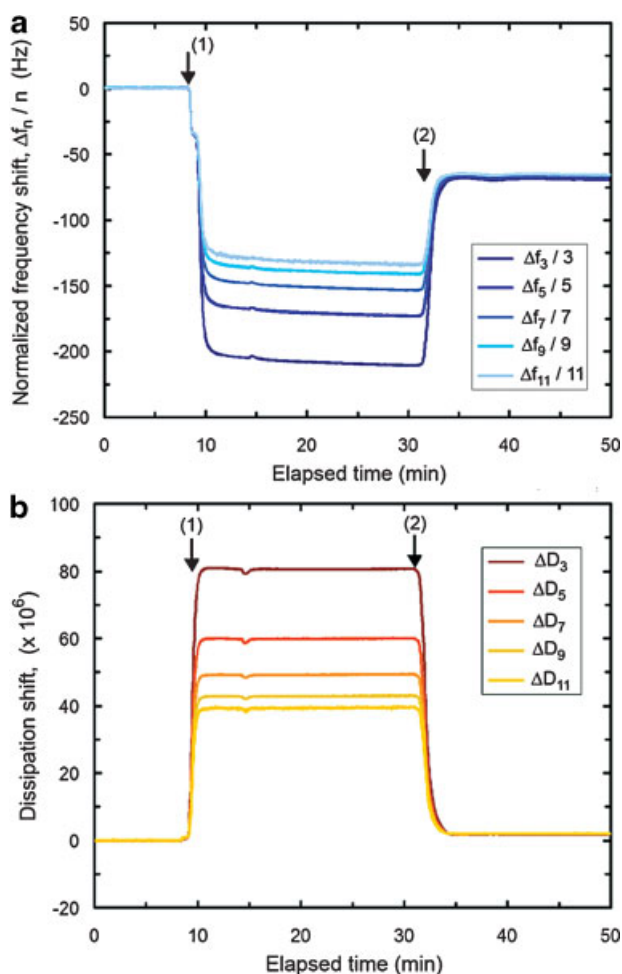
## Data Analysis Methodology

In this work, the viscoelastic properties of the solution (i.e., the storage modulus  $G'$  and loss modulus  $G''$ ) were obtained from the measured

QCM-D frequency and dissipation shifts through the application of Eq. (4). It is important to note that  $\Delta f$  and  $\Delta D$  terms represent the absolute change relative to vacuum. Thus, the theoretical changes due to water as given by the Kanazawa and Gordon result (Eqs. 2 and 3) were added to the measured changes in frequency and dissipation factor relative to water. The treatment accounting for the adsorbed protein film is described below.

## RESULTS AND DISCUSSION

Figure 1 presents the time course of a typical experiment and shows the changes in both resonant frequency and dissipation factor at each



**Figure 1.** The changes in (a) resonant frequency and (b) dissipation factor of a quartz sensor due to the (1) introduction and (2) subsequent purging of a 70 mg/mL solution of mAb 1 in 10 mM sodium acetate, pH 5.0 above the surface of a 5 MHz quartz sensor crystal initially equilibrated in water.

of the measured resonances when protein solution replaces MilliQ water above the surface of a clean, gold-coated quartz sensor crystal. After demonstrating a stable baseline in water, the protein sample was introduced into the flow chamber at approximately  $t = 10$  min. This resulted in an immediate change in both  $\Delta f_n$  and  $\Delta D_n$  at each of the resonances with higher overtones registering larger changes in resonant frequency and smaller changes in dissipation factor than lower order resonances. Note the data in Figure 1a are scaled by harmonic number to demonstrate that the initial changes in resonant frequencies did not scale linearly with  $n$ . Furthermore, a closer examination of the data also revealed that the changes in resonant frequency and dissipation factor do not scale as  $n^{1/2}$  and  $n^{-1/2}$ , respectively. This suggests that the observed changes are not attributable to simply differences in solution viscosity and density. Rather, a more significant change in the rheological properties of the solution and/or the adsorption of species in solution onto the surface of the sensor must account for the complexity of the measured changes.

To determine if the latter was occurring and to distinguish between these two effects, water was introduced at approximately  $t = 30$  min. This water rinse allowed the measurement of the adsorbed film in the absence of the protein solution. Upon the introduction of water, the resonant frequency at each harmonic increased to differing extents such that the net change in frequency from the water baseline scaled linearly with harmonic number. Concurrently, the net change in dissipation factor relative to the baseline became  $< 2 \times 10^{-6}$ . The presence of a finite net change in the resonant frequencies from the baseline values when the protein solution is replaced with water is strong evidence that solution properties alone do not account for the observed changes, and a significant amount of protein has adsorbed from the solution onto the crystal surface.

Since the frequency changes scaled linearly with harmonic number and the net changes in dissipation factor were small, the protein film was approximated as a Sauerbrey film. By applying Eq. (2), we estimated the protein surface density as  $1.17 \pm 0.02 \mu\text{g}/\text{cm}^2$  at pH 5.5. Assuming a protein density of  $1.38 \text{ g}/\text{mL}$ ,<sup>26</sup> this corresponds to a film  $8.5 \pm 0.1 \text{ nm}$  thick. The amount of adsorbed protein was similar for pH 5.0–6.0. However, while the amount of adsorbed protein at pH 4.0 was the same as that for pH 4.5, it was lower than

for the higher pH values ( $0.97 \pm 0.02 \mu\text{g}/\text{cm}^2$  at pH 4.0 and 4.5). This indicates that the interactions between the protein and gold surface change significantly between pH 4.5 and 5.0. It seems likely that the protein assumes a larger projected area on the surface at lower solution pH decreasing the areal density of protein in this pH range possibly due to the greater net charge on the molecule as the pH is moved further away from the pI of the protein (the pI was 7.4). The deposition is surface dependent and different surfaces and proteins may yield non-Sauerbrey behavior.

Because the protein film could be treated in a Sauerbrey manner in this case, the contributions of the solution properties to the QCM-D response can easily be separated from those of the adsorbed protein film by simply subtracting the shifts due to film in water from the shifts due to the presence of both the film and protein solution. It was assumed that the protein film was largely unchanged when the antibody solution above the crystal was replaced with MilliQ water. The resonant frequencies reached their values almost immediately upon the introduction of water and did not change over time, which indicated that the adsorbed protein film was stable and material was not desorbing from the surface. Thus, the following equations were used to determine the solution properties while accounting for the protein film:

$$G' = \frac{\pi^2 \rho_q \mu_q}{f_0^2 \rho_{\text{liq}}} \left[ \frac{n^2 f_0^2}{4} (\Delta D_n - \Delta D_n^{\text{film}})^2 - (\Delta f_n - \Delta f_n^{\text{film}})^2 \right] \quad (6a)$$

$$G'' = -\frac{\pi^2 n \rho_q \mu_q}{f_0 \rho_{\text{liq}}} (\Delta f_n - \Delta f_n^{\text{film}}) (\Delta D_n - \Delta D_n^{\text{film}}) \quad (6b)$$

In the above expressions,  $\Delta f_n$  and  $\Delta D_n$  represent the measured changes when the crystal is loaded with both the film and high concentration protein solution while  $\Delta f_n^{\text{film}}$  and  $\Delta D_n^{\text{film}}$  represent the changes measured from the baseline after the crystal has been rinsed with water.

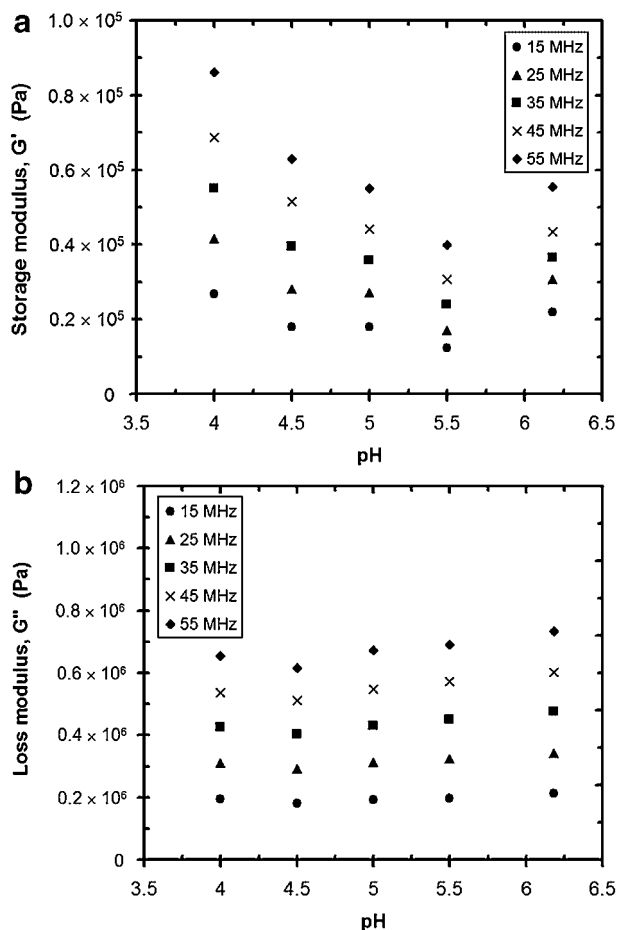
The ability to isolate the effect of the protein solution greatly simplified data analysis. Subtracting the contribution of the protein film allowed the system to be modeled as a quartz resonator operating in a homogeneous viscoelastic medium (i.e., in the absence of a mechanically distinct surface film). This approach allowed the use of Eqs. (6a) and (6b) to directly determine the viscoelastic properties of the solution instead of an iterative data fitting approach using a more

general model capable of modeling multilayered viscoelastic films. Caution must be exercised when utilizing this approach as the frequency shifts due to the film can only be subtracted when the film can be assumed to behave in a Sauerbrey manner (i.e.,  $\Delta D_n^{\text{film}}$  is negligibly small). For systems in which the protein film results in high net changes in dissipation factor and/or normalized frequency shifts ( $\Delta f_n/n$ ) dependent on harmonic number, the QCM-D response attributed to the film and to the viscoelastic solution are convoluted. A more general, multilayered model able to describe the presence of a viscoelastic protein film in addition to a viscoelastic solution must be used to determine the physical properties of such a system. Alternatively, several surface passivation methods exist<sup>27,28</sup> capable of minimizing protein adsorption though care must be taken to ensure the passivation layer does not significantly affect the dissipation factor of the crystal in water.

The storage and loss moduli calculated using Eq. (4) for each of the resonances monitored are plotted in Figure 2 as a function of antibody solution pH. Sodium acetate was used for the pH range from 4.5 to 5.5. However, due to limitations in the buffering capacity of acetate, MES buffer was used for pH 6.2. In all cases, the concentration of the buffering species was 10 mM. These formulations did not include polysorbate or sucrose in order to minimize any effects these excipients may have with interactions between the surface and the antibody.

It is important to note that the storage and loss moduli show a significant frequency dependence. Thus, it is not appropriate to model the responses at multiple harmonics using a single common value for  $G'$  and  $G''$ . The advantage of measuring multiple overtones to determine the unknown viscoelastic properties of multi-layered systems is only realized if it is assumed that the properties are relatively frequency independent.

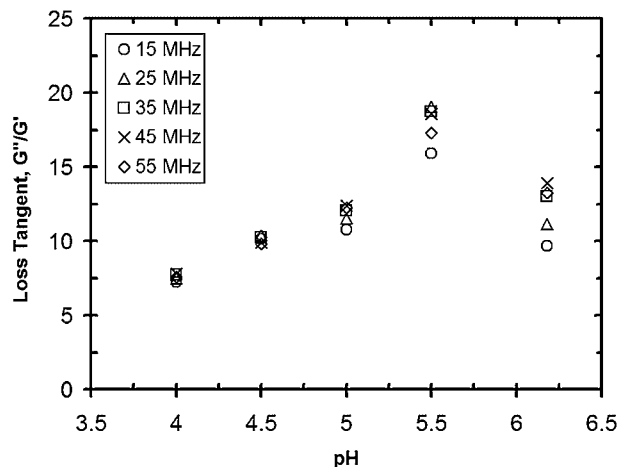
As shown in Figure 2a, the storage modulus,  $G'$ , showed a minimum at all the resonances at pH 5.5. On the other hand, the loss modulus,  $G''$ , at each of the measured resonances went through a minimum at pH 4.5 (Fig. 2b). In all cases, the values for  $G''$  were approximately one order of magnitude higher than for  $G'$ . The moduli calculated from measurements at the lower harmonics (i.e., lower frequencies) were lower in magnitude than those calculated from the higher order resonances. The storage modulus appeared more sensitive to changes in pH than the loss



**Figure 2.** (a) Storage and (b) loss moduli of 70 mg/mL solutions of mAb 1 at different pH calculated using QCM-D data from the 3rd, 5th, 7th, 9th, and 11th harmonics.  $G'$  shows a minimum at pH 5.5 with higher frequencies showing a sharper minimum, while  $G''$  shows a shallow minimum at pH 4.5.

modulus at each resonance with  $G'$  exhibiting a larger range of values than  $G''$  over the pH range examined. Further, the storage modulus was more sensitive to changes in pH at higher frequencies than at lower frequencies. Though the protein film was not measured by Saluja et al., this result corroborates their observation that a crystal with a fundamental resonance frequency of 10 MHz is more sensitive to changes in  $G'$  of high concentration antibody solutions than a crystal with a fundamental frequency of 5 MHz.<sup>9</sup>

The ratio of the loss modulus to the storage modulus,  $G''/G'$ , is a convenient way to describe the behavior of a viscoelastic material. In Figure 3, this viscoelastic ratio, also known as  $\tan(\delta)$ , is plotted as a function of solution pH and quartz resonance. Since  $G''$  is proportional to the

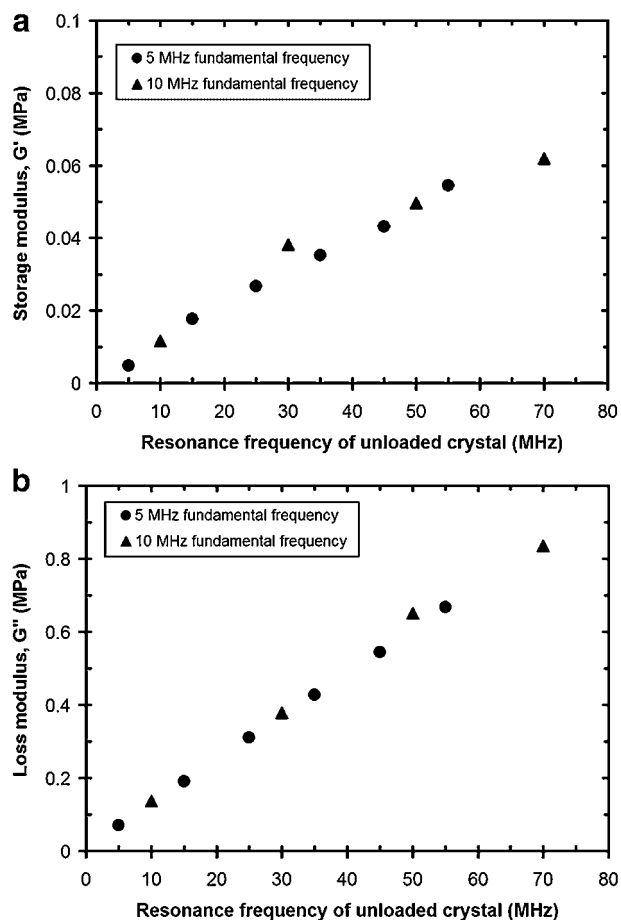


**Figure 3.** The loss tangent,  $G''/G'$ , shows a maximum at pH 5.5 indicating that this formulation behaves the most liquid-like over the frequency regime studied.

energy lost and  $G'$  is a measure of the energy stored per oscillation of the quartz crystal, a value greater than one indicates that the material behaves more as a liquid than as a solid. Indeed, over the pH range studied,  $\tan(\delta)$  for the protein solutions was always  $>1$ . This coincides with the observation that the solutions always appeared to be liquid and did not appear to form a gel. The value of the viscoelastic ratio at all of the resonances was largest at pH 5.5, indicating that this solution was the most liquid-like in character exhibiting the greatest amount of viscous energy losses compared with the energy stored during deformation.

As pH was increased,  $\tan(\delta)$  became a stronger function of frequency. Since dynamic rheological measurements probe molecular processes occurring on similar time scales as the frequency of measurement, this may indicate changes in protein-protein interactions and conformational rearrangements as well as rotational and translational diffusion, which occur on time scales of  $10^{-7}$ – $10^{-9}$  s.<sup>13</sup>

The frequency dependence of the  $G'$  and  $G''$  are presented in Figure 4 for the pH 5.0 solution. The data represent measurements made using two different sensor crystals. One had a fundamental resonance at 10 MHz and the other had a fundamental resonant frequency of 5 MHz. By measuring the QCM-D response of two crystals with differing fundamental resonant frequencies at multiple harmonics, it was possible to determine the values of  $G'$  and  $G''$  over a larger range of frequencies than would be possible with a single



**Figure 4.** The (a) storage and (b) loss moduli for a 70 mg/mL solution of mAb 1 in 10 mM sodium acetate at pH 5.0 is relatively linear with frequency of the sensor crystal.  $G''$  is roughly one order of magnitude larger than  $G'$ .

type of crystal. Over the range studied, both  $G'$  and  $G''$  increase approximately linearly with frequency. However,  $\tan(\delta)$  stays relatively constant suggesting that the overall qualitative behavior of the solution does not change with the frequency of applied strain over the range investigated at this solution condition. The larger range and higher resolution may have the potential to distinguish between molecular processes occurring on slightly different time scales.

## SUMMARY

In summary, a simple, analytical methodology to determine the storage and loss moduli of protein solutions using commercially available QCM-D

technology was provided. The adsorption of protein onto the surface of the sensor was measured and accounted for in the analysis. Using this method, the viscoelastic properties of an IgG<sub>2</sub> antibody showed a maximum in  $G''/G'$  at solution pH 5.5 at each of the resonances investigated, indicating the solution is most liquid-like at this pH. While differences were noted between these measurements and others reported in literature, the cause is ascribed to the difference in treatment with regard to the protein film as well as to differences in the molecules investigated. It was found that both  $G'$  and  $G''$  were dependent on frequency, but  $\tan(\delta)$  was relatively frequency independent at lower pH.

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