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Humidity scanning quartz crystal microbalance with dissipation monitoring setup for determination of sorption-desorption isotherms and rheological changes

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A new method to determine water sorption-desorption isotherms with high resolution in the complete range of water activities (relative humidities) is presented. The method is based on quartz crystal microbalance with dissipation monitoring (QCM-D). The QCM-D is equipped with a humidity module in which the sample film is kept in air with controlled humidity. The experimental setup allows for continuous scanning of the relative humidity from either dry to humid conditions or vice versa. The amount of water sorbed or desorbed from the sample is determined from the resonance frequencies of the coated quartz sensor, via analysis of the overtone dependence. In addition, the method allows for characterization of hydration induced changes of the rheological properties from the dissipation data, which is closely connected to the viscoelasticity of the film. The accuracy of the humidity scanning setup is confirmed in control experiments. Sorption-desorption isotherms of pig gastric mucin and lysozyme, obtained by the new method, show good agreement with previous results. Finally, we show that the deposition technique used to coat the quartz sensor influences the QCM-D data and how this issue can be used to obtain further information on the effect of hydration. In particular, we demonstrate that spin-coating represents an attractive alternative to obtain sorption-desorption isotherms, while drop-coating provides additional information on changes of the rheological properties during hydration. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4920919>]

I. INTRODUCTION

Quartz crystal microbalance (QCM) is an ultrasensitive method for mass determination of materials adsorbed on a piezoelectric quartz sensor. Traditionally, QCM has been employed as a weighing device in vacuum or gaseous environments according to the methodology described by Sauerbrey in 1959.¹ Further, this technique has been employed as (i) a tool to determine the dew point of water vapor at various temperatures,² (ii) a tool to estimate the enthalpy of sublimation of different molecules,³ (iii) a tool to detect deposition of atomic layers transported over the sensor in gas,⁴ and (iv) a humidity sensor.⁵ In particular, the special variation of this technique called the quartz crystal microbalance with dissipation monitoring (QCM-D) has been widely employed due to its capacity to provide additional information on the viscoelastic properties of the adsorbed material.⁶ Taken together, these studies illustrate the versatility of QCM-D (operated in gaseous atmosphere, which is also the case for the present work). In this work, we expand the versatility of QCM-D by presenting a novel methodology to investigate the influence of hydration and dehydration of thin films of various materials. The sample film is deposited on the sensor and kept in contact with a gaseous environment with controlled relative humidity (RH), while the QCM-D measures the mass of water that is either sorbed to or desorbed from the film. In other words, with

the present method, it is possible to determine water sorption-desorption isotherms.

Water sorption-desorption isotherms describe the relationship between the water content of a sample when in equilibrium at a given RH (or water activity, a_w) and constant temperature. Most often, sorption-desorption isotherms are determined by the isopiestic method where the sample is equilibrated at a given RH (usually set by saturated salt solutions with known water vapor pressure⁷) and the water mass measured gravimetrically after equilibration. Unfortunately, establishment of hydration levels in equilibrium is time demanding with the isopiestic method, possibly several days, which is likely due to the relatively large sample size needed (typically above 100 mg). Previously, QCM-D has been employed in a few cases to determine the water uptake at a few points of RH. Usually, this has been achieved by step-wise hydration at different RH levels by flowing close to saturated salt solutions through the humidity module⁸ or by flowing N₂ gas with defined amounts of water vapor above the sensor.⁹ However, to fully characterize the effect of hydration, it is beneficial to investigate a wide range of RH values due to that multiple transitions between different dynamical or structural states can occur when the system is subsequently hydrated or dehydrated. In other words, dynamical or structural transitions that occur between the investigated RH levels may go unnoticed in the step-wise hydration-dehydration approach. This important aspect is typically not considered in investigations performed merely on the dry and fully hydrated system or a few selected humidities only. Another significant aspect to

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consider is the potential occurrence of hysteresis between the sorption and desorption isotherms, which can provide additional information on how the properties of the system are influenced by hydration and dehydration. Recently, a humidity scanning (HS) QCM-D method was developed in our laboratory and successfully employed to obtain water sorption isotherms of lysozyme¹⁰ and starch films.¹¹ In the present work, we present further developments of this method, which allow for humidity scanning in both sorption and desorption modes by continuously adjusting the RH from either low to high humidities or in the opposite direction. In other words, the presented method takes into account the important aspects mentioned above and represents an appealing alternative to the conventional techniques for hydration studies, especially since the method gives complementary information on hydration induced changes of rheological properties during the sorption-desorption process. In addition to the new method, we also present new insights of the deposition technique used to coat the sensor and how the sample preparation influences the QCM-D hydration data. In particular, it is demonstrated that spin-coating is beneficially used to obtain sorption-desorption isotherms, while drop-coating provides additional information on changes of the rheological properties during hydration.

The outline of this paper will be as follows. First, we explain the QCM-D theory and data analysis. Then, we present the novel humidity scanning QCM-D setup that allows for continuous scanning of the RH in sorption or desorption mode. Finally, we evaluate the method by performing hydration and dehydration experiments on thin films of different biopolymers to obtain sorption-desorption isotherms and characterization of hydration induced rheological changes.

II. MATERIALS AND METHODS

A. Materials

Partially purified pig gastric mucin, PGM, type III (cat. no. M1778) and lysozyme from chicken egg white (cat. no. 62971) were purchased from Sigma Aldrich and used without further purification. All water used in this work was of ultrapure grade. Close to saturated LiCl solution was prepared from anhydrous LiCl (p.a. quality, Sigma Aldrich) by mixing excess amounts of LiCl in water for several days. The saturated solution was then filtered two times to remove excess LiCl salt. Thicknesses of dry films were calculated with density values of $\rho = 1.08 \text{ g cm}^{-3}$ and $\rho = 1.20 \text{ g cm}^{-3}$ for PGM⁸ and lysozyme,¹⁰ respectively.

B. QCM-D instrument and preparation of QCM-D sensors

A q-sense QCM-D E4 combined with the humidity module QHM 401 and AT-cut SiO₂ sensors (QSX 303, 5 MHz) from Biolin Scientific AB was used in this work. The humidity module was equipped with a Gore® membrane, which separates the flowing solution from the sensor. New sensors were washed with water and ethanol before use. Reused sensors were cleaned according to the guidelines described in the

q-sense manual (cleaning protocol B). The film was deposited on the sensor by either spin- or drop-coating from an aqueous solution. The optimal thickness for deposited films for determination of sorption-desorption isotherms is between ~ 200 and 1000 nm . The main reason for avoiding thin films is that mounting and remounting the sensor usually result in a small alteration of the resonance frequency due to differences in clamping of the sensor in the QCM-D module. This frequency change introduces an error in the determination of the areal mass of the dry film, which is more pronounced for thin films. To estimate this inaccuracy, we performed control experiments with the same sensor, which was mounted and remounted in between each experiment. The results are summarized in Fig. S1 of the supplementary material¹² and show that the error corresponds to about $\pm 20 \text{ Hz}$ (or below $\pm 3 \text{ nm}$ in film thickness). On the other hand, thick films are associated with unstable resonance signals and disappearing of overtones (especially at high levels of hydration).⁸ Therefore, to coat the sensors accordingly, we used the following procedures: for spin-coating, the sample was applied by adding $20 \mu\text{l}$ of aqueous solution and most often repeating the procedure 3-10 times in total during which the sensor was rotated at 1200 rpm (spin-coater developed in-house). The concentration of the aqueous solutions used for spin-coating varied between 2 and 20 wt. % (lower concentrations resulted in inappropriately thin films). In the case of drop-coating, $20 \mu\text{l}$ of solution was applied on the sensor and dried overnight. The concentration of the aqueous solutions used for drop-coating varied between 0.1 and 0.2 wt. %.⁸

C. QCM-D data analysis

1. Mass determination

The QCM-D technique operates by applying an oscillating potential field across a quartz crystal sensor, which induces an oscillating shear motion of the crystal. This oscillating motion generates an acoustic wave, and resonance condition occurs when the wave is an odd integer of the thickness of the quartz sensor. The resonance frequency is monitored and gives information on the adsorbed mass on the quartz crystal sensor. Under the assumptions that the material is rigidly adsorbed and homogeneously distributed over the active area of the sensor, and that the mass of the film is small relative to the mass of the quartz crystal, the Sauerbrey equation (Eq. (1)) can be used to calculate the mass of the adsorbed material,¹

$$\Delta f_n = -\frac{2f_0^2 m_f}{Z_q}. \quad (1)$$

The Sauerbrey equation (Eq. (1)) describes the proportionality between the negative frequency change, normalized per overtone, $\Delta f_n = \Delta f/n \text{ (Hz)}$, and areal film mass $m_f \text{ (kg m}^{-2}\text{)}$. In Eq. (1), Z_q is the acoustic impedance of quartz ($8.8 \times 10^6 \text{ kg m}^{-2} \text{ s}^{-1}$) and f_0 is the fundamental resonance frequency of the quartz sensor ($\approx 5 \text{ MHz}$). In many cases, the coating of the quartz sensor with a film leads to deviations from the Sauerbrey equation due to the rheological and/or surface properties of the applied film. In this work, the QCM-D data were analyzed according to the single viscoelastic film in

air model,¹³ which previously has been used for QCM-D hydration studies on pig gastric mucin,⁸ lysozyme,¹⁰ and starch films.¹¹ According to this model, the frequency change, normalized per overtone, corresponding to the total mass of the coated sensor m_f , is proportional to the overtone in square n^2 by the following relation:

$$\Delta f_n = -\frac{2f_0^2 m_f}{Z_q} \left(1 + \frac{Z_q^2 m_f^2 \pi^2 n^2}{3Z_f^2 m_q^2} \right), \quad (2)$$

where m_q is the areal mass density of quartz (kg m^{-2}) and Z_f is the acoustic impedance of the film ($\text{kg m}^{-2} \text{s}^{-1}$), while f_0 and Z_q have the same meaning as in Eq. (1). The total mass of the film can be derived from Eq. (2) by extrapolating the frequency shift to the zeroth overtone. For clarity, we modify Eq. (2) into Eq. (3) by introducing parameters $a = 2f_0^2/Z_q$ and $b = Z_q^2 \pi^2 / (3Z_f^2 m_q^2)$ according to

$$\Delta f_n = -am_f(1 + bm_f^2 n^2) = -am_f - abm_f^3 n^2. \quad (3)$$

Equation (3) gives a linear relationship between the frequency shifts, normalized per overtone, as a function of n^2 . Thus, the mass of the film m_f can be obtained by linear extrapolation to the intercept where n^2 approaches zero, at which $\Delta f_{n(n \rightarrow 0)} = -am_f$.

The water content of the film is quantitatively determined by first measuring the frequency change corresponding to the mass of the dry film. If the Sauerbrey equation is valid, it is expected that the normalized frequency shifts, from each overtone, are equal. In this case, the mass of the dry film can be calculated directly from Eq. (1) from the mean value of Δf_n . However, in some cases, Δf_n varies between the overtones and instead depends on the overtone number. For example, if Δf_n depends on the overtone number according to Eq. (3), it suggests that the dry film can be treated as a single viscoelastic film in air.¹³ A more complex situation arises when the overtone dependence deviates from either Eq. (1) or (3), which most likely occurs due to surface roughness of the dry film. This problem can be treated empirically by extrapolating Δf_n to zero overtone according to Eq. (3), but with the term n^2 replaced by n^β , where β is an exponent that is adjusted to give the best linear fit. The mass of the dry film is then determined in the same manner as described above, i.e., by extrapolating to zeroth overtone where $\Delta f_{n(n \rightarrow 0)} = -am_f$.

Next, the mass of water in the film is calculated from the dry mass and the total mass according to $m_{f,w} = m_{f,\text{total}} - m_{f,\text{dry}}$, while the water content X_w (wt. %) is given by $X_w/100\% = m_{f,w}/m_{f,\text{total}}$. In the described calculations, it should be noted that the normalized frequency change Δf_n in all cases is determined with respect to the uncoated sensor as reference state.

In the present work, we describe the films by their estimated thicknesses, which we believe is more illustrating as compared to their corresponding areal masses. The film thickness of the dry film d can be calculated from the areal mass of the dry film according to $d = m_f/\rho$, assuming that the density ρ of the dry sample is known. We stress that the value of the density used in this calculation does not influence the data analysis used to obtain sorption-desorption isotherms in any way (only the estimated film thickness).

2. Rheological characterization

In addition to the frequency change, the QCM-D technique also monitors the dissipation D , which is related to the decay time of the oscillating resonator when the alternating potential is turned off. The decay time is proportional to the energy dissipation of the quartz resonator and greatly depends on the viscoelastic properties of the film that coats the sensor. Thus, the dissipation gives information on the rheological properties of the film. The dissipation can also be quantified in terms of Γ , which is half the bandwidth at half maximum of the resonance peak and related to D according to $\Gamma = Df/2$. Additional information on the rheological properties of the film can be extracted by treating the data according to the model of a single viscoelastic film in air,¹³ as previously shown.^{8,10} A relevant starting point for this is to analyze the slope of Eq. (3), which corresponds to $\text{slope} = -abm_f^3$ and thus contains information on the rheological properties of the film (see definitions of parameter b above). More specifically, the slope is related to the acoustic impedance of the film Z_f , which in turn depends on the shear modulus G_f of the film according to $Z_f = \sqrt{\rho G_f} = \sqrt{\rho(G'_f + iG''_f)}$. Here, G'_f and G''_f are the storage and loss moduli, respectively, and ρ is the film density. In addition, the rheological properties of the film can be evaluated through the ratio between the storage and loss moduli, which describes the elastic behavior (G'_f) in relation to the viscous properties (G''_f) of the film.^{10,14} This is achieved by incorporating the complex frequency, which is defined as $\Delta \tilde{f} = f + i\Gamma$, where f is the real part and Γ is the imaginary part. Equation (2) can now be rewritten according to Eq. (4) (see supplementary material¹² for a more detailed derivation),

$$\frac{G'_f}{G''_f} = \frac{-\Delta f_n + \Delta f_{n(n \rightarrow 0)}}{\Delta \Gamma_n}. \quad (4)$$

In Eq. (4), the bandwidth, normalized per overtone, $\Delta \Gamma_n = \Delta \Gamma/n$, is defined as the difference between the hydrated film and the uncoated sensor, which is consistent with the corresponding values of Δf_n .

D. HS QCM-D

The sorption-desorption method is based on controlled dilution or concentration of a LiCl solution, to adjust the water activity, a_w , in a continuous manner. A schematic illustration of the setup is shown in Fig. 1 where bottle A contains a LiCl solution with continuously adjusted a_w . To ensure that the LiCl concentration is close to uniform in the entire volume of bottle A, the LiCl solution is magnetically stirred at 400 rpm. Bottle B contains either pure water (sorption mode) or close to saturated LiCl solution (desorption mode), while bottle C is used to collect the LiCl solution that has passed the QCM-D humidity module. The incoming and outgoing solutions are pumped with the same peristaltic pump. In sorption mode, the experiment starts with a close to saturated LiCl solution in bottle A, which is continuously diluted with incoming pure water. For desorption, the experiment starts with an appropriately diluted LiCl solution that is continuously concentrated with incoming close to saturated LiCl solution. To describe the working principles of the HS QCM-D method, we start by

FIG. 1. Schematic illustration of the HS QCM-D setup. The LiCl solution in bottle A is stirred at 400 rpm during the whole experiment to ensure close to uniform concentration.

accounting for the calculation of the a_w . Then, we describe our experimental approach to validate the calculations.

1. Calculation of the water activity

The a_w in the LiCl solution kept in bottle A is calculated from experimental data on a_w as a function of LiCl molal concentration.¹⁵ In turn, the molal concentration of the solution in bottle A can be derived by defining how the mass of LiCl salt (m_s) and the mass of water (m_w) change as a function of time according to Eqs. (5) and (6), respectively,

$$\frac{dm_s}{dt} = C_{s,in}r_{in} - C_{s,out}(t)r_{out}, \quad (5)$$

$$\frac{dm_w}{dt} = C_{w,in}r_{in} - C_{w,out}(t)r_{out}. \quad (6)$$

In Eqs. (5) and (6), r_{in} and r_{out} are the flow rates ($\text{cm}^3 \text{s}^{-1}$) of the incoming and outgoing solutions, respectively. The concentration is defined as $C = m/V$ (g cm^{-3}) where the volume of the solution V (cm^3) can be derived according to $V = m/\rho = (m_s + m_w)/\rho$, assuming that the density of the solution ρ is known. The incoming and outgoing concentrations can now be rewritten as

$$C_{i,in} = \frac{m_{i,in}}{V_{in}} = \frac{m_{i,in}}{m_{s,in} + m_{w,in}} \rho_{in} = X_{i,in} \rho_{in}, \quad (7)$$

$$C_{i,out}(t) = \frac{m_{i,out}(t)}{V_{out}(t)} = \frac{m_{i,out}(t)}{m_{s,out}(t) + m_{w,out}(t)} = X_{i,out}(t) \rho_{out}(t), \quad (8)$$

where i corresponds to either LiCl salt (s) or water (w) and X is the mass fraction. Equation (7) reflects the fact that the concentration of the incoming solution is constant (irrespective of time), while Eq. (8) shows that the concentration of the outgoing solution changes continuously during the sorption-desorption experiment. For clarity, we revise Eqs. (7) and (8) to describe how the mass of LiCl salt and water changes in the solution kept in bottle A according to the following:

$$\frac{dm_s}{dt} = X_{s,in} \rho_{in} r_{in} - X_{s,out}(t) \rho_{out}(t) r_{out}, \quad (9)$$

$$\frac{dm_w}{dt} = X_{w,in} \rho_{in} r_{in} - X_{w,out}(t) \rho_{out}(t) r_{out}. \quad (10)$$

Equations (9) and (10) can now be integrated into the final equations used for calculating the change of masses of LiCl salt (m_s) and water (m_w) as a function of time, which are given

by Eqs. (11) and (12), respectively,

$$m_s(t) = m_s^0 + \int_{t_p}^t [X_{s,in} \rho_{in} r_{in} - X_{s,out}(t) \rho_{out}(t) r_{out}] dt, \quad (11)$$

$$m_w(t) = m_w^0 + \int_{t_p}^t [X_{w,in} \rho_{in} r_{in} - X_{w,out}(t) \rho_{out}(t) r_{out}] dt. \quad (12)$$

In Eqs. (11) and (12), the integration starts from the initial conditions when the pump is turned on (t_p) and continues over time (t) as the experiment evolves. To utilize these equations for calculating the change of masses in the LiCl solution in bottle A, it is necessary to characterize the solution densities ρ_{in} and $\rho_{out}(t)$ and the two flow rates r_{in} and r_{out} . Because the incoming and outgoing solutions are transported in different tubes (cf. Fig. 1) and have constant or varying density, respectively, it is suitable to treat them separately. Starting with the former, the flow rate of the incoming solution, which in all cases has a constant and known density, can be determined by

$$r_{in} = \Delta V / \Delta t = \Delta m_{in} / (\rho_{in} \Delta t). \quad (13)$$

Here, Δt refers to the time interval during which solution is pumped from bottle B into bottle A (cf. Fig. 1), while Δm_{in} is the corresponding mass of the solution added to bottle A during this time period. Due to the variation of the density of the outgoing solution, it is not possible to calculate r_{out} in the same manner. Instead, we use the following iteration procedure. First, we numerically solve Eqs. (11) and (12) by assuming that r_{out} is equal to r_{in} . In this way, we acquire a first approximation of $m_s(t)$ and $m_w(t)$ and thus the molal composition of the outgoing LiCl solution as a function of time. Then, from the molal composition as a function of time, we can derive $\rho_{out}(t)$ for the outgoing LiCl solution. This is achieved by fitting the molal composition to experimental data on density of LiCl solutions as a function of its molal concentration at 25 °C.^{10,16} After calculating the variation of the solution density from this fitting procedure, we can finally calculate the flow rate r_{out} according to Eq. (14), which accounts for the fact that the density varies as a function of time,

$$r_{out} = \frac{\Delta m_{out}}{\int_{t_p}^{t_e} \rho_{out}(t) dt}. \quad (14)$$

The term $\rho_{out}(t)$ in Eq. (14) is integrated between the start (t_p) and end (t_e) points of the time interval during which LiCl solution is pumped out from bottle A towards the humidity module. The difference in mass Δm_{out} is the corresponding mass of the LiCl solution that has left bottle A during this time period. Subsequently, Eqs. (11) and (12) are solved again by employing the new values of $\rho_{out}(t)$ and r_{out} from the iteration procedure. The iteration is repeated until no significant change of these parameters occurs, which is true after three iterations. The data from the last iteration accurately describe how the molal composition of the LiCl solution changes over time (as demonstrated below). This allows for a precise determination of a_w in the LiCl solution of bottle A by fitting the LiCl molal composition to experimental data on a_w as a function of molal concentration.^{10,15}

2. Control experiments

To confirm that the calculated values of LiCl molality and a_w of the LiCl-solution were correct, we performed control experiments. The masses of LiCl salt and water in the final LiCl-solution, together with its total mass, were determined gravimetrically. For this, about 20 g of the LiCl solution was taken from bottle A (see Fig. 1) after ending the QCM-D experiment and loaded into a weighed glass flask. Next, the filled flask was weighed and dried at 150 °C for 24 h and finally weighed again. The masses of water and LiCl were then calculated from the loss of mass. The a_w of the final LiCl solution kept in bottle A (see Fig. 1) was measured with a NovaSina LabMaster- a_w apparatus at 25 °C. The instrument was calibrated with saturated salt solutions (SAL-T standards provided with the instrument) at 11.3%, 75.3%, 83.3%, and 97.3% RH (corresponding to saturated LiCl, NaCl, KCl, and K₂SO₄, respectively) before measurements. If the a_w of the LiCl solution was above 0.973 (i.e., out of the calibration range), the instrument was also calibrated with ultrapure water, which in theory has $a_w = 1$. The LiCl solution was allowed to come into thermal equilibrium and then a stable value was recorded for a minimum time period of 30 min. The procedures described above are routinely used to determine the precise molality and water activity of the LiCl solutions used in all experiments.

III. RESULTS AND DISCUSSION

A. Evaluation of the water activity calculation

A crucial factor in the calculation of the a_w is the determination of the LiCl molal concentration, from which the calculated values of a_w are based on. In this work and ongoing projects, the LiCl molality is routinely determined by gravimetric analysis and the relative standard deviation of this procedure is less than 0.1%. The calculated values of a_w can therefore be expected to fall within the same range of precision. To validate that the procedure to calculate a_w results in accurate values, we compared calculated data with experimental values of the initial and final composition of the LiCl solution, together with the measured a_w of the corresponding solutions. For this comparison, we measured a_w

at 25 °C and determined the composition by a gravimetric analysis of the initial and final LiCl solutions from sorption and desorption experiments. Representative results are shown in Fig. 2 from both (a) sorption and (b) desorption experiments. The calculated values of a_w are within the uncertainty ($a_w \pm 0.003$) of the instrument used to determine this parameter. The relative deviation between the calculated and the experimentally determined masses is below 0.5%. Thus, there is excellent agreement between the calculated values of a_w and the experimentally determined ones.

B. Practical procedures

The quantification of a_w in the sorption-desorption method relies on gravimetric determination of the ingoing and outgoing solution masses and that the corresponding time intervals of inward and outward flow are precisely established. Likewise, it is important that the initial molal concentrations of the LiCl solutions are accurately determined. For these reasons, it is suitable to clarify the experimental procedure by referring to the setup shown in Fig. 1 and a representative set of sorption-desorption data that clearly mark the relevant time events during the experiment. In the following text we describe the experimental procedure, from start to end, with respect to the sorption-desorption experiment presented in Fig. 3. Here, it should be pointed out that the method is very flexible in terms of hydration conditions that are desirable to investigate (i.e., sorption or desorption starting and ending at different levels of RH).

Fig. 3 shows a set of data from an experiment on a 132 ± 3 nm thick PGM film where the humidity was initially scanned from low to high values (sorption mode), followed by the opposite scan direction (desorption mode). Before starting the sorption experiment, it is required to determine the dry mass of the film. This is done by first measuring the resonance frequency of the uncoated sensor under the flow of N₂ gas. Next, the sensor is removed from the QCM-D module, coated with the sample, dried, and put back for measurement of the resonance frequency of the coated sensor under the flow of N₂ gas. Before the onset of the pump, the tubing from bottle B to bottle A (cf. Fig. 1) is filled with solution taken from bottle B, while the outgoing tubing from bottle A is kept empty. The experiment starts when the pump is turned on (cf.

FIG. 2. Comparison of calculated data on masses (left y-axis) and water activities (right y-axis) with experimentally determined masses and water activities of the initial (circles) and final (squares) LiCl solutions. Results in (a) are from a sorption experiment, while (b) shows results from a desorption experiment.

FIG. 3. HS QCM-D sorption-desorption experiment. The figure shows frequency shifts, normalized per overtone, as a function of time for a representative sorption-desorption experiment performed on a 132 ± 3 nm thick film of PGM (spin-coated with $3 \times 20 \mu\text{l}$ of 2.5 wt. % aqueous solution). The time region between A and B corresponds to sorption mode, while the time period between B and C corresponds to desorption mode. Note that Δf_n is virtually identical under dry conditions at the start and end of the experiment. Explanations: t_p is when the pump is turned on, t_0 corresponds to the time point when the solution enters the humidity module (resulting in a sharp frequency response), and t_e is the end of the sorption-desorption experiment when the pump is turned off, while s and d indicate sorption and desorption, respectively.

t_p in Fig. 3) so that the solution from bottle B starts to enter bottle A at the same time as solution starts to leave bottle A into the tubing leading to the humidity module. During the experiment, the continuously diluted, or concentrated, LiCl solution is flowing through the module and finally collected in bottle C. The experiment is terminated when the pump is turned off (cf. t_e in Fig. 3). To obtain the correct masses (i.e., Δm_{in} and Δm_{out}) at the end of the experiment, the solution in the tubing from bottle B to bottle A (cf. Fig. 1) is collected in bottle B, while the solution in the outgoing tubing from bottle A to bottle C, via the humidity module (cf. black tubing in Fig. 1), is collected in bottle C. Subsequently, the flow rate of the incoming solution r_{in} is determined from Eq. (13). In this equation, Δt refers to the time between the onset (t_p) and offset (t_e) of the pump, while Δm_{in} is the gravimetrically determined mass of the incoming solution during Δt (i.e., the mass loss of bottle B). The determination of the outgoing flow rate r_{out} from Eq. (14) is similar in that the term $\rho_{\text{sol,out}}(t)$ is integrated over the time interval between the onset (t_p) and the offset (t_e) of the pump, while Δm_{out} is the mass of the solution that has left bottle A during the corresponding time interval. Note that Δm_{out} should include the mass of the solution in the humidity module at the end of the experiment (the volume of humidity module is $120 \mu\text{l}$).

C. Deposition technique

Previous hydration studies with QCM-D from our laboratory have been performed on films that were drop-coated on the sensor.^{8,10,11} These studies suggest that the surface roughness of the drop-coated film affects the QCM-D hydration data.^{8,10,11} To explore this matter in more detail, we employed two different deposition techniques: drop-coating and spin-coating. The conclusion from these experiments is that spin-coating is more suitable for studies where the aim is to obtain water sorption-desorption isotherms. On the other hand, drop-coating is favorable to acquire data with additional information on rheological changes induced by hydration. We suggest that

the reason for this behavior is related to variations of the surface roughness due to the type of deposition technique employed. This difference in film distribution and surface roughness is clearly observed in optical microscopy (see Fig. S2¹²). In other words, spin-coated films are evenly distributed over the sensor as smooth films, while the distribution of drop-coated films is relatively irregular with more pronounced surface roughness. To investigate how this difference affects the dependence of Δf_n on overtone number, we performed multiple experiments where lysozyme films were deposited by either spin- or drop-coating, followed by QCM-D measurements at dry conditions. The results from these measurements are presented in Fig. S3¹² and clearly show differences between the two data sets. The data set corresponding to the spin-coated films shows that Δf_n is relatively constant, independent of overtone number, which is in agreement with the Sauerbrey equation that assumes homogeneous film distribution. This is in contrast to the data set corresponding to drop-coated films, which shows that Δf_n depends on the overtone number. In both cases, the lysozyme films are dry, rigidly adsorbed, and have similar thicknesses, which implies that the observed difference of Δf_n is related to different distributions of the lysozyme film on the QCM-D sensor.

Taken together, these aspects related to the type of deposition technique were taken into account in the present study where sorption-desorption isotherms are primarily obtained from experiments on spin-coated samples, while data corresponding to drop-coated samples are more demonstrative for hydration induced rheological changes. For comparison, we show this difference in data from measurements on spin- or drop-coated samples.

D. Water sorption-desorption isotherms

The HS QCM-D method was evaluated by performing measurements on protein films to obtain water sorption-desorption isotherms. For this purpose, we selected two different proteins, viz., PGM and hen lysozyme. PGM is a

glycoprotein consisting of a hydrophilic glycosylated protein core and terminal domains with segments of hydrophobic residues and low number of carbohydrate moieties.¹⁷ The effect of hydration of PGM has been investigated by means of AFM, differential scanning calorimetry (DSC), sorption calorimetry, and QCM-D^{8,14} and from these studies, it has suggested that PGM undergoes a glass transition around 60%–70% RH at 25 °C. Hen lysozyme, on the other hand, has globular shape and is perhaps the most common model protein.¹⁸ Hydration of lysozyme in the solid state (i.e., below the limit of full hydration) has been extensively studied.^{10,19–22} Based on these studies, it has been proposed that lysozyme undergoes a broad glass transition between roughly 67% and 91% RH, which is caused by an interplay between hydration induced structural and dynamical changes.¹⁰

Starting with PGM, Fig. 3 shows the normalized frequency shifts during a set of sorption-desorption experiments performed on a 132 ± 3 nm thick PGM film. A detailed evaluation of the data in Fig. 3 reveals that the hydrated film is behaving according to the viscoelastic film in air model with the overtone-normalized frequency Δf_n being linearly dependent on n^2 (cf. Eqs. (2) and (3)), which is illustrated in Figs. 4(a) and 4(b) for sorption and desorption modes, respectively.

In Fig. 4, the frequency shift, corresponding to the total mass of the film (i.e., mass of dry film plus mass of water), is given by the intercept of the regression lines at the zeroth overtone. The mass of the dry film was calculated according to Eq. (3) with the term n^2 replaced by n^β , where β was adjusted to give the best linear fit. This empirical approach to determine the dry film mass is justified in Fig. S4.¹² In brief, due to the fact that Δf_n , for the dry film, becomes more negative as a function of n , it is not appropriate to use the mean value of Δf_n in this case. In addition, the regression analysis is less adequate for $\beta = 2$ as compared to when β is adjusted to give the best fit.

The sorption-desorption isotherms, corresponding to the data in Figs. 3 and 4, are presented in Fig. 5, together with previously published results,¹⁴ obtained with sorption calorimetry, for comparison.

FIG. 5. Sorption and desorption isotherms at 25 °C of a 132 ± 3 nm thick film of PGM (spin-coated with $3 \times 20 \mu\text{l}$ of 2.5 wt. % aqueous solution, corresponding to the data in Figs. 3 and 4). Arrows give direction of the sorption and desorption traces. For comparison, the corresponding isotherm obtained by sorption calorimetry is included (dashed line, data from Ref. 14).

In Fig. 5, a first observation is that the sorption isotherms from QCM-D and sorption calorimetry are in good agreement, which confirms that the HS QCM-D method is accurate. In addition, the sorption and desorption isotherms are in agreement at high water activities, while a clear hysteresis between the isotherms is observed below $a_w \sim 0.75$. This value coincides with the upper limit of the glass transition water activity of PGM at 25 °C.^{8,14} These results can be rationalized by considering the dynamical properties of PGM above and below the glass transition water activity. Above this transition, the PGM polymers are in a flexible state, in which water diffusion is expected to be sufficiently fast to allow for equilibrium conditions. However, below $a_w \sim 0.75$, the PGM polymers are in a glassy state where water diffusion is more restricted, which acts to retard water desorption leading to higher water content in the desorption branch.

FIG. 4. Determination of film mass by extrapolation of Δf_n to zeroth overtone. Data show frequency shift normalized per overtone as a function of overtone in square during (a) sorption mode (a_w increases) and (b) desorption mode (a_w decreases). Experiments performed on a 132 ± 3 nm thick film of PGM (spin-coated with $3 \times 20 \mu\text{l}$ of 2.5 wt. % aqueous solution, corresponding to the data in Fig. 3). For clarity, only a minor part of the data set is shown.

FIG. 6. Sorption and desorption isotherms at 25 °C of a 439 ± 3 nm thick film of lysozyme (spin-coated with $5 \times 20 \mu\text{l}$ of 20 wt. % aqueous solution). The corresponding sorption-desorption isotherm from calorimetry is included for comparison (dashed lines, data from Ref. 23).

The results for water sorption-desorption of lysozyme are presented in Fig. 6, together with previously published data,²³ obtained by sorption-desorption calorimetry, for comparison. Again, the water content of the lysozyme film from the QCM-D experiment was calculated from Eq. (3) by extrapolating Δf_n versus n^2 to the zeroth overtone (see Fig. S5¹²). The data from the dry film showed insignificant overtone dependence (i.e., in accordance with the Sauerbrey equation) and the mass of the dry film was therefore calculated from the mean value of Δf_n according to Eq. (1) (cf. Fig. S5¹²). Fig. 6 shows that the HS QCM-D method provides results that are in good agreement with the corresponding data from calorimetry. In the case of lysozyme, the sorption-desorption hysteresis starts at a very high a_w where the water content is roughly 35 wt. %. The suggested reason for this is that above this concentration limit there is sufficient amount of water to fill the space between the protein molecules, which enables the globular protein to adopt its native structure.^{22,23} In other words, removal of water below approximately 35 wt. % results in distortion of the lysozyme molecular structure, which induces hysteresis.^{22,23} In addition,

the hysteresis at lower a_w is suggested to depend on slow kinetics of structural changes of the lysozyme molecule in combination with slow diffusion of water in the glassy lysozyme matrix leading to higher water content in the desorption isotherm.

E. Hydration induced rheological changes

A valuable feature of the presented QCM-D method is the possibility to obtain additional information on changes of rheological properties induced by the hydration process. This is achieved through analysis of the dissipation data, which is closely connected to the viscoelastic properties of the film. In other words, if the viscoelasticity of the sample film changes during the hydration process, this is expected to change the energy dissipation of the coated quartz resonator. Interestingly, large differences are observed between measurements performed on lysozyme films that were deposited by either spin- or drop-coating.

This feature is illustrated in Fig. 7 where the dissipation is plotted as a function of water activity for (a) spin- and (b) drop-coated lysozyme films. The dashed lines in Fig. 7 mark the a_w levels between which it has been established that changes, related to the glass transition, occur.^{10,21,23} The initial transition expected at $a_w \sim 0.67$ is not observed in the results in Fig. 7(a), which shows data from a spin-coated sample. In fact, in this case, the dissipation is close to zero up to the end point of the broad glass transition at $a_w \sim 0.91$, where the dissipation data start to increase (Fig. 7(a)). This is in contrast to the results in (b) from a drop-coated sample, which display a sharp increase around $a_w \sim 0.67$ and a second transition at $a_w \sim 0.91$, in agreement with previous data obtained from drop-coated lysozyme films.¹⁰ It should be noted that this clear difference in dissipation data, between spin- and drop-coated lysozyme films, is reproducible as confirmed in several experiments (see Fig. S6¹²). We suggest that in all cases (i.e., both for spin- and drop-coated samples), the lysozyme film is undergoing a glass transition, but due to differences of the surface roughness, the dissipation data are not the same for spin- and drop-coated films. In other words, the more irregular distribution of the drop-coated film is likely associated with peaks and valleys (cf. Fig. S2¹²), giving rise to a less stable

FIG. 7. Dissipation data as a function of water activity for (a) spin- and (b) drop-coated lysozyme films. The vertical dashed lines mark the water activities where changes related to the glass transition occur.^{10,21,23} Film thicknesses: (a) 158 ± 3 nm (spin-coated with $5 \times 20 \mu\text{l}$ of 10 wt. % aqueous solution) and (b) 189 ± 3 nm (drop-coated with $20 \mu\text{l}$ of 0.2 wt. % aqueous solution).

FIG. 8. G'/G'' as a function of water activity for (a) spin- and (b) drop-coated lysozyme films. The vertical dashed lines mark the water activities where changes related to the glass transition occur.^{10,21,23} Film thicknesses: (a) 158 ± 3 nm (spin-coated with $5 \times 20 \mu\text{l}$ of 10 wt. % aqueous solution) and (b) 189 ± 3 nm (drop-coated with $20 \mu\text{l}$ of 0.2 wt. % aqueous solution).

film structure that collapses at the on-set of the broad glass transition. This results in a large response in the dissipation data (see Fig. 7(b)). On the other hand, the structure of the more evenly distributed spin-coated film is less sensitive towards hydration induced rheological changes, which results in that the dissipation increases first after the glass transition is completed.

Additional information on changes of the viscoelastic properties is obtained by analyzing the QCM-D data according to Eq. (4), which gives the ratio of the storage and loss moduli G'_f/G''_f . For rigid films, in the glassy state, G'_f/G''_f is expected to take on high values due to dominance of the elastic behavior, while soft films give rise to values of the G'_f/G''_f ratio close to zero due to dominance of viscous-like properties.^{8,10} Fig. 8 shows the results from this analysis on the data corresponding to the experiments in Fig. 7. The results from the spin-coated sample (Fig. 8(a)) show that the G'_f/G''_f ratio decreases between $a_w \sim 0.67$ and $a_w \sim 0.91$ (between the dashed lines), implying that the elastic properties of the film decrease at the expense of viscous properties during the glass transition. The data in Fig. 8(a) are, however, in contrast to the sharp transitions observed in the data from the drop-coated sample (Fig. 8(b)). The clear changes of the G'_f/G''_f ratio at $a_w \sim 0.67$ and $a_w \sim 0.91$ are in agreement with the dissipation data and consistent with hydration induced changes of the viscoelastic properties of the film from a rigid glassy state to a more fluid-like state (cf. Fig. 7(b)).

Finally, it should be noted that the viscoelastic film in air model¹³ implies that the slope of the linear extrapolation according to Eq. (3) contains information on the rheological properties of the film via the shear modulus of the film, G_f . This is confirmed by Fig. S7,¹² which shows the slope as a function of water activity for the data corresponding to the experiments in Figs. 7 and 8.

Taken together, the implication of the lysozyme results obtained from the two different deposition techniques (Figs. 7 and 8) is that drop-coated samples are to prefer if the primary aim is to investigate hydration induced changes of viscoelastic properties. On the other hand, lysozyme samples prepared by spin-coating are more suitable if the purpose is to obtain sorption-desorption isotherms (cf. Fig. S8¹²).

IV. CONCLUSIONS

In this work, we have presented a novel HS QCM-D method for determination of sorption-desorption isotherms and investigations of hydration induced changes of rheological properties of thin films. The HS QCM-D is equipped with a humidity module in which the sample film is kept in air with controlled RH (water activity). The experimental setup allows for continuous scanning of the water activity from either dry to humid conditions or vice versa. The method is validated by control experiments confirming that the water activity is regulated with precision and accuracy in both sorption and desorption modes. Subsequently, the methodology is proven to be sound by obtaining sorption-desorption isotherms of spin-coated biopolymer samples that are in good agreement with previously published results.

A major advantage of the present method is that it is possible to acquire insights of hydration induced rheological changes of the film. As shown, a complete characterization of hydration induced changes of the viscoelastic properties should consider a combination of data on dissipation, frequency, and G'_f/G''_f . In particular, this characterization is advantageously performed on data from drop-coated samples.

Finally, we emphasize that with the presented method, it is possible to obtain high-resolution sorption-desorption isotherms in short time using small amounts of material. For example, the isotherms presented in Figs. 5 and 6 required approximately $20 \mu\text{g}$ of sample material in the coating procedure.

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