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Determination of Swelling of Responsive Gels with Nanometer Resolution. Fiber-Optic Based Platform for Hydrogels as Signal Transducers

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A novel technique for detection of hydrogel swelling intended for use as a chemical or biological sensor, but also generally applicable for obtaining high-precision hydrogel swelling data, is described. The underlying design principle is that a hydrogel bound to the tip of an optical fiber constituting the environmental sensing element makes up a Fabry−Perot cavity for high-resolution detection of the optical length. The interference of light guided by the optical fiber and reflected at the two interfaces, fiber−gel and gel−solution, enables optical detection of the optical path length within the gel and degree of swelling of the gel. Acrylamide-based hydrogels with various molar fractions of the cationic monomer, *N*-(3-dimethylaminopropyl)acrylamide, were fabricated at the end of the fiber to demonstrate the feasibility of the approach. These sensors were investigated in solutions of varying ionic strength and pH. Relative gel length changes of the ∼50-μm half-spherical gels were determined with a precision of ∼2 nm. Moreover, the combination of good reproducibility and resolution of determination of swelling supports measurements of ionic strength changes in the millimolar range. Kinetic measurements for gel swelling induced by changes in ionic strengths had a time constant of ∼2 s (half-spherical gel with 60-μm radius), whereas the time constants for gel swelling induced by changes in pH were observed in the range 90−130 s. Thus, different processes dictate the swelling rate in the two different cases. The results show that hydrogel equilibrium swelling and kinetics can be determined by the optical interference method with nanometer resolution, thus providing a unique platform for characterization of hydrogels swelling in general, and using functionalized hydrogels as biological sensors in particular.

Hydrogels are three-dimensional networks that are formed by cross-linking hydrophilic polymers capable of imbibing large amounts of water. Over the past few decades, hydrogels have been subject to intense investigation due to their ability to undergo volume change in environmental changes including pH,[1–4] temperature,[5,6] ionic strength,[1,2,7,8] electric fields,[9] and surfactants.[10] This has given these materials potential applications in a variety of fields such as controlled drug release,[11,12] wound dressing,[13] and molecular separations.[14]

Traditionally, hydrogel swelling has been detected either optically, i.e., by imaging using a light microscope, or simply by weighing the water content in the gel. More accurate detection methods have been developed over the years for incorporating hydrogels into sensors such as conductometric,[15] liquid column length,[16] or optical sensing.[17–19] Of the optical sensors, inverse opal hydrogels have proven to be both popular and promising.[17,20] This technique is based upon diffraction from crystals within the hydrogel or particles deposited on the gel surface. As the gel swells or shrinks, the diffracted wavelength changes allowing sensitive measurements for small variations in the swelling degree.[17] The evanescent mode configuration is another employed sensing scheme.[19] Here a gel is deposited around the core of an optical fiber, and volume changes in the gel induce microbends in the fiber, which in turn are measured by the transmitted optical intensity.[19]

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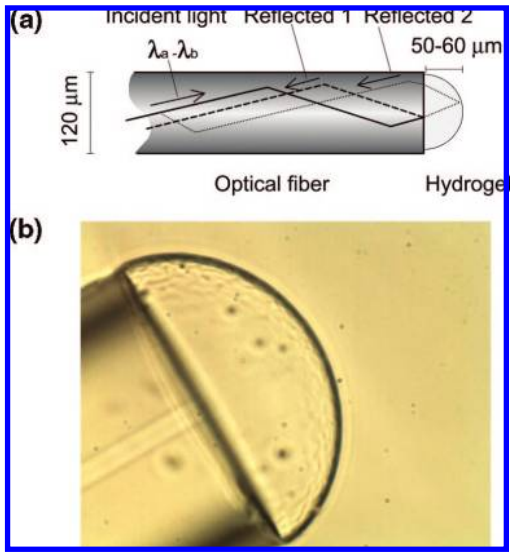

Figure 1. (a) Schematic illustration of the instrumental technique. The incident light (wavelength range λ_a–λ_b from 1530 to 1560 nm) sent through the optical fiber (diameter 120 μm) is reflected at both the fiber–gel (reflected 1) and the gel–solution (reflected 2) interfaces. The interference wave is the basis for the measuring technique. The hydrogel covalently linked to the end of the optical fiber adopts a near half-spherical geometry with radius of the order 50–60 μm. (b) Micrograph of a polymerized gel attached to a fiber. The gel adopts a near half-spherical form.

In this paper, we present a novel instrument developed by InvivoSense (formerly Optomed)[21] utilizing an interferometric technique to measure gel swelling. More specifically, a hydrogel is chemically bound to the end of an optical fiber and makes up a Fabry–Perot cavity, which optical length can be determined with high precision. Light is sent through the fiber; light reflected from the fiber–gel and the gel–solution interfaces forms an interference wave from which the optical length of the gel can be determined (Figure 1). The resolution of the method is one of the most superior techniques so far applied for the study of swelling of supported hydrogels. The sensitivity of the responsive hydrogel platform by this interferometric readout is demonstrated by determination of gel equilibrium length and swelling kinetics of an acrylamide-based hydrogel. Acrylamide-based hydrogels with a cationic monomer *N*-(3-dimethylaminopropyl)acrylamide were prepared as supported half-spherical probes attached to the end of the waveguide. These supported gels were investigated in salt solutions of varying concentrations, and also the swelling degree has been examined as a function of pH.

BACKGROUND: EQUILIBRIUM GEL SWELLING AND SWELLING KINETICS

A brief account of the basic theory for hydrogel equilibrium swelling and swelling kinetics is provided as a background for the relation to the interferometric technique for determination of gel length/volume parameters. For an unsupported hydrogel in an excess of solvent, the equilibrium swelling volume is determined by equal chemical potential μ_l of the diffusible components outside (superscript s) and inside (superscript g) the swollen polymer network:[22,23]

$$\mu_l^s = \mu_l^g \tag{1}$$

This can alternatively be expressed by zero osmotic pressure (Π) due to the fact that the osmotic pressure equals the derivative of the chemical potential with respect to the gel volume. The osmotic pressure of an ionic hydrogel is assumed to consist of three additive contributions in the simplest form of the theory. These are due to (1) the free energy of mixing of the polymer with the solvent, Π_{mix}, (2) the elastic retractive force, Π_{el}, and (3) the difference in mobile ion concentration between the inside and outside of the gel, Π_{ion}. Collectively the total osmotic pressure can then be given as

$$\Pi_{mix} + \Pi_{el} + \Pi_{ion} = \frac{RT}{V_1}(\ln\phi_1 + \phi_2 + \chi\phi_2^2) + \frac{\upsilon RT}{V_0}\left(\frac{\phi_2}{2\phi_{2,0}} - \left(\frac{\phi_2}{\phi_{2,0}}\right)^{1/3}\right) + RT\Delta C_{tot} \tag{2}$$

Here subscripts 1 and 2 of the volume fractions ϕ are used to denote the solvent and polymer phase respectively. V_1 is the molar volume of the solvent, υ is the molar number of elastic active polymer chains in the gel at the reference volume fraction $\phi_{2,0}$, V_0 is the gel volume for the reference state, R is the molar gas constant, T is the absolute temperature, and χ is the Flory–Huggins interaction parameter. The total difference in molar concentration of mobile ions between the gel and the surrounding aqueos solution, ΔC_{tot}, is for 1:1 valence electrolytes given by[8]

$$\Delta C_{tot} = 2\left[c_s - \sqrt{c_s^2 + \left(\frac{\rho\phi_2}{2M_2}\right)^2}\right] \tag{3}$$

where c_s is the external molar salt concentration, ρ the density of the dry polymer, and M_2 is the molar mass of the polymer per free counterion. Elaboration of this simplest form of the theory to include finite extensibility of the elastically active networks chains, and swelling in aqueous solutions with electrolytes of various valencies, including a description of effects associated with counterion condensation, is described.[8]

A brief account of the theoretical model of kinetics of swelling of a polymer gel network developed by Tanaka and co-workers[25] is presented in the following to provide a first-order approximation for analyzing the corresponding experimental data. The equation of motion for the swelling process of a spherical symmetric polymer gel network was derived to be

$$\frac{\partial u}{\partial t} = D\frac{\partial}{\partial r}\left\{\frac{1}{r^2}\left[\frac{\partial}{\partial r}(r^2 u)\right]\right\} \tag{4}$$

In eq 4, u is the displacement from its final equilibrium position ($u(t \to \infty) = 0$), D the gel diffusion coefficient, and r the radius

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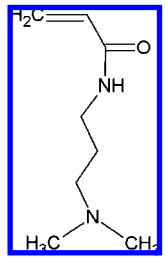
Scheme 1. Chemical Structure of the Cationic Monomer *N*-(3-Dimethylaminopropyl) Acrylamide (DMAPAA) Used as a Comonomer in the DMAPAA−Acrylamide−Bisacrylamide Hydrogels



within the spherical coordinate system. This equation was solved using Fourier series yielding

$$a(t) = a - \left(\frac{6}{\pi^2}\right)\Delta a_0 \sum_{n=1}^{\infty}\left(\frac{1}{n^2}\exp(-n^2 t/\tau)\right) \quad (5)$$

where the characteristic swelling time τ is given by the equilibrium gel radius a and the diffusion coefficient of the gel D:

$$\tau = \frac{a^2}{\pi^2 D} \quad (6)$$

Parameter Δa_0 (eq 5) is the total radius change in the actual swelling process; i.e., the swelling starts from an initial radius $a-\Delta a_0$ and attains the new equilibrium with radius a.

MATERIALS AND METHODS

Materials. The various materials were obtained from the various companies as specified in the following: acrylamide (99%, Sigma), (AAM), *N*,*N*-methylene-bisacrylamide (99%, Acros organics), (Bis), 1-hydroxycyclohexylphenylketone (99%, Aldrich), ethylene glycol (Acros Organics, >99,9%), 3-(trimethoxysilyl)propyl methacrylate (Sigma, >98%), *N*-(3-dimethylaminopropyl)acrylamide (TCI Europe NV) (DMAPAA, Scheme 1), sodium chloride (99%), sodium hydroxide (99%, Merck), hydrochloric acid (37%, Merck), water (resistivity 18.2 MΩ/cm using a Millipore setup), sodium hydrogen phosphate monohydrate (Merck, P.A), and disodium hydrogen phosphate dehydrate (Merck, P.A). An aqueous 30 wt % acrylamide stock solution in 1 mM phosphate, pH 6.7, buffer was prepared and used as the aqueous solvent for all syntheses. A stock solution of Bis was prepared in the same buffer, and both stock solutions were degassed with nitrogen.

Gels. The synthesized gels were AAM based, with Bis as a cross-linker (3 mol % relative to AAM), and the cationic monomer DMAPAA with added to a mole percent concentration relative to AAM of 0, 2, 3, 5, and 7 mol %, respectively, of the various gels. In all cases, the monomer to solvent ratio was held constant (15 wt % monomer) as was the photoinitiator (1-hydroxycyclohexylphenylketone) to monomer ratio (0.125 mol % to monomer).

EQUIPMENT AND INSTRUMENTATION

Detection. The equilibrium gel swelling and kinetics of swelling was determined using an interferometric technique (Figure 1). The fabricated gel at the tip of the optical waveguide adopts a near half-spherical geometry. A change in the equilibrium gel volume, expressed in terms of the volume fraction of the gel ($\varphi_2^{1/3}$) results in a change in the optical path length l_{opt} inside the gel. Additionally, the change in the volume fraction of the polymer yields a change in concentration with concomitant change in the optical properties of the material, i.e. the refractive index of the gel, n_{gel}. This requires that the physical length of the gel, l is calculated from the detected optical length:

$$\Delta l_{opt} = \Delta l n_{gel} + l\Delta n_{gel} \quad (7)$$

The interferometric measurement is carried out using a tunable source based on a superfluorescent Er-doped optical fiber source covering the wavelength range $\lambda_a-\lambda_b$ = 1530−1560 nm. The optical signal reflected from the hydrogel sensor is digitized and normalized by the optical source spectrum to yield an interference signal with constant amplitude. The optical length within the hydrogel, l_{opt}, is related to the frequency difference between two neighboring frequency peaks in the interference spectrum, Δf:

$$l n_{gel} = l_{opt} = \frac{c}{2\Delta f} \quad (8)$$

where l is the physical length of the hydrogel and c is the speed of light. The dynamic length change (relative length) is monitored by measuring the phase of the interference signal. The change in the optical path length Δl_{opt} within the gel is calculated from change in the phase of the interference wave, $\Delta\varphi$, as

$$\Delta l_{opt} = \Delta\varphi\lambda_0/4\pi \quad (9)$$

where λ_0 is the center wavelength of the light source. Both the dynamic length change and the length are extracted from reflected signal in real time.

Conversion of the optical length increments due to changes in physical length of the gels due to changing conditions of the solvent was based on eq 7. The following Taylor series expansion for the effect of changes in ionic strength and gel concentration due to various swelling degrees on the refractive index of the gel was used as the basis:

$$n_{gel} = n_w + \frac{\partial n}{\partial c_s}\Delta c_s + \frac{\partial n}{\partial c_p}\Delta c_p \quad (10)$$

In eq 10, n_w is the refractive index of water, $(\partial n/\partial c_s)$ and $(\partial n/\partial c_p)$ are the refractive index increments of the gel phase due to salt and polymer, respectively, and Δc_s and Δc_p are the concentration difference in salt and polymer, respectively. Equation 10 was applied in conversion of the experimentally determined Δl_{opt} (eq 9) to the corresponding changes in physical length of the gel, Δl, according to eq 7 starting from the reference condition for making the gel with a known polymer concentration and ionic strength and using incremental changes due to ionic strengths and associated change in polymer gel concentration due swelling to recalculate n_{gel} (eq 10). The parameter values of $(\partial n/\partial c_s)$ = 0.008 8077 mol^{-1}, [26] and $(\partial n/\partial c_p)$ = 0.165 mL/g = 1.17×10^{-2} mol^{-1},[27] were employed, and a hemispherical model of the

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swelling gel geometry was applied as a first approximation for the estimation of the polymer gel concentration at other swelling states than the reference.

Instrument Setup. The instrument setup consists of an optical fiber with a gel chemically bound at one end and a connector/adaptor system at the other (connector: FOC2 STD-A600, Huber + Suhner fiber optics. Adaptor: FOC2 FOC2-D001, Huber + Suhner fiber optics). An optical cable (108163/02 Suhner Fiberoptic) was attached to the detector, which was controlled by a computer. A LabView program (implemented by InvivoSense) was used for instrument readout. The phase, amplitude, and dc signal of the reflected light signal are determined and used as the basis for deriving changes in the optical length of the gel cavity. The changes in the relative length derived from the phase information (eq 9) are the most precise and, therefore, used as the main basis for the characterization of the swelling of gels attached to the optical fibers.

Fiber Preparation. First the fiber was stripped of the jacket, cleaned with 96% ethanol, and cut (Fitel model S323, Furukawa Electric Co. Ltd.). Next the fiber was prepared for silanization.[28] This consisted of dipping the fiber in a solution of NaOH (1 M) for 20 min to remove impurities on the surface and then rinsing with MQ water. The fiber was immersed in a solution of 0.01 M HCl to activate the surface for silanization and finally soaked in a solution of 3-(trimethoxysilyl)propyl methacrylate (0.02 M, nitrogen-purged MQ water pH 3.5) for 1 h, chemically binding the methacrylate groups to the fiber tip.

Sensor Fabrication. The appropriate amounts of monomers in the two stock solutions (AAM and Bis) were mixed together with the buffer (phosphate buffer 1 mM, pH 6.7), and then DMAPAA was added. Then the initiator was dissolved in ethylene glycol (0.1 M) and added to a concentration 0.125 mol % relative to the amount of monomer, yielding the pregel solution. The optical fiber with surface-bound methacrylate groups was placed in a small plexiglass chamber where a mixture of wet and dry nitrogen, adjusted by flowmeters, was flushed through the chamber. The flow of the wet and dry nitrogen was used to adjust the humidity to maintain constant size of the polymer gel droplet during the polymerization. A drop of the prepolymer solution was guided to the fiber by pipet, and due to surface tension, a half-spherical drop was formed following deposition of the droplet on the end of the fiber. This was carried out while the fiber and pipet were observed using an optical stereomicroscope. Photoinitiation was used to start the gelation reaction by exposing the pregel solution to UV light for the duration of 90 s (Dymax Bluewave 50 equipped with a light guide). Finally the gel was washed in the aqueous buffer (1 mM phosphate buffer, pH 6.7) for at least one day to eliminate unreacted monomers and other impurities. Micrographs of the gels connected to the fiber were obtained employing an Olympus IX70 inverted optical microscope equipped with a Motic Moticam 1000, 1.3-megapixel CCD camera.

Measurements. For the measurements of hydrogel swelling, the half-spherical gel at the end of the optical fiber was immersed into the buffer solution of an appropriate volume where the desired amounts of the pH/saline solution were pipetted into the solution. The gel was left until equilibrium was reached, manifested by a constant phase of the interference wave as monitored by the software. After each experiment, the gels were washed (until constant phase) before repeating the gel swelling experiments. All experiments were carried out at room temperature with the gels at the fiber tip immersed in the aqueous solution under constant agitation using a magnetic stirrer.

The determination of gel swelling dynamics and equilibrium as a function of ionic strength at constant pH were carried out for stepwise increases of the ionic strength starting at 0.01 M by adding fixed amounts of a 2 M NaCl stock solution. For the decreasing ionic strength experiments, the experiments started at an ionic strength of 0.3 M, and the buffer solution was added to stepwise decrease the salt concentrations. A similar procedure was also employed for the pH measurements where a 0.1 M solution of acid/base was added to the base solution, which was a 0.050 M NaCl solution in this case. The experiments were performed in the pH interval ~5−~9.6, and in contrast to the saline measurements, varying amounts of the acid/base solution were added instead of a fixed amount. The pH was controlled by PHM92 laboratory pH meter immersed in the solution during the entire experiment. Furthermore, the ionic strength was monitored with a pH−electrical conductivity 340i (WTW) meter. The conductivity was held constant throughout the experiment, ensuring that only the pH effect was measured.

RESULTS AND DISCUSSION

Sensor Fabrication. Following the 90 s of UV exposure to initiate the cross-linking polymerization reaction, the sensors were immediately immersed into solution giving an indication of whether the polymerization reaction was completed: If the phase of the reflected light remained constant, the reaction was assumed to be complete, whereas for gels that were not completely polymerized, the phase fluctuated erratically. For the gels investigated here, a constant phase was seen following the UV exposure, although there was an initial change in the phase of the reflected interference wave for the ionic gels due the excess osmotic pressure of the gel. This latter effect was larger for the gels with higher fraction of ionic groups. The excess osmotic pressure arising from the polyelectrolyte character of the elastically active network chains was easily distinguished from the failing polymerization of the pregel solution to yield the gel sensor since the phase change quickly stabilized after an initial increase for the former whereas the phase fluctuated eratically without stabilization for the latter. The optical micrograph of a polymerized gel attached to the end of a fiber (Figure 1b) indicates that the gel adopts a near hemispherical geometry.

Both water and ethylene glycol were investigated as possible solvents when preparing the gel matrixes. The motivation for exploring ethylene glycol as a solvent was due to encountered challenges in maintaining an apparent constant volume of the small aqueous droplet during polymerization by controlling the H_2O vapor pressure of the surrounding atmosphere. Ethylene glycol has a lower vapor pressure than water, and it was easier to maintain a constant drop size. Using ethylene glycol as a solvent resulted in a situation where the acrylamide/Bis did not polymerize very well, an effect most likely due to chain transfer during the radical polymerization retarding the cross-linking−polymerization reaction.[29] In addition, as chain transfer could occur

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for many different species, this could also occur with comonomers especially with increasing content in solution.

Another constraint in selecting gel parameters originates from the refractive index difference relative to the surrounding solvent to obtain a sufficient reflectivity at the gel−solvent interface. If the difference between the refractive index of the solution and gel was not above a certain level, the measurements became unreliable, which can be accounted for by a decrease in the reflectivity from the gel−solution interface. This facet represents a practical constraint with respect to concentration and amount of cross-linker of the pregel solution to obtain a sufficient optical signal.

Similarly, if large amounts of ionic groups were incorporated into the gel, the equilibrium swelling degree could be too large for reproducible results especially at low ionic strengths. For the gels used here, the pregel solution was 15 wt %, and the cross-linker was 3 mol % as this composition proved to be appropriate for the experiments carried out. It was, however, possible to fabricate less concentrated gels that could be utilized as sensors. Moreover, the total polymer concentration and the cross-linking density are factors that influence the pore size of the resulting network and, therefore, transport properties of various molecules in the gel network. When fabricating a particular sensor for recognition of a specific biological macromolecule using, for example, the reported principles,[30,31] the possible influence of the gel network on the macromolecule transport properties is an additional factor that needs to be taken into account when optimizing design parameters of the gel network.

SENSOR TESTING

Hydrogel Swelling in Aqueous Solutions of Various Ionic Strengths. When measuring with the sensor using the phase signal (eq 9), the relative optical length of the gel is determined with reference to the selected initial state due to the initial arbitrary phase; i.e., the data are presented to start at 0 nm for this state. Changes in the relative length are directly proportional to the phase change (eq 9). On the other hand, the absolute length can be worked out from the start of the experiment (eq 8). All the ionic strength measurements are carried out at pH 6.7. This is well below the reported pK_a of DMAPAA equal to 8.5,[32] thus there is a high degree of dissociation for the ionic groups.

Figure 2a shows the changes in the optical length within the gel for the cationic hydrogels with varying ionic strengths. In all cases, there is an initial decrease in the swelling degree with increasing ionic strength. The gels with the highest relative content of DMAPAA show the largest change in the relative length. This can be qualitatively accounted for by the osmotic pressure difference in these gels due to the ionic groups: As the salt concentration in the solution increases, the difference between the ion concentration inside and outside the gel diminishes, and thus, the gel shrinks. The same trend, but smaller, also observed for pure acrylamide gel, is a result that is not straightforwardly intuitive.

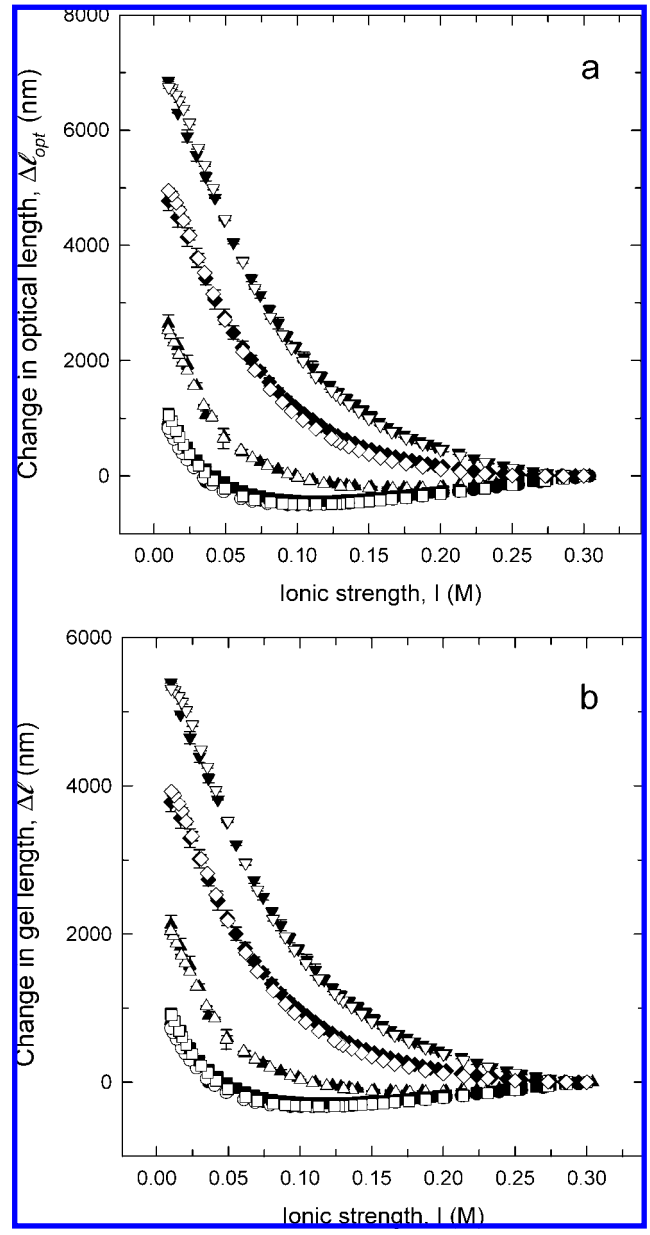

Figure 2. (a) Change in optical lengths within acrylamide−DMAPAA hydrogels of various molar compositions versus ionic strength of the aquous NaCl solution (pH 6.7) at room temperature. The changes in the optical lengths and gel lengths were determined as the equilibrated plateau following stepwise changes of the ionic strength for increasing (filled symbols) and decreasing ionic strength (open symbols). Data were collected for gels with the following mole percent DMAPAA relative to acrylamide: 0.0 (●, ○), 2.0 (■, □), 3 (▲, △), 5 (◆, ◇) and 7 mol % (▼, ▽), respectively. Data points and their standard deviation collected at or after the equilibration. (b) The same measurements are shown as in (a), but for the physical length change of the gel. The physical length is calculated by equation 7 taking into account the refractive index increments for both the polymer concentration and the salt concentration.

The gel with the 2 mol % DMAPAA (ionic) monomer has a virtually identical swelling curve as the nonionic gel, which indicates that the ionic contribution is already screened for this gel at these concentrations. For the gels with the lowest amount

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of ionic groups (0−3 mol % DMAPAA), there is a slight increase for the relative length toward higher ionic strengths. As the instrument determines the optical length within the gel (eq 7), it is expected that the increase in the refractive index of the solution contributes to this. A charge screening effect appears not to be the main molecular mechanism underlying the increased Δl_{opt} observed for ionic strength beyond 0.1 M, due to the monotonic relation between the electrostatic contribution to the persistence length and Debye screening length (see ref 33 for a recent review). All experiments were carried out two times, and good reproducibility of determination of Δl_{opt} was observed. The data (Figure 2a) show good reproducibility of Δl_{opt} of the gel by the very good correspondence between the data obtained for increasing and decreasing ionic strength. Such a lack of hysteresis in the swelling indicates that the gels attain their equilibrium state following the equilibration period used. As explained above, the data logging starts at 0 nm; therefore, the increasing ionic strength experiments are offset so that the end point is zero. In Figure 2b, the changes in ionic strength-induced swelling are presented as changes in physical length of the gel, Δl converted from Δl_{opt} (Figure 2a) using the procedure stated above. The calculated refractive indices of the gel vary from 1.361 (ionic strength 0.3 M) to 1.352 at the lowest ionic strength (0.01 M), e.g., less than 0.65%. In accounting for the impact of various polymer concentrations at different swelling ratios, the gel is modeled as a free swelling hemisphere, and the concentration is estimated to 15 wt % at the highest salt concentration, i.e., the monomer concentration of the pregel solution. The data show the same trends as for the presentation based on Δl_{opt}, but with a smaller absolute values. The conversion of the data of Δl_{opt} to Δl is necessary if quantitative modeling of gel swelling based on a molecular model is aimed at, but the general features of the data obtained are well conveyed when they are presented as the Δl_{opt}being the primary physically observable.

Hydrogel Swelling in Aqueous Solutions of Various pH. Figure 3 depicts the measurements of the equilibrium gel swelling as a function of the pH in the aqueous solvent for hydrogels of various molar fractions of cationic monomer. Each experiment was repeated twice. Various volumes of acid/base solution were added to yield a stepwise change in pH of similar magnitude. The data (Figure 3) display that the pure acrylamide gel does not show any swelling change within this pH interval due to the lack of ionic groups in this gel. For both the 3 mol % DMAPAA and the 7 mol % DMAPAA gels, there is a decrease in the swelling ratio when increasing pH that arises from neutralizaton of the cationic groups of DMAPAA. Similar to the equilibrium gel swelling as a function of ionic strength (Figure 2), good reproducibility between different experiments was observed (Figure 3). For the 7 mol % DMAPAA gels, the experiments carried out for both increasing and decreasing pH show that there is no large degree of hysteresis. However, one of the decreasing-pH measurements deviates slightly from the others. For both the 3 and 7 mol % DMAPAA, the swelling degree leveled out with increasing pH of the solution at pH 9.5. Based on these equilibrium gel swelling data, a pK_a for DMAPAA was estimated from the midpoint of swelling as function of pH to pK_a ~8.6. This is in agreement with

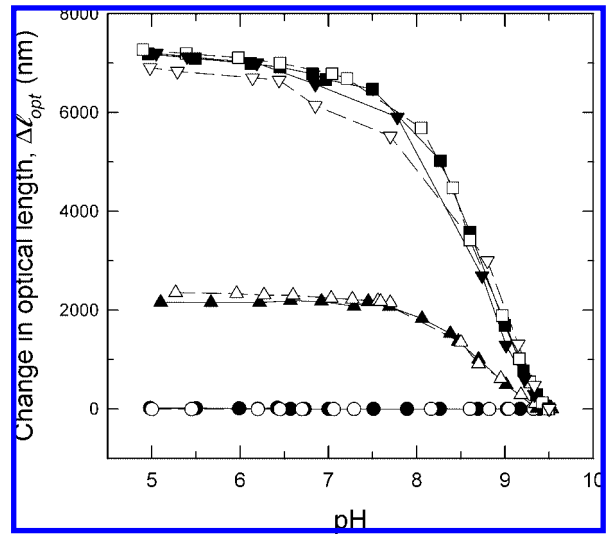

Figure 3. Change in optical lengths within acrylamide−DMAPAA hydrogels of various molar compositions versus pH of the aquous solution at room temperature. The changes in the optical lengths were determined as the equilibrated plateau following stepwise changes of the pH. Data were collected for two parallell preparations for increasing pH for gels with the following mole percent DMAPAA relative to acrylamide: 0.0 (●, ○), 3 (▲, △), and 7 mol % (■, □) and for deceasing pH for the 7.0 mol % DMAPAA gel (▼, ▽, respectively. Data points and their standard deviation collected after the equilibration.

results reported by others.[32] The swelling of the gels as a function of pH is presented based on the primary physically observable Δl_{opt} because the conversion to Δl in this case only represents a scaling of the values of the swelling due to a nearly constant refractive index.

Resolution in the Determination of Gel Swelling. Figure 4 depicts time series of changes in optical path length within a 3 mol % DMAPAA gel versus time following a stepwise increase in the ionic strength of the aquoues solution. This series of data provides an experimental basis for determination of the sensitivity of the measuring technique. The experimental data show that the technique readily supports optical length changes of less than 11 nm, corresponding to the difference in stationary values of the optical lengths at ionic strengths 0.151 and 0.163 M. For the selected gel and range of ionic strength, such changes are associated with differences in gel equilibrium swelling for differences of a few millimolar in ionic strength. In Table 1, the average of the relative length, and the standard deviation of these values, and average of the absolute length are shown for the plateau regions from the time series reported (Figure 4). The differences in relative length of 11 nm for the plateau of the time series collected from solutions of 6 mM difference in ionic strength is clearly distinguishable. Since the standard deviation of the measurements only varies by 1−2 nm, the resolution of the instrument is at least 4 nm, and as the absolute length of the gel is ~58 μm, we can conclude that a 0.007% relative length change is detectable. For lower ionic strengths or gels with more ionic groups, the sensitivity toward changes in ionic strength will be enhanced since the difference in ion concentration between the gel and solution dictates the swelling degree for the gel in this experiment.

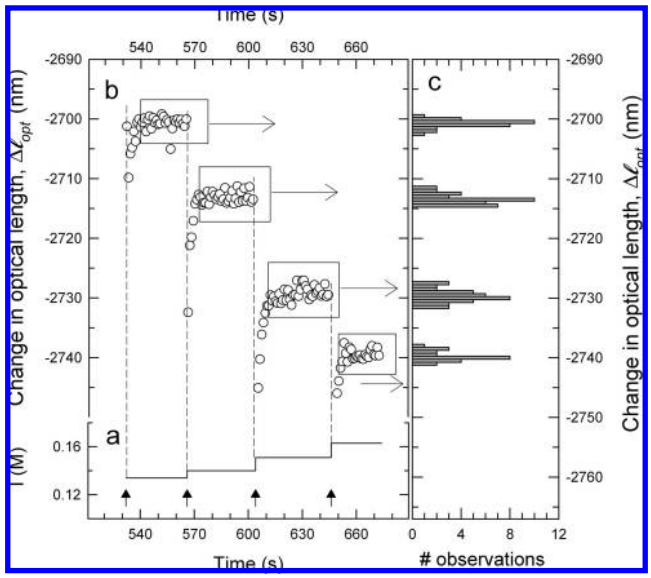

Figure 4. Change in optical lengths within acrylamide−DMAPA hydrogels versus time for stepwise increase in ionic strengths of the immersing aqueous solution for a 3 mol % DMAPAA gel. (A) A stepwise increase in ionic strength of the immersing aqueous solution (continuous line) of 0.134, 0.140, and 0.151 M and to 0.163 M by adding aliquots of 2 M NaCl (↑). (B) Change of optical length of the hydrogels versus time (○) as response to the stepwise increase in ionic strength (indicated by the dotted lines). (C) Distribution of the change in the optical length following the initial period at the various levels of ionic strengths. The data included in these various distributions are indicated by the boxes and arrows in (B).

Table 1. Average of the Absolute Optical Length, Average of the Relative Optical Length, Δl_{opt}, and Standard Deviation of Δl_{opt} for the Data from Figure 4 (3 mol % DMAPAA Gel)[a]

ionic strength, M	absolute length, μm	relative length, nm
0.134	58.74	2701 ± 1
0.140	58.67	2713 ± 1
0.151	58.55	2729 ± 1
0.163	58.48	2740 ± 2

[a] The uncertainty is the standard deviation calculated from the equilibrium swelling data collected for at least 30 s (Figure 4).

KINETICS OF GEL SWELLING

A time series of deswelling of a 3 mol % DMAPAA hydrogel induced by stepwise increase in ionic strength from 0.03 to 0.074 M (NaCl) is used to illustrate the determination of kinetics of hydrogel swelling accessible by the fiber-optic platform (Figure 5). The raw data are collected versus time from a reference of optical length at even lower ionic strength (Figure 5b). The response data (Figure 5b) following the stepwise increase in the ionic strength (Figure 5a) show equilibration to the new plateau level of Δl_{opt} almost instantaneously (~3−4 s). This can be accounted for by a decrease in the difference of the ion concentration inside and outside of the gel. In some cases (e.g., see data in Figure 4), an initial dip occurs in the swelling degree before equilibrium is reached.

Quantitative determination of parameters of the swelling kinetics was performed by fitting the theoretical model for swelling of a spherical gel due to step changes in the surrounding chemical

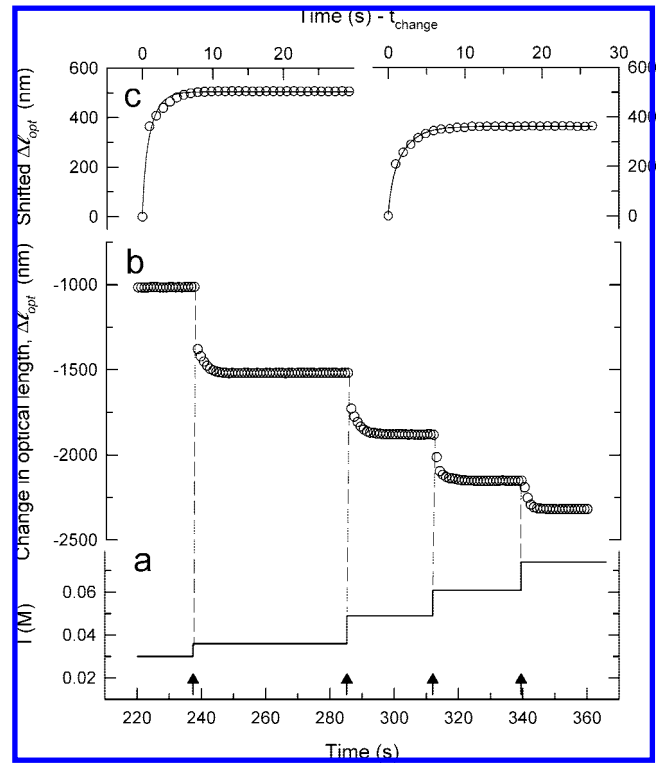

Figure 5. Response kinetics of swelling of acrylamide−DMAPAA hydrogels for stepwise increase in ionic strengths of the immersing aqueous solution of a 3 mol % DMAPAA gel. (A) A stepwise increase in ionic strength of the immersing aqueous solution (continuous line) of 0.030, 0.036, 0.049, and 0.061 M and to 0.074 M by adding aliquots of 2 M NaCl (↑). (B) Change of optical length of the hydrogels versus time (○) as response to the stepwise increase in ionic strength (indicated by the dotted lines). (C) Fit of the theory for swelling of gels (continuous line) to the experimental data (○) for plateau at 0.036 (left panel) and 0.049 M (right panel). For the kinetic analysis, the data are shown with origin at the time of inducing the step change, and with absolute amplitude.

potential (eq 5) to the experimentally determined data. This is applied as a first approximation for modeling of the half-spherical gels bound to the surface. It is assumed that the constraint introduced by the binding to the surface introduces not too serious deficiences for the application of this theory in view of the relative small changes of the gel dimensions (a few μm) compared to the overall size (60 μm). For step changes in ionic strengths of the aqueous immersing solution of 12−14 mM in the range of ~50 mM, time constants of τ =1.3−1.9 s were determined (Figure 5c). This range of values for τ for a half-spherical gel of radius of 50−60 μm compares well with the kinetic constant for swelling of spherical gels of the same size[34] in the case of gel network diffusion being the limiting factor. The use of the primary observable Δl_{opt} data for kinetic analysis estimating τ of the swelling yields the same values of τ as a procedure based on the physical length changes Δl. This is due to the fact that the refractive index is practically constant for the imposed step changes in the ionic strength, and hence, this will represent only a constant scaling factor in the amplitude of the parameter representing the gel swelling.

Similarily, step changes in pH induces gel swelling responses (Figure 6), from which an apparent kinetic constant of hydrogel swelling can be determined. These swelling data display a large difference for the equilibration time in comparison to the swelling

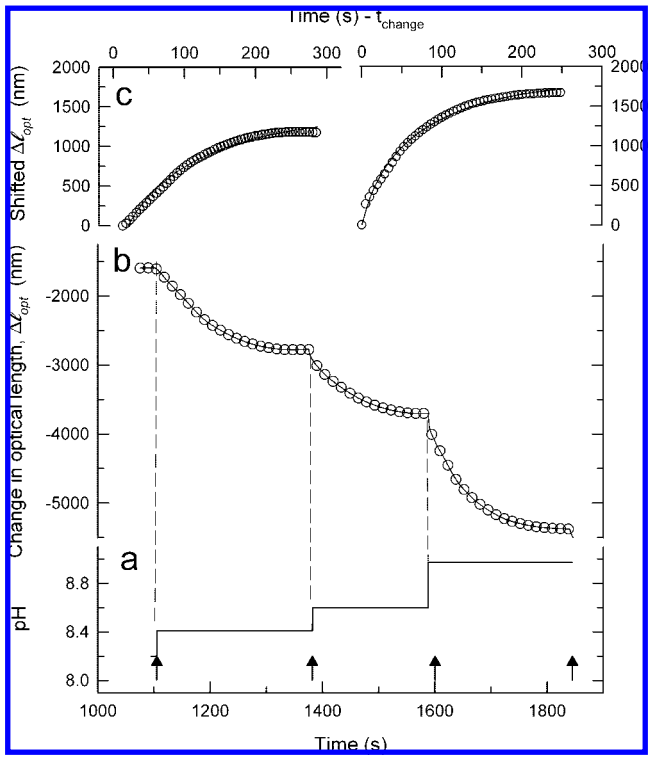

Figure 6. Response kinetics of swelling of acrylamide−DMAPAA hydrogels for stepwise increase in pH of the immersing aqueous solution of a 3 mol % DMAPAA gel. (A) A stepwise increase in pH of the immersing aqueous solution (continuous line) from pH 8.2, to 8.41, 8.60, and 8.97 by adding aliquots of NaOH (↑). (B) Change of optical length of the hydrogels versus time (○, shown for every 10th data point) as response to the stepwise increase in pH (indicated by the dotted lines). (C) Fit of the theory for swelling of gels (continuous line) to the experimental data (○, shown for every 5th data point) for plateau at pH 8.41 (left panel) and pH 8.97 (right panel). For the kinetic analysis, the data are shown with origin at the time of inducing the step change, and with absolute amplitude.

kinetics induced by stepwise changes in ionic strength. The apparent time constants for the swelling of the network induced by stepwise changes in pH were determined to $\tau = 130$ s for a step change of pH of the solution from 8.30 to 8.41, and 95 s for a pH change from 8.60 to 8.97. These values of τ of 95−130 s are nearly a 100-fold larger τ than estimated for the gel swelling induced by step changes in ionic strength. The rather large difference compared to the values of τ observed for the change in the ionic strengths suggests that diffusion of polymer network strands to adjust to the new equilibrium swelling volume is not the rate-limiting factor. Furthermore, since the diffusion coefficients of H_3O^+/OH^- and Na^+/Cl^- in water are all in the range of 10^{-5} cm/s,[26] it is unlikely that the negligible differences have an impact on the rate of gel swelling. It is therefore more plausible that the rate-limiting factor is due to the readjustment of the equilibrium concentration of the ammonium groups when the pH is changed, i.e., the chemical reaction. Note also that the validity of using the gel swelling model in eq 5 is less for the stepwise change in pH than the ionic strength. This is due to the resulting change in network parameters associated with the reaction with H_3O^+/OH^- in the case of the stepwise change in pH. Despite this limitation, the application of eq 5 also in the case of the stepwise change in pH provides an estimate of a characteristic time for gel swelling that can be compared directly to that obtained using the identical model for the step change in ionic strength, where network diffusion appears to be the rate-limiting factor.

The kinetics of swelling induced by stepwise changes in ionic strength or pH highlight a major advantage of the instrument in being able to continuously sample changes of optical path length within the hydrogel with a resolution of 2 nm (optical length) and a concomitant temporal resolution of ∼1 s. Thus, the raw data show that the instrument has the potential for use in real-time experiments due to this short detection time.

CONCLUSIONS

Acrylamide-based hydrogels with ionic groups, *N*-(3-dimethylaminopropyl)acrylamide, were synthesized to demonstrate a novel optical technique for high-resolution detection of gel swelling. Hydrogels of ∼60 μm were bound to the end of an optical fiber, where light sent through the fiber reflected at the fiber−gel and gel−solution interface formed an interference wave, which was the basis for detection. Some capabilities of this fiber-optic based platform were investigated by measuring the swelling ratio of the various gels in solutions of varying ionic strength and pH-intervals. Results showed a high resolution (2 nm, optical length), which is only 0.007% of the absolute gel length. Furthermore, there was a very good degree of reproducibility with negligible hysteresis. The gels with the largest amount of ionic groups displayed the largest relative swelling ratio when changing either the ionic strength or pH as expected, and all gels decreased in length in increasing ionic strength. For the nonionic gel and the gels with lesser amounts of ionic groups, an increase in the swelling ratio was seen at higher concentrations, which is probably is due to an increase in the refractive index. Experiments showed that ionic strength changes as small as 6 mM were detectable at an ionic strength of ∼0.15 M, and due to the resolution, it should be possible for even smaller ionic strength changes. The change in the optical path length of the hydrogels as a function of pH showed that the apparent pK_a for DMAPAA was ∼8.6. From the ionic strength and pH experiments, it became apparent that there is different swelling kinetics in the two cases. In the pH experiments, the gel swelling seemed to be dictated by the chemical reaction of the DMAPAA groups associated with changing pH, whereas in the ionic strength experiments, swelling of the gel network itself appeared to be the rate-limiting step. Such data are accessible due to the sampling rate of ∼0.98 s of the setup. In future projects, we aim to use the instrument in more complex systems such as detection of biological molecules. The results presented here show good promise for such measurements where the sample rate could prove helpful for real-time experiments. Also, due the miniature size of the sensor instrument, it should be applicable for in vivo detection.

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