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In-vitro investigations of a pH- and ionic-strength-responsive polyelectrolytic hydrogel using a piezoresistive microsensor

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Abstract

Environmental responsive or smart hydrogels show a volume phase transition due to changes of external stimuli such as pH or ionic strength of an ambient solution. Thus, they are able to convert reversibly chemical energy into mechanical energy and therefore they are suitable as sensitive material for integration in biochemical microsensors and MEMS devices. In this work, micro-fabricated silicon pressure sensor chips with integrated piezoresistors were used as transducers for the conversion of mechanical work into an appropriate electrical output signal due to the deflection of a thin silicon bending plate. Within this work two different sensor designs have been studied. The biocompatible poly(hydroxypropyl methacrylate-*N,N*-dimethylaminoethyl methacrylate-tetra-ethyleneglycol dimethacrylate) (HPMA-DMA-TEGDMA) was used as an environmental sensitive element in piezoresistive biochemical sensors. This polyelectrolytic hydrogel shows a very sharp volume phase transition at pH values below about 7.4 which is in the range of the physiological pH. The sensor's characteristic response was measured in-vitro for changes in pH of PBS buffer solution at fixed ionic strength. The experimental data was applied to the Hill equation and the sensor sensitivity as a function of pH was calculated out of it. The time-dependent sensor response was measured for small changes in pH, whereas different time constants have been observed. The same sensor principal was used for sensing of ionic strength. The time-dependent electrical sensor signal of both sensors was measured for variations in ionic strength at fixed pH value using PBS buffer solution. Both sensor types showed an asymmetric swelling behavior between the swelling and the deswelling cycle as well as different time constants, which was attributed to the different nature of mechanical hydrogel-confinement inside the sensor.

Keywords

biochemical microsensor; polyelectrolytic hydrogel; pH-sensitive; swelling behavior

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1. INTRODUCTION

The rapidly expanding field of biotechnology and medicine in the recent years provides a great need for advanced biocompatible sensor systems for on-line monitoring and control of specific analytes in physiological environments. A great variety of potential analytes such as glucose, enzymes, bacteria as well as pH, partial pressure of carbon dioxide and oxygen and the concentration of specific ions such as sodium, potassium, calcium or chloride strongly determine the properties of biophysiological systems [1,2]. Since all biophysiological systems are water-based-systems, they heavily depend on acid-base equilibria and thus on pH value. To prevent perilous reactions the pH value of the human blood can just vary in a very narrow range around pH 7.4, also enzyme activity as well as cell function, growth and development are highly pH-sensitive [3]. Therefore, the development of new customized microfabricated reliable chemical and biosensors, specific for particular species, is highly required [4]. The goal behind the sensor design is the accurate, fast, cheap and simple quantitative determination of the concentration of the analyte by detecting physical and/ or chemical signals proportional to the analyte concentration [5]. The application of polymer films makes it possible to chemically design the polymer in such a way that it is sensitive to the specific analyte of interest. In particular, a special class of polymers, the so called “stimuli-responsive” or “smart” hydrogels, has recently attracted much attention for use as functional materials in sensor and actuator applications [6-9]. Their ability to absorb or desorb large quantities of water resulting in a volume phase transition in response to environmental triggers such as temperature, pH, light or concentration of certain analytes have brought them in the focus of research for chemical and biological sensors as well as for autonomous drug delivery devices [7-11].

A biocompatible hydrogel consisting of the comonomer *N,N*-dimethylaminoethyl methacrylate (DMA) was used in this study. This type of hydrogels with different copolymer compositions were widely studied in the literature [12-14]. This cationic hydrogels are pH sensitive due to basic moieties, namely the tertiary amine groups, which are uncharged at high pH and charged at low pH values. The addition of the copolymer hydroxypropyl methacrylate (HPMA) leads to a shift of the transition pH towards the physiological range. The combination of this hydrogel with a piezoresistive pressure transducer allows the on-line detection of the analyte concentration and the conversion into an appropriate electrical output signal.

2. EXPERIMENTAL METHODS

2.1 Materials and hydrogel synthesis

The investigated hydrogel is based on three monomers. The monomer *N,N*-dimethylaminoethyl methacrylate (DMA) contains a pH-sensitive tertiary amine, hydroxypropyl methacrylate (HPMA) was included to obtain a transition pH close to the physiological range [12,13] and tetra ethyleneglycol dimethacrylate (TEGDMA) acts as crosslinker. All monomers were obtained from Polysciences, Inc. and used as received. Ammonium peroxydisulfate (APS, Sigma-Aldrich), *N,N,N',N'*-tetra-methylethylenediamine (TEMED, Sigma-Aldrich) and phosphate-buffered saline (PBS, Sigma-Aldrich) were used as received without further purification.

The polyelectrolyte hydrogels containing HPMA/DMA/TEGDMA at a nominal mole ratio 70/30/02, respectively, were synthesized by free radical cross-linking copolymerization as described in [13]. The pregel solution, containing measured amounts of the monomers HPMA, DMA and crosslinker TEGDMA, was degassed by bubbling nitrogen gas. Thereafter, the pregel solution was brought in a well-defined 400 μm cavity between two

square glass plates using a Teflon spacer. The side walls were held together by metal clamps. Shortly after, free radical polymerization was initiated at room temperature by injection of the initiator APS and the reaction accelerator TEMED. After 4 hours at room temperature the hydrogel was removed from the mold and cut into square samples of 1...2.3 cm in length. Before use the cutted samples were washed in PBS buffer (pH 7.4) for at least two days. The samples were exposed to a bath of changing ionic strength between 0.05 M and 0.15 M in order to induce swelling/deswelling and thus acceleration of the cleaning process.

2.2 Piezoresistive chemomechanical microsensors

Two different types of piezoresistive microsensors were used for investigating the swelling behavior of the hydrogel. Although the sensor principles are similar, the sensor configuration differs with respect to hydrogel confinement and the supply of the bath solution (external stimulus). Nevertheless, both sensor types under investigation consist of two principal components: A chemo-mechanical transducer, namely the stimuli-responsive hydrogel, and a mechano-electrical transducer

2.2.1 Sensor type 1 - Sensor with partly constrained hydrogel—The sensor design is based on a commercially available micromachined piezoresistive silicon pressure sensor chip (Aktiv Sensors GmbH, Stahnsdorf, Germany) with a thin flexible square bending plate and four integrated piezoresistors at the edges connected to a Wheatstone bridge (Fig. 1) [15]. The backside of the chip was wet-etched using a silicon nitride mask as etch resist. The deformation of the thin bending plate causes a mechanical stress state in the piezoresistors, thus a change in the resistivity of the bridge circuit and proportionally affect the electrical output voltage (bridge voltage). This passive device has been employed as mechano-electrical transducer to convert the force-induced deflection of the bending plate into an appropriate electrical output signal. The silicon chip was bonded to a socket with fluidic channels for solution inlet and outlet. The force was generated due to the volume phase transition of the hydrogel which operates as a chemo-mechanical transducer. The hydrogel layer is enclosed in the chip cavity between silicon chip and socket. The smart hydrogel was synthesized and cleaned as described above and cut into $1 \times 1 \text{ mm}^2$ samples using a scalpel. Subsequently, one sample was glued on the chip holder in the dry state (thickness $\approx 330 \text{ }\mu\text{m}$) between the two fluidic channels and was therefore mechanically constrained at one side. The bath solution to be measured was pumped through the chip cavity coated with a 200 nm PECVD silicon nitride layer to guarantee chemical protection. This design proves to be advantageous due to the strict separation between chemical solutions on the chip backside and the electronic components on the top side of the chip.

2.2.2 Sensor type 2 - Sensor with fully constrained hydrogel—For this type of sensor a piezoresistive pressure transducer (EPB-501-5P, Entran, Inc., Fairfield, NJ, USA) as mechano-electrical transducer was used [16]. The transducer consists of a cylindrical stainless steel sensing area. The sensing area was completely covered with the hydrogel layer of thickness 400 μm in the pre-conditioned state (equilibrium degree of swelling in PBS buffer with pH 7.4 and ionic strength 0.15 M) as shown in Fig. 2. In order to hold the hydrogel in place a cap was used. The top surface of the cap consists of a porous membrane which is replaceable; mass transfer can occur through the open pores. The membrane is a stainless steel wire cloth mesh (type 304, Small Parts, Inc., Miramar, FL, USA). In this arrangement the hydrogel is mechanically confined at all its surface areas through the contact with the cap, the porous membrane and the pressure transducer. The pressure transducer was calibrated using a water column and an air manometer (PCL-200 D, Omega, pressure range 0-65 kPa). The synthesized hydrogel was cut into a disc-shape sample with a

circular biopsy tool to a diameter of 3.18 mm and transferred to the sensing area using tweezers. The sensor cap and the porous membrane were adjusted to the sensor using three screws. The screws were tightened to impose axial compressive stress to the hydrogel layer inside the sensor.

The sensor was then exposed into a covered environmental bath containing PBS buffer of specific ionic strength and pH value at room temperature. In order to support mass transfer to the sensor the bath solution was stirred with a magnetic stirrer.

3. RESULTS AND DISCUSSION

3.1 Swelling kinetics

The use of hydrogel-based piezoresistive sensors for the reliable detection of solvent properties and composition (e. g. pH and ionic strength) requires the evaluation of the sensor output signal, when the hydrogel layer is in its equilibrated state. Beside this use as sensor itself it is an excellent tool for in-situ measurements of the swelling properties and kinetics of the particularly used hydrogel film. The deflection of the thin silicon bending plate and the accompanied change of the electrical output voltage (see section 2.2.1) are directly related to the time-dependent change in the degree of swelling during the volume phase transition of the gel layer. In this work the polyelectrolyte HPMA/DMA/TEGDMA hydrogel layer with an initial thickness in the dry state of about 330 μm was studied and kinetic measurements have been performed. For swelling kinetic measurements sensor type 1 was used. As described in the previous section, the hydrogel layer was integrated in the dry state inside the chip cavity of the sensor. For the conditioning of the hydrogel layer, which denotes the transition from dry state to the swollen state, PBS buffer with initial pH 7.4 and ionic strength 0.15 M were pumped through the sensor at room temperature. The sensor signal during the conditioning cycle is shown in Fig. 3.

The curve in Fig. 3 obviously has sigmoidal shape and mainly consists of three parts. At time $t = 0$ min the hydrogel is in its initial glassy dry state. Then, the gel is exposed to PBS buffer solution. Nevertheless, no sensor response was detected in the first 27 minutes. This characteristic lag time of the electrical signal results from the gel-solution interaction. In this early phase (1) of the swelling process the difference between the chemical potential of the hydrogel and the surrounding buffer solution and the accompanied osmotic pressure difference cause diffusion of the solution molecules into the outer hydrogel layers. The outer faces of the gel layer are now in a rubbery swollen state carrying fixed positively charged tertiary amine groups, whereas the inner core is still in a dry glassy unionized condition. The latter tend to impose a swelling constrain to the hydrogel. Swelling can only progress through the transport of protons from the outer solution to uncharged amines at the swelling front. Protons may either transported by bounding to the water as hydronium ions or to the acidic form of the buffer. A similar behavior has been found in [17,18] for free swelling MMA/DMA copolymer gels in different buffer solutions. Since hydrogel dimensions and hydrogel thickness are in the same order of magnitude, several swelling fronts will arise corresponding to the surface areas contacting the buffer medium. The swelling fronts move to the inner part of the gel layer. When these fronts meet, then the solid core disappears and passes into the rubbery gel phase. Thus, the swelling constrain vanishes and “free” swelling can occur. However, it is noteworthy that free swelling in this context means swelling of a hydrogel layer which is mechanically constrained at one surface and which is later on exposed to the counterforce from the deflected plate (see Fig. 1). Although the solid core hinders swelling, the hydrogel layer is penetrated by solvent molecules and, thus, one would expect small changes in the degree of swelling. However, no increase in the sensor output voltage can be recorded in phase (1). During this time there is still a small gap of about 50

μm between the upper hydrogel face and the undeflected bending plate. Therefore, after “free” swelling is initiated the hydrogel has additionally to fill the gap before an appropriate output signal can be detected. This behavior explains the fast increase of the sensor signal after the time $t_1 = 27$ min. This second part (2) is governed by diffusion of analyte molecules into the hydrogel and electrostatic repulsion of charged amino groups which lead to an additionally tremendous expansion of the gel network. This stage shows a linear characteristic during a time period of $t_2 = 20$ minutes and subsequently passes over to the third stage (3) that exhibits an exponential increase which can be attributed to the mechanical counterforce of the elastic network chains. This elastic force counteracts the network expansion caused by water uptake and electrostatic repulsion until force balancing sets in and the hydrogel reaches quasi-equilibrium. The time constant for that process was found to be 21 minutes applying an first order exponential least square fit to the experimental data ($R^2 = 0,99$).

3.2 Sensing of pH

3.2.1 Sensor output characteristic and sensitivity—In order to realize a pH-sensor the ionic HPMa/DMA/TEGDMA hydrogel was used as sensitive sensor element. The presence of a pendant tertiary amine on the comonomer DMA is responsible for a reasonable pH-response of the gel. The hydrogel contains basic moieties which are charged at low pH and uncharged at high pH values. Fig. 4 shows the output characteristic of the pH-sensor. The electrical output voltage of sensor type 1 was measured during the swelling and shrinking process of the hydrogel layer under the influence of different pH values of PBS buffer solution at a fixed ionic strength of 0.15 M. The measurements were carried out using a pre-conditioned hydrogel film as described in section 2.1.

The sensor voltage (triangle symbols) exhibits two plateaus, even if the one in the basic region is only adumbrated. In basic media ($\text{pH} > 8$) all tertiary amine groups on the polymer backbone are in their unionized state leading to gel collapse. Therefore, no sensor response has been measured. For $\text{pH} < 7.8$ a cumulative protonation of the amine groups leads to the accumulation of fixed charges along the polymer backbone and thus to a temporarily increase in the osmotic pressure which is compensated by hydrogel swelling. This gives rise to a very sharp transition region between pH 7.8 and pH 6.8 where a signal increase of about 250 mV has been found. This pH range is of particular interest for the monitoring of physiological and biological processes [1,2]. The approximately linear sensor behavior within this range enables a reliable detection of pH and makes it a good candidate for use in biomedical devices. The signal slope and magnitude as well as the transition pH depend on the copolymer composition [12]. When altering the pH-value towards acidic conditions then more and more functional groups become ionized until all of them are protonated. This is the case for sufficiently low pH values. Hence, the sensor signal levels off in a steady state and nearly no signal change occurs.

Due to the overall strongly nonlinear behavior of the sensor transfer characteristic, the determination of the sensor sensitivity from the experimental data is not possible. For that reason a phenomenological functional approach based on the Hill equation [19] has been made to describe the sensor response. The model was successfully applied to the experimental data using least-square-method ($R^2 = 0.99$). The model equation is given below and the corresponding graph is illustrated in Fig. 4.

$$\Delta V_{out}(pH) = \Delta V_{out,min} + \frac{\Delta V_{out,max} - \Delta V_{out,min}}{1 + 10^{(pH_T - pH)\eta}} \quad (1)$$

This four-parameter model describes a sigmoidal curve characteristic and is widely used to analyze biochemical and medical data [20,21]. In particular, it was derived to describe the response of a species saturated by ligands as function of the ligand concentration. Certain parallels can be drawn to hydrogels, in the sense that a certain change in hydrogen ion concentration leads to a change in ionization of the functional groups on the polymer backbone and consequently to a response of the hydrogel network. Nevertheless, the parameters are of phenomenological nature but can be well related to their chemo-physical background. The parameters $\Delta V_{out,min}$ and $\Delta V_{out,max}$ describe the sensor response when the gel is in its unionized collapsed or fully ionized swollen state, respectively. Whereby, $\Delta V_{out,max}$ is a function of the copolymer composition (polymer chain charge density) and subchain length [12,22]. The transition point of the output characteristic (pH at which the maximal increase in the gel volume occurs) is described by $pH_T (= 7.34)$. This parameter can be ascribed to the copolymer composition again and the equilibrium constant of the charged groups. The slope of the characteristic curve is described by η (here $\eta = -1.16$) the so-called hill slope. The sign of this parameter indicates whether the studied hydrogel contains cationic (negative sign) or anionic (positive sign) functional groups. A high value of the hill slope indicates a sharp transition that takes place over a small range of pH whereas a small value stands for wide transition occurring over a large pH range.

The sensor sensitivity as a function of pH can be calculated from Eq. (1):

$$S_{pH}(pH) = \frac{\partial \Delta V_{out}(pH)}{\partial pH} \quad (2)$$

As expected, the sensor sensitivity increases dramatically within the transition region and reaches its maximum value at the transition point (pH_T) which is marginally below the physiological pH. At this point a sensor sensitivity S_{pH} of 265 mV/pH has been found.

3.2.2 Time-dependent sensor response—The response time of the piezoresistive biochemical sensor during the detection of pH in PBS solutions is determined by the polymer layer thickness as well as by the swelling kinetics of the hydrogel. The latter depends on the diffusion rate of the respective buffer solution in the hydrogel network, the buffer properties as well as on interactions between buffer molecules and gel structure. It has to be noted that the kinetics of swelling of charged hydrogels in buffered solutions substantially differ from those in aqueous media. The time-dependent sensor response for swelling in PBS buffer for different steps in pH value is demonstrated in Fig. 5. All three curves were measured based on an initially swollen gel in PBS buffer at pH 7.45 and ionic strength $I = 0.15$ M. The sensor response times are notoriously long, what can be attributed to the large hydrogel layer thickness. Nevertheless, an analysis of the sensor kinetics is possible. As expected from the sensor transfer function the change in the sensor output voltage increases with decreasing pH value. In other words, the change in the electrical output signal for decreasing pH steps also decreases. For small pH steps less gel amine groups become ionized compared to larger changes in pH, resulting in a smaller magnitude of the sensor signal. Nevertheless, even for a small pH change of 0.1, a well-detectable signal change of about 27 mV has been measured. Beside different magnitudes of the sensor signal for different small pH changes, another obvious characteristic turns out from Fig. 5. It has been found that the sensor response time also depends on the pH changes. The response time increases with increasing pH. A similar behavior has been found in [23] for the time-dependent resistance change of a hydrogel-coated electrode array. The time response of the sensor is determined by a complex interaction between the hydrogel and the buffer system. Since the fraction of ionizable tertiary amines within the hydrogel and the buffer

concentration are preset in our experiments, the rate of swelling depends on the desired change of the ionization state of the charged groups to reach a new equilibrium and the capability of the buffer to transport protons. Both factors are a function of pH. For a larger change in the ionization of the hydrogel a larger number of protons is required to be transported into or out of the gel to establish a new equilibrium state. Therefore, one could expect that a greater change in pH value results in a longer response time. On the contrary, Fig. 5 indicates that the opposite is true, which gives rise to the conclusion that the ionization of the gel amines is not the rate-limiting step for the sensor response. The buffer pH also affects the ionization state of the buffer medium depending on its equilibrium constant and thereby its ability to transport protons [17,18]. For smaller pH values a greater number of protons is available which permits faster proton transfer from the bath solution to the hydrogel. Hence, an increase in the pH value leads to an increase in the sensor response time because proton transport as a function of the ionization state of the buffer seems to be the rate-limiting step.

3.3 Sensing of ionic strength

Since the hydrogel inside the sensor is of a polyelectrolyte type, it responds to changes in pH as well as in ionic strength. If the pH of a solution is constant, sensors with the same sensitive hydrogel layer can be used for the detection of ionic strength. Now, both types of sensors have been used to study the kinetic sensor response caused by changes in salt concentration. As described above, they mainly differ in the confinement of the gel layer inside the sensor and the supply of the swelling agent. For both sensor types the time-dependent change in the output signal for changes in ionic strength from $I = 0.15 \text{ M}$ to $I = 0.05 \text{ M}$ and vice versa at fixed pH 6.8 has been measured. The measurement results are illustrated in Fig. 6.

Obviously, the ordinates of both graphs exhibit different physical quantities. For sensor type 1 the change in electrical voltage is used as output signal, whereas for sensor type 2 a pressure change was measured. However, this should not cause confusion since the kinetic sensor response is independent of the kind of sensor output and hence comparable. Fig. 6 demonstrates that lowering the ionic strength results in hydrogel swelling. In that case, the chemical potential of the surrounding water and therefore the osmotic pressure is increased. This temporary increase in osmotic pressure is compensated by hydrogel swelling, directly leading to an increase in the sensor output signal. A subsequent increase of ionic strength and the consequently temporary decrease of the osmotic pressure in the solution, compared to the osmotic pressure inside the hydrogel, induce gel collapse and a concomitant decrease in the sensor signal. The same cycle was repeated three times. Fig. 6 indicates that both sensor signals are reversible and reproducible.

Both sensor types show a similar response for cyclic variations in environmental ionic strength. However, kinetics differ with respect to the sensor response time. Furthermore, it can be observed from Fig. 6 that the time-dependent response curves differ in their characteristic from a strict first-order behavior. Hence, for comparison of response kinetics, we define τ_{50} as the time at which the change in the sensor output signal (ΔV_{out} , ΔP) reaches 50% of its equilibrium or long time value. For the response time τ_{50} in Fig. 6a values of 8.8 min for swelling and 5 min for deswelling have been found, which can be compared to 6.4 min for swelling and 2.3 min for deswelling in Fig. 6b. The observed asymmetry between the kinetics of swelling and deswelling seems to be induced through hydrogel confinement in the sensor, since free swelling experiments of the same hydrogel under the same environmental conditions showed equal response times for both half-cycles [6]. Inside the sensors the hydrogels are exposed to mechanical forces. In sensor type 1, forces on the hydrogel are induced by the socket and the bending plate, whereas in sensor type 2 the

piezoresistive diaphragm and the porous membrane lead to a counterforce which opposes swelling and accelerate deswelling. When the swelling process is initiated then the hydrogel starts to swell laterally before moving in its axial direction, tending to fill the free space in the sensor. Due to the mechanical confinement of the gel at its bottom face in sensor type 1, the lateral expansion is just possible to a limited extend, but still occurs. The hydrogel in sensor type 2 also will tend to fill the eventually existing free space before the deflection of the piezoresistive diaphragm is complete. However, lateral expansion is much more limited in the latter case because of the fully enclosed gel layer (see Fig. 2). In contrast, during the deswelling half-cycle the bending plate of the transducer returns to its equilibrium position before the lateral contraction of the gel layer is complete, leading to a faster deswelling cycle. Albeit this explanation confirms the observed asymmetry for the different swelling cycles and the different response times for both sensor types, it has to be noted that the initial loading pressure, which was existent at the beginning of the measurements, is not known for both sensors but might affect response kinetics.

4. CONCLUSIONS

In the present work the operational principal of a smart hydrogel-based microsensor has been demonstrated. A biocompatible polyelectrolytic HPMA/DMA/TEGDMA hydrogel has been used as chemo-mechanical transducer inside the sensor. The swelling of the hydrogel layer leads to a deflection of a silicon bending plate and thus to the conversion of the induced force into an electrical output signal, using the piezoresistive effect. Two types of sensors have been studied. In sensor type 1 the hydrogel layer is only confined at one side. This sensor was used to study the swelling kinetics in PBS buffer solution at pH 7.4 and ionic strength $I = 0.15$ M, initially starting from the dry state. Three phases of the swelling process have been found, which could be attributed to the movement of several swelling fronts into the dry gel, a subsequent free-swelling process and the final interaction between hydrogel expansion and the elastic counterforce of the gel network. The same sensor design was used as pH sensor. The sensor output characteristic as a function of pH at fixed ionic strength showed a sharp increase for pH values in the physiological range near 7.4. The Hill equation as a phenomenological sensor model was successfully applied to the experimental data and allowed the calculation of the sensor sensitivity as a function of pH. It has been found, that the sensor shows its maximal sensitivity marginal below pH 7.4. For time-dependent measurements at different pH values it turned out that the magnitude of the sensor output signal decreases with increasing pH. Furthermore, for increasing pH the time constant of the sensor signal increased, which is due to the influence of the buffer properties. The proton transport as a function of the ionization state of the buffer was found to be the rate-limiting step for the sensor response. This is essential because using the sensor in different buffer solutions will affect the sensor response kinetics, which can either lead to faster or slower response times. Sensor type 2 which is characterized by a fully constrained hydrogel film was compared to sensor type 1 using both sensor designs for the sensing of ionic-strength of PBS buffer solution. The time-dependent sensor response has been measured for variations in ionic strength at fixed pH value. It has been found that hydrogel confinement has an impact on the time-dependent sensor response. For both sensors an asymmetry between the swelling and deswelling cycle was figured out. Furthermore, both sensor types exhibited different time constants, whereby the sensor with fully constrained hydrogel layer showed smaller response times for both half-cycles compared to sensor type 1.

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Fig. 1. Chemomechanical sensor with partly constrained hydrogel. (1) thin bending plate, (2) integrated piezoresistor, (3) responsive hydrogel layer, (4) silicon pressure sensor chip, (5) socket, (6) solution inlet/outlet fluidic channels, (7) bonding wire, (8) bath solution.

Fig. 2. Chemomechanical sensor with fully constrained hydrogel. (1) Entran pressure transducer, (2) disc-shape pre-conditioned hydrogel layer, (3) cap, (4) porous membrane, (5) bath solution.

Fig. 3. Electrical output signal of the hydrogel-based piezoresistive sensor (sensor type 1) during the conditioning cycle with PBS buffer at fixed pH 7.4 and $I = 0.15$ M.

Fig. 4. Transfer characteristic $\Delta V_{out}(pH)$ of the hydrogel-based piezoresistive pH-sensor (triangle symbols), Hill equation (solid line) and sensor sensitivity relative to pH-change S_{pH} derived from the Hill equation (dashed line) at fixed ionic strength $I = 0.15$ M.

Fig. 5. Time-dependent change in the sensor output voltage ΔV_{out} for the swelling process of the HPMA/DMA/TEGDMA hydrogel in PBS buffer for different pH steps, initially starting from pH 7.45 at fixed ionic strength $I = 0.15$ M.

Fig. 6. Time-dependent change in the sensor output signal for cyclic variations in environmental ionic strength from $I = 0.15 \text{ M}$ to $I = 0.05 \text{ M}$ at fixed pH equal 6.8 for (a) sensor type 1, (b) sensor type 2.