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Glucose-responsive polymeric hydrogel materials: From a novel technique for the measurement of glucose binding towards swelling pressure sensor applications

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Abstract

This paper presents new insights into material design and physicochemical interactions which are relevant for the use of glucose responsive polymeric hydrogels in continuously operating biosensor systems. Investigated hydrogels were based on either acrylamide or *N*-isopropylacrylamide, covalently crosslinked by *N,N'*-methylenebis(acrylamide), and *3*-acrylamidophenylboronic acid and (*N*-(*3*-dimethylaminopropyl)) acrylamide were the comonomers to enable selective glucose binding at physiological pH. A novel assay for the determination of the amount of bound glucose inside the hydrogel was developed, enabling the direct recording of these receptor effects parallel to the determination of the change of water content, i.e. free swelling. Binding isotherms, affinity constants and maximum degree of complexation of boronic acid groups with glucose were determined. The affinity towards glucose could be increased three-fold compared to literature values for phenylboronic acid free in solution by use of a suitable hydrogel composition. The library of differently composed materials was then evaluated in a pressure sensor setup. Thereby, the long-term use of the hydrogels was established, the hydrogels could be analyzed for a period of three months without the reduction of the pressure signal sensitivity. Based on all results a composition which is suitable for efficient glucose recognition was identified, at which up to 25 % water was released at 37°C and pH 7.4 and a change of the glucose concentration from 0 to 10 mM. In the physiologically relevant range (3 - 10 mM) a linear dependence of the

swelling pressure on the glucose concentration was found, allowing accurate determination of glucose concentration. Overall, the obtained results provide significant progress in efforts to enable glucose detection by a robust sensor setup.

Keywords:

hydrogels, glucose recognition, pressure sensor, binding isotherm, polyacrylamides

1. Introduction

In 2017, 422 million adults worldwide were reported to suffer from diabetes with medication costs of approximately 727 billion USD. The prevalence is increasing rapidly, it will rise by about 50 % in the next 30 years.[1] Besides the high costs, the personal complications, e.g. premature death, heart attack, kidney failure, or leg amputation demand an effective treatment and medical care.[2] Avoiding too high or too low blood glucose level along with keeping the required amount of medication as low as possible is a recommended strategy for prevention of such complications.[2][3] Monitoring the blood glucose level by a continuously measuring sensor combined with effective medication would be required to improve the quality of the patient's life significantly.[3]

Sensors are used to detect a certain kind of analyte, e.g. a chemical substrate. For this purpose, these sensors are comprised of two units, a receptor and a transducer unit. The receptor unit is able to interact with the analyte in dependence of its concentration. The transducer unit transfers the effect of the analyte-receptor interaction in a physical signal, which can be measured.[4]

Many efforts have been made to construct a sensor, which can detect glucose.[5][6] In particular, polymeric colloidal crystalline arrays,[7][8] holographic sensors,[9] optical fibers,[10]-[12] electrochemical sensors[5][13] or pressure sensors[14]-[16] have been proposed. The latter are advantageous, because these systems are scalable and could be employed in medical implants.[17] Moreover, pressure sensors are robust, established for long-term use and are therefore advantageous over other sensing concepts.[17] In our previous research we had already established the use of sensor setups measuring the swelling pressure of hydrogels.[17]-[19] Thereby, the representation of measurement conditions of a medical application was considered by the construction of the sensing setups. The hydrogels shall in the future be available as sensor material in medical implants. Novel studies report on the suitability of implanted pressure sensors for the continuous measurement of blood pressure.[20]-[22] By use of stimuli-responsive hydrogels integrated with such pressure sensor systems (analogous to the version used in our present work), the range of medical sensor applications can be tremendously expanded, thus facilitating the recording of important physiological parameters for a better control of the patient's health.

While concanavalin A[23] and glucose oxidase[13][24] are highly selective receptors for glucose, they are limited in their long term use due to the risk of protein denaturation. In contrast, synthetic receptors like boronic acid can overcome this drawback.[4][23] Boronic acid can reversibly form covalent bonds with glucose yielding a boronate ester; these bonds are even stable in aqueous media.[4][23] Due to its rigid structure, boronic acid is not selective, it can bind to any *cis*-diol.[4][23] The selectivity of this receptor can be targeted towards glucose by an adjustment of its pK_a and the incorporation of a tertiary or quaternary amine in proximity to the boronic acid is a known strategy.[4][10][11][25] Without the presence of the amine, the binding constant of fructose is 40 times higher than that of glucose.[15] Despite the 500 times lower concentration of fructose compared to glucose in human plasma,[26] fructose would significantly interfere with boronic acid.[4][25]

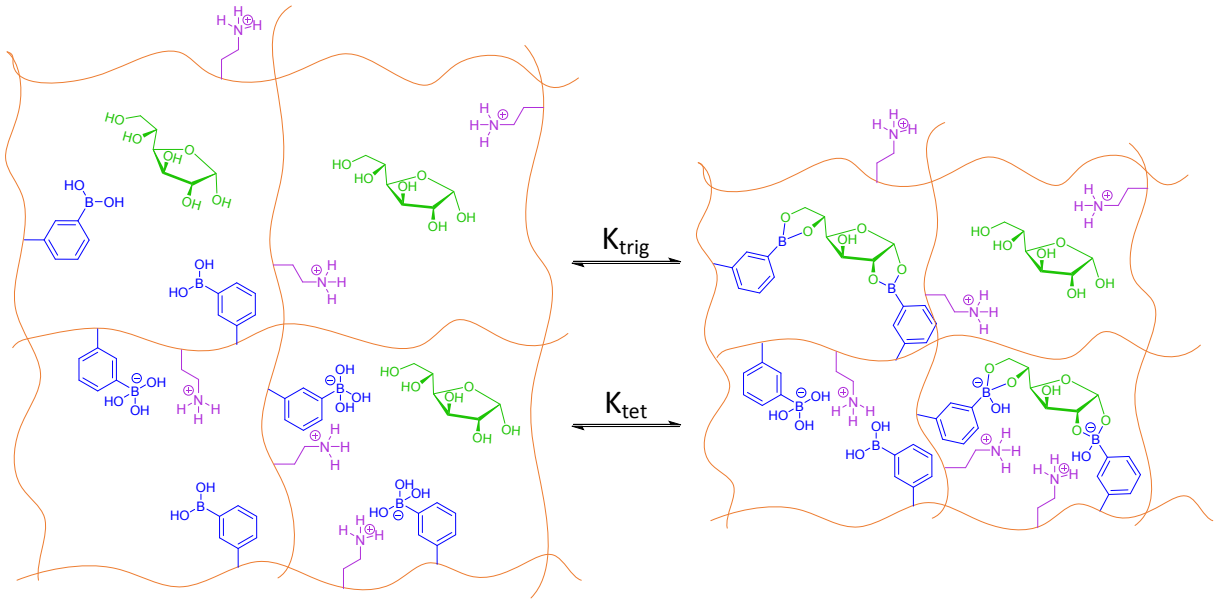

Figure 1. Two pathways for phenylboronic acid (blue) complexing glucose (green) inside the respective hydrogel network (orange). The first (upper pathway), starting from the neutral form (in absence of a tertiary amine as a charge stabilizing group), glucose binding leads to the uncharged form. The second is based on the dissociated form of phenylboronic acid, here no change of charge takes place (lower pathway). The first pathway is not favored; several studies indicate that the affinity constant K_{trig} is rather small in comparison to K_{tet} (K_{trig} = complex formation with trigonal (trig), i.e. neutral, PBA; K_{tet} = complex formation with tetragonal (tet), i.e. dissociated, PBA).[4][28] The following monomers were used to synthesize glucose responsive hydrogels: *3*-acrylamidophenylboronic acid (PBA, blue) as glucose receptor unit; (*N*-(*3*-dimethylaminopropyl)) acrylamide (DMAPAA, purple) to ensure glucose sensitivity at physiological pH. As transducer unit a

poly-acrylamide (PAAm) or poly-*N*-isopropylacrylamide (PNIPAAm) based hydrogel covalently crosslinked by the aid of *N,N'*-methylenebis(acrylamide) (MBAAm) was chosen.

Due to the hemiacetal ring opening and re-closing glucose undergoes isomerizations in aqueous media.[4] Thus, it can exist in five different isomeric forms; the fraction of the different forms depends on the surrounding conditions, like temperature or solvent.[4][27] These different forms can interact differently with phenylboronic acid.[27] It was found, that especially the furanose form builds stable complexes with phenylboronic acid despite its low abundance in solution (0.14 %, D_2O, 27°C).[27] Additionally, this form can build stable 2:1 complexes while the binding of the pyranose form is sterically hindered by the synclinal position of the hydroxyl groups (**Figure 1**).[4][27] Furthermore, glucose is able to form complexes with the neutral and the dissociated form of phenylboronic acid. However, its reaction with the dissociated form is much more likely, which is also expressed in the affinity constants ($K_{trig} << K_{tet}$, **Figure 1**).[4][28]

Stimuli-responsive hydrogels are well-known transducer materials.[29][30] In contrast to conventional hydrogels like PAAm, they can react in a very pronounced and reversible manner to different stimuli, like temperature, pH or the concentration of a chemical substance, dependent on the physical interactions in the network.[30]

PNIPAAm is a prominent example for a temperature responsive polymer.[29] Due to the interaction of its hydrophilic and hydrophobic segments with water in dependence on temperature, the linear polymer possesses a lower critical solution temperature (LCST), and the crosslinked polymer has a volume phase transition temperature (VPTT), in both cases at about 32°C.[30] The LCST of PNIPAAm or VPTT of PNIPAAm-based hydrogels can be tuned by the incorporation of either hydrophilic or hydrophobic comonomers.[29][31][32][33][34] For example, the glucose receptor phenylboronic acid introduces more hydrophobicity into the polymer due to its phenyl ring causing a decrease of LCST or VPTT.[35] In contrast, *N*-(*3*-dimethylaminopropyl))-acrylamide (DMAPAA) is hydrophilic due to its positive charge and, thus, leads to a strong increase of the LCST, which even disappears, if the fraction of DMAPAA in the copolymer is high enough.[35][36]

If positive or negative charges are incorporated into a hydrogel, for example by DMAPAA or PBA, it may become pH responsive in case the charge is neutralized at a certain pK_a. When only one kind of charge is incorporated into the hydrogel network, the material is a

polyelectrolyte, which swells if the groups are dissociated and collapses in neutral state. In an ampholytic network, the hydrogel shrinks in case both groups are dissociated (what causes compensation of charges) and swells if one kind of charge is predominant in the network.[30]

By the incorporation of substrate selective functional groups, hydrogels can become responsive towards a chemical stimulus like glucose.[29][30][37][38] The binding of substrates to a thermo-responsive polymer based on PNIPAAm can shift the LCST or the VPTT, which may amplify the transducer effect.[29][37][38] This is expected to enhance the sensitivity of the respective hydrogel-based sensor at a suited temperature.

Great efforts had been made to create hydrogels which are glucose responsive under physiological conditions, i.e. a temperature of 37°C, pH 7.4 and a background of 150 mM sodium chloride.[37][39] Especially the behavior towards glucose has been thoroughly studied.[10][11],[14]-[16],[37][39] However, these studies have been focused only on the improvement of the transducer signal, this is the change of swelling degree in dependence on the glucose concentration. To the author's best knowledge, there is no study existing, which explains in depth the interactions between the substrate glucose and the receptor inside the hydrogel. Furthermore, only PAAm-based hydrogels have been used in glucose sensing applications. The authors wondered why this base material was preferred although the transducer signal of PNIPAAm-based hydrogels can be enhanced by its VPTT.

In this work, we analyzed three different aspects regarding the use of glucose responsive hydrogels. First, we assumed PNIPAAm being the better transducer material because of its possibility to enhance the physical signal in case the hydrogels are analyzed close to their volume phase transition temperature. To gain quantitative data for a comparison, PAAm-based hydrogels were analyzed in parallel. Using data from free swelling and methodologies from our previous report,[29] also the performance coefficients of these materials were determined. These data were used for a detailed comparison of the different factors influencing the response of the hydrogels towards glucose. For this purpose, glucose responsive hydrogels were synthesized using *3*-acrylamidophenylboronic acid (PBA) as receptor. *N*-(*3*-dimethylaminopropyl))-acrylamide (DMAPAA) was added to enable glucose recognition at physiological pH (7.4).[10][11] Second, an unambiguous sensor response would be obtained when the change of equilibrium water content would be caused only by glucose

binding to the receptor. To investigate this hypothesis, a new method was developed for the quantitative determination of bound glucose inside the hydrogels. This method was further used for the determination of the binding isotherms and, derived from that, for the calculation of affinity constants and maximal degree of complexation of boronic acid groups. The determination of these vital parameters yields direct insight into the complex formation, which has not been reported so far, allowing the determination of the glucose sensitivity of the hydrogels. The quantitative analysis of glucose binding to the receptor further allows for the quantitative comparison of these isotherms with the swelling data. For this purpose, the swelling data are fitted with the same mathematical model used for the determination of binding isotherms. This quantitative comparison reveals to which extend the transducer effect is directly influenced by glucose binding. In case, glucose binding would be the only factor influencing the transducer effect, this is the release of water in dependence of glucose concentration, both isotherms would be identical except for a conversion factor. Finally, we anticipated the results from swelling measurements in free volume being transferrable to the swelling pressure measurements. Although the polymer volume fraction has a high influence on the absolute swelling pressure,[17]-[19] the general behavior of the hydrogels should be the same. The swelling pressure as function of glucose concentration was analyzed, revealing that with some of the synthesized hydrogels, glucose can be analyzed quantitatively under physiologically relevant conditions by using a pressure sensor setup. Moreover, the stability of the hydrogels was proven for long-term use in the swelling pressure setup, experimentally demonstrated by a reproducible sensor response for up to three months.

2. Experimental

2.1. Materials

Acrylamide (AAm; 99 %), 3-acrylamidophenylboronic acid (PBA; 98 %), *N,N'*-methylenebis(acrylamide) (MBAAm; 99 %), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES; > 99.5 %), iron(II) sulfate heptahydrate (purity > 99.5 %), glucose oxidase from *aspergillus niger*, peroxidase from *horseradish* and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS; purity > 98 %) were purchased from Sigma Aldrich, St.

Louis, USA. *N*-isopropylacrylamide (NIPAAm; 99 %) stabilized with 4-methoxyphenol (MEHQ), triethylamine (99 %), and tris(hydroxymethyl)aminomethane (TRIS; 99.8 %) were received from Acros Organics, Geel, Belgium. *N*-(3-dimethylaminopropyl)-acrylamide (DMAPAA; purity > 98 %) stabilized with MEHQ was purchased from TCI Ltd., Tokyo, Japan. The photoinitiator IRGACURE 2959 was received from Ciba Specialty Chemicals Inc., Basel, Switzerland. Glucose (anhydrous, pure Ph. Eur.), potassium dihydrogen phosphate, disodium hydrogen phosphate and dimethyl sulfoxide (DMSO; 98 %) were purchased from AppliChem GmbH, Darmstadt, Germany. Sodium chloride, 1 M potassium hydroxide solution, 1 M hydrochloric acid and acetic acid were purchased from Bernd Kraft, Duisburg, Germany. Sodium acetate was received from Fluka Chemicals, Buchs, Germany. NIPAAm was recrystallized from *n*-hexane prior to use. MEHQ was removed from DMAPAA using an inhibitor remover before use. If not further mentioned the other chemicals were used as received. The water used for all syntheses and analyses was purified by an Arium Pro system from Sartorius AG, Göttingen, Germany.

2.2. Methods

2.2.1. Synthesis of hydrogels – PAAm-based hydrogels

14.1 mmol AAm, 2.3 mol% MBAAm [a], 1.4 mol% IRGACURE 2959 [b] and the corresponding amount of PBA [a] ([a] referred to the amount of AAm; [b] referred to the sum of all co-monomers; *cf.* **Table 1**) were dissolved in 1 mM HEPES buffer at pH 7.4. In parallel, a stock solution containing 21.1 mmol DMAPAA in 10 mL 1 mM HEPES buffer at pH 7.4 was prepared. A volume of the DMAPAA solution was added to obtain a total solution volume of 9 mL (*cf.* **Table 1**). Additionally, 500 – 600 µL triethylamine and 1 mL DMSO were added as co-solvents. The solutions were stirred at room temperature (rt) until all monomers were dissolved, then degassed with argon at rt for 15 min prior to use. The reaction mixtures were irradiated with UV light for 15 min at 20°C in a self-made reactor, which was connected to a heating bath (F25-EH, Julabo GmbH, Seelbach, Germany), by use of an UVA Print 100-200 HPV (Dr. Hönle AG, Gräfeling, Germany). Then the resulting hydrogels were taken out of the reactor and washed with 200 mL 1 mM HEPES buffer solution to remove unreacted monomers and the co-solvents. After approximately 6 washing steps, the buffer solution was exchanged by pure water to ensure the complete removal of all substances. The washing

process was finished when the TOC content was three times below the detection limit of 1 mg/L. The data could further be used for the calculation of the yield of the polymerization toward the hydrogel. The clean hydrogels were either cut into disks of 10 mm diameter or were granulated, via squeezing them through a mesh with help of a press, for further analyses. These samples were freeze-dried prior to use.

2.2.2. Synthesis of hydrogels – PNIPAAm-based hydrogels

PNIPAAm-based hydrogels were in general prepared according to the procedure described in **section 2.2.1**. Here, 14 mmol NIPAAm, 3.7 mol% MBAAm [a] 2.8 mol% IRGACURE 2959 [b] and 300 µL triethylamine were used ([a] referred to the amount of NIPAAm, [b] referred to the sum of all comonomers). PBA and DMAPAA were added as described above. DMSO was not needed as co-solvent, it was replaced by 1 mL HEPES buffer. Since PNIPAAm is a thermo-responsive polymer a reaction temperature of 5°C had to be used to generate the desired mechanical properties.

2.2.3. Characterization of hydrogels

Total Organic Carbon (TOC) – Analysis

During the washing procedure, a TOC sample was taken at each washing step. In dependence of the TOC concentration the sample was either not diluted or diluted with pure water with a ratio of 1:10. TOC content was measured by a Total Carbon Analyzer TOC-V_{CPN} of Shimadzu Europa GmbH.

The TOC content was determined indirectly by the measurement of the amount of total carbon (TC) and inorganic carbon (IC) (**equation 1**)

$$c_{TOC} = c_{TC} - c_{IC} \qquad (1)$$

c_{TOC}: concentration of TOC

c_{TC}: concentration of TC

c_{IC}: concentration of IC

The yield was calculated according to **equations 2** - **5**. Here, it must be considered, that the TOC content of co-solvents and HEPES buffer have to be subtracted from the total TOC amount, because these substances do not take part in the reaction (**equation 3**)

$$m_{TOC,total} = \sum_{i=1}^{n} c_{TOC,i} \cdot V_i \quad (\mathbf{2})$$

$$m_{TOC,HG} = m_{TOC,total} - \sum_{i=1}^{n} m_{TOC,HEPES,i} - m_{TOC,NEt_3} - m_{TOC,\,DMSO} \quad (\mathbf{3})$$

$$m_{TOC,HG,total} = \sum_{k=1}^{n} C\%_k \cdot m_k \quad (\mathbf{4})$$

$$yield[\%] = \left(1 - \frac{m_{TOC,HG}}{m_{TOC,HG,total}}\right) \cdot 100 \quad (\mathbf{5})$$

$m_{TOC,total}$: total mass of TOC in all washing steps

V_i: volume of washing water

$m_{TOC,HG}$: mass of TOC in washing water from unreacted monomers

$m_{TOC,HEPES}$: mass of TOC in washing water from HEPES buffer

$m_{TOC,\,NET_3}$: mass of TOC in washing water from triethylamine (NEt_3)

$m_{TOC,DMSO}$: mass of TOC in washing water from DMSO

$m_{TOC,HG,total}$: total mass of TOC in the monomers according to their initial amount in the reaction solution

$C\%_k$: carbon content of each monomer k

m_k: mass of monomer k

2.2.4. Swelling degree measurements

The swelling degree was determined gravimetrically by use of an analytical balance XT 220A (Precisa Gravimetrics AG, Dietikon, Switzerland). Before each measurement, the dry hydrogel disks were weighted to gain the mass of the pure polymer (mass(dry)) Each hydrogel sample was soaked in a separate vial in 20 mL 50 mM HEPES buffer at pH 7.4 for a minimum of 48 h and was equilibrated at the desired temperature in an ED-33 closed heating bath (Julabo GmbH, Seelbach, Germany). These parameters were kept constant

during the whole experiment. HEPES buffer was chosen, because it is proved not to change the binding constant between PBA and glucose significantly in contrast to other Lewis bases like phosphate buffer.[4][28] To analyze the swelling behavior, the glucose concentration was increased after each equilibration step, therefore fresh solutions were prepared. After each equilibration step the samples were weighted to obtain the mass of the wet hydrogels (mass(wet)). Therefore, the remaining water drops were removed thoroughly with precision paper. Dry and wet masses could be used to calculate the swelling degree SD according to **equation 6**. Thereby, each condition was tested by analyses of three independent samples. For a better comparison of the results, also the relative change of water content ΔSD was determined (**equation 7**).

$$SD = \frac{mass(wet)}{mass(dry)} \qquad (\mathbf{6})$$

$$\Delta SD = \left(\frac{mass(wet)}{mass(dry)} - 1\right) \cdot 100\% \qquad (\mathbf{7})$$

For a quantitative comparison, the initial slope K_{SD} and the maximal change of water content ΔSD_{max} were calculated using the Langmuir model[40] in an adapted way (**equation 8**). In **equation 8**, c is the glucose concentration in thermodynamic equilibrium, which is equal to the glucose starting concentration here. Due to the large solution volume, glucose uptake by the hydrogel does not change the glucose starting concentration significantly. For the calculation, the equation was used in the linear form (**equation 9**).

$$\Delta SD = \frac{\Delta SD_{max} K_{SD} c}{1 + K_{SD} c} \qquad (\mathbf{8})$$

$$\frac{1}{\Delta SD} = \frac{1}{\Delta SD_{max} K_{SD}} \cdot \frac{1}{c} + \frac{1}{\Delta SD_{max}} \qquad (\mathbf{9})$$

2.2.5. Determination of the degree of complexation

This experiment is comprised of different steps; it can be divided into the loading and the leaching steps (*cf.* **Figure S1**). In detail, it consists of two parts: the quantitative determination of glucose via enzymatic reaction and the gravimetric determination of the water content (*cf.* **Figure S1**). The gravimetric measurements were conducted as described for the swelling degree determination (*cf.* **section 2.2.4**). The enzymatic reaction was adapted to the concentration range. For a concentration range of 2 - 50 mM, the Fenton's

test[41] was used. In case the glucose concentration was above the linear range of the calibration curve, the sample was appropriately diluted. Below 2 mM, the glucose oxidase – peroxidase test was conducted by use of ABTS as chromogen.[42] For the Fenton's test the glucose oxidase concentration was adjusted to 46 U/mL and 0.5 mM ferrous sulfate was used. Two separate solutions were prepared with a background of 50 mM sodium acetate at pH 4.0.[41] Also for the ABTS test, a concentration of 46 U/mg was used for each enzyme. Both enzymes were mixed in one solution, the other solution contained 0.9 mM ABTS. Here, a background of 66.7 mM phosphate buffer saline (PBS) was used at pH 6.0. For the glucose concentration determination, 20 µL glucose solution, 100 µL enzyme solution and 100 µL dye solution were mixed in a microtiter plate. To guarantee the accuracy, a calibration curve comprised of 6 - 8 different glucose concentrations was measured in parallel to each set of samples. The absorbance was measured 30 min after preparation to ensure a complete reaction[41] with a µQuant UV/vis plate reader spectrometer of the company BioTek Instruments Inc., Winooski, USA.

The enzyme assay can be used to retrieve only the glucose concentration in the solution surrounding the hydrogel. At starting concentrations above 500 µM, the leaching of glucose is used to determine the glucose concentration inside the hydrogel. Glucose exists in two different forms inside the hydrogel, that is the glucose bound to PBA (bound glucose) and the free glucose (**equation 10**). Since the hydrogel is an absorber material that is comprised of 90 - 95 wt% water, glucose can diffuse inside the hydrogel network without being bound (free glucose). The glucose concentration is determined in thermodynamic equilibrium, so it can be assumed that the concentration of free glucose inside the hydrogel is the same as outside.[43] Therefore, the mass of free glucose can be calculated if the mass of water inside the hydrogel is known (**equation 11**). That is the mass of the wet hydrogel minus the polymer mass (dry hydrogel).

$$amount_{bg} = \sum_{i=1}^{n} amount_{fg} - \sum_{i=0}^{n} amount_{lg} \quad (\mathbf{10})$$

$amount_{bg}$: amount of bound glucose

$amount_{fg}$: amount of free glucose

$amount_{lg}$: amount of leached out glucose

$$amount_{fg} = \frac{mass_{solution,\,HG}}{density_{water}} \cdot c_{G,solution} \quad (\mathbf{11})$$

$mass_{solution,\,HG}$: mass of solution inside hydrogel

$c_{G,solution}$: concentration of glucose in solution surrounding hydrogel

According to **equation 12**, the amount of bound glucose is referred to the amount of PBA in the hydrogel sample to obtain the degree of complexation Θ.

$$\Theta = \frac{amount\ of\ glucose}{amount\ of\ PBA} \quad (\mathbf{12})$$

The measurements were conducted at different glucose starting concentrations in the range of 0.23 – 50.0 mM. The resulting degree of complexation was fitted with the Langmuir absorption model[40] (**equation 13**); by the linearization of this equation, the maximal degree of complexation Θ_{max} and the affinity constant K_{aff} were obtained (**equation 14**). Here, c is the glucose concentration in thermodynamic equilibrium. It was measured using the enzyme assay.

$$\Theta = \frac{\theta_{max} K_{aff} c}{1 + K_{aff} c} \quad (\mathbf{13})$$

$$\frac{1}{\Theta} = \frac{1}{\theta_{max} K_{aff}} \cdot \frac{1}{c} + \frac{1}{\theta_{max}} \quad (\mathbf{14})$$

2.2.6. Swelling pressure measurements

A detailed description of the setup for pressure sensor measurements can be found in the Supporting Information including a complete list of the suppliers for the custom made equipment. Also, the scheme of the measurement setup and the sensor housing are shown there (**Figure S2**).

All components were placed in a cooled incubator (**Figure S2**). The test solutions were put in 1 L bottles. A peristaltic pump was circulating the solution through the tubing system. First, the temperature was measured by a PT-100 temperature sensor, then the solution passed the pump and afterwards was transported through the hydrogel containing sensor housings. Computer switchable pinch valves allowed the selection of the respective solution. Additionally, the solution could bypass the sensor housings and was either recycled into its bottle or disposed in the waste container. A computer was controlling the valves and the

pump and was recording the data from pressure and temperature sensors. Besides the temperature sensor in the solution, the incubator contained an own temperature sensor measuring the temperature of the air inside the device. This system could operate at temperatures up to 40°C. For the measurement of the swelling pressure piezo resistive pressure sensors were used (XPM5, Measurement Specialties, TE Connectivity Ltd., Hampton, USA), which could be used for pressures between 0 - 12 bar. These pressure sensors contained an oil-filled cavity, which was sealed with a metal membrane on top of the cavity. This membrane transmitted changes of the outer pressure to the oil, which then changed its density and thereby the resistance of the pressure sensor.

The sensor housings were made of different stainless-steel plates with the same diameter (**Figure S2**). The exact description of the assembling is included in the Supporting Information. The first plate was the holder for the pressure sensor. The second plate contained the swelling chamber, i.e. the cavity the hydrogel was filled into. It was centered on top of the pressure sensor and had a diameter of 3.34 mm and a height of 1 mm. On the other side, the cavity was closed by a pressure-stable metal membrane with pores of 5 µm diameter, preventing the hydrogel from leaching and allowing the test solutions to be transported inside the hydrogel. By use of this, the volume of the cavity was fixed. A channel for the solution transport was embedded in the last plate. This plate also contained the adapters for the tubes. The gap between the metal plates was sealed with polyethylene foil.

The dry hydrogel granulate was filled in the swelling chamber and compressed by a pestle to increase the hydrogel mass, then the sensor housing was closed. Water was pumped through the sensors to moisten the hydrogels and to induce swelling. Hereby, the pressure was controlled and adjusted to a range of 3 – 7.5 bar by adding or removing some hydrogel for ensuring comparable results. For a homogenous hydration of the hydrogels they were conditioned prior to use. In dependence of the further analysis this was done with pure water or 50 mM HEPES buffer at pH 7.4. During the conditioning, the temperature was switched from 40°C to 28°C every 12 h while the solution was recycled through the sensors, these temperature cycles were repeated 7 times.

The conditions were kept constant for 36 h for the measurement with glucose containing solutions. The glucose concentration in the 50 mM HEPES solution at pH 7.4 was increased according to the following two sequences:

Sequence 1: 0, 0.1, 2.3, 10.9 mM;

Sequence 2: 0, 2.5, 5.0, 7.5, 10.0 mM.

Sequence 1 was used for a rough characterization of the hydrogels and sequence 2 for further use of the data for the mathematical analysis of the transducer effect. At every change of the solutions, the tubing system was washed for 4 min with the new solution bypassing the sensor housings and disposing the solution. The hydrogels were analyzed at different temperatures depending on the base material, i.e. 20°C, 30°C and 37°C. Overall, hydrogels were kept in the swelling pressure setup for 3 months until the pressure signal showed a reduction and there was a need for hydrogel replacement. The swelling pressure SP was recorded every 5 min, before plotted the onset pressure was subtracted from the measured value. The relative change of swelling pressure ΔSP was calculated according to **equation 15**.

$$\Delta SP = \left(\frac{pressure(glucose\ solution)}{pressure(buffer\ solution)} - 1\right) \cdot 100 \qquad (\mathbf{15})$$

3. Results and discussion

3.1. Synthesis of the hydrogels

An overview of the synthesized hydrogels and their key properties is given in **Table 1**. As we could show for previously synthesized hydrogels, TOC measurement is a suited method for the determination of the yield of hydrogels from monomer mixtures.[29] For PAAm-based hydrogels a yield of 116 ± 6 % was obtained. It was observed, that the TOC content inside the washing water was smaller than the TOC content of HEPES buffer plus DMSO plus triethylamine, which are the unreactive components. This indicates that some of the co-solvents were remaining inside the hydrogel. If none of the unreactive species was washed out, the apparent yield would have been risen to 200 %. For PNIPAAm-based hydrogels yields of 95 ± 5 % were reached.

Besides, the swelling measurements (*cf.* **chapter 3.2**) show the expected changes in dependence on PBA and DMAPAA contents.[10][25] Derived from both methods it can be assumed, that the monomers are incorporated almost quantitatively in the hydrogel

structure. Therefore, the desired ratios of the functional groups can be adjusted by the amounts of PBA and DMAPAA in the reaction solutions.

Table 1. Nomenclature and composition of all hydrogels and their corresponding properties (i.e., VPTT and initial swelling degree at 0 mM glucose, pH 7.4 and a background of 50 mM HEPES). PBA is the glucose receptor, DMAPAA (quaternary amine) enables glucose recognition at physiological pH. For a better overview, the notation of the hydrogel names is chosen as follows: A – acrylamide (AAm), N – *N*-isopropylacrylamide (NIPAAm) P – 3-acrylamido-phenylboronic acid (PBA), D - *N*-(3-dimethylaminopropyl)acrylamide (DMAPAA).

Hydrogel	Base material	P content [a] [mol%]	D content [a] [mol%]	Molar ratio D to P [-]	VPTT [°C]	Initial SD [-]	Yield [%]
14A_8P_10D	AAm	8	10	1.25	-	11.4 ± 0.6	114 ± 2 [b]
14A_8P_5D	AAm	8	5	0.63	-	9.6 ± 0.5	117 ± 5 [b]
14A_12P_15D	AAm	12	15	1.25	-	11.4 ± 0.6	113 ± 6 [b]
14A_12P_7.5D	AAm	12	7.5	0.63	-	7.3 ± 0.3	120 ± 4 [b]
14N_8P_10D	NIPAAm	8	10	1.25	50 ± 4	10.9 ± 0.3	95 ± 6
14N_8P_5D	NIPAAm	8	5	0.63	34 ± 4	5.6 ± 0.2	94 ± 5
14N_12P_15D	NIPAAm	12	15	1.25	62 ± 4	11.6 ± 0.6	88 ± 2
14N_12P_7.5D	NIPAAm	12	7.5	0.63	38 ± 4	5.8 ± 0.2	99 ± 11

[a] Amount of substance relative to the amount of base material (AAm or NIPAAm)

[b] Overestimation due to residual co-solvent in the thoroughly washed hydrogels (cf. synthesis; **sections 2.2.1**, **2.2.2** and **2.2.3**).

3.2. Determination of swelling degree

3.2.1. Results of swelling degree measurements

For further discussion it is important to note, that PBA introduces more hydrophobicity into the hydrogel network. Therefore, it is able to decrease the VPTT of PNIPAAm-based hydrogels. DMAPAA is positively charged at pH < 9. Hence, it introduces more hydrophilicity into the hydrogel; it is able to shift the VPTT of PNIPAAm-based hydrogels towards higher temperature (*cf*. **Table 1**).

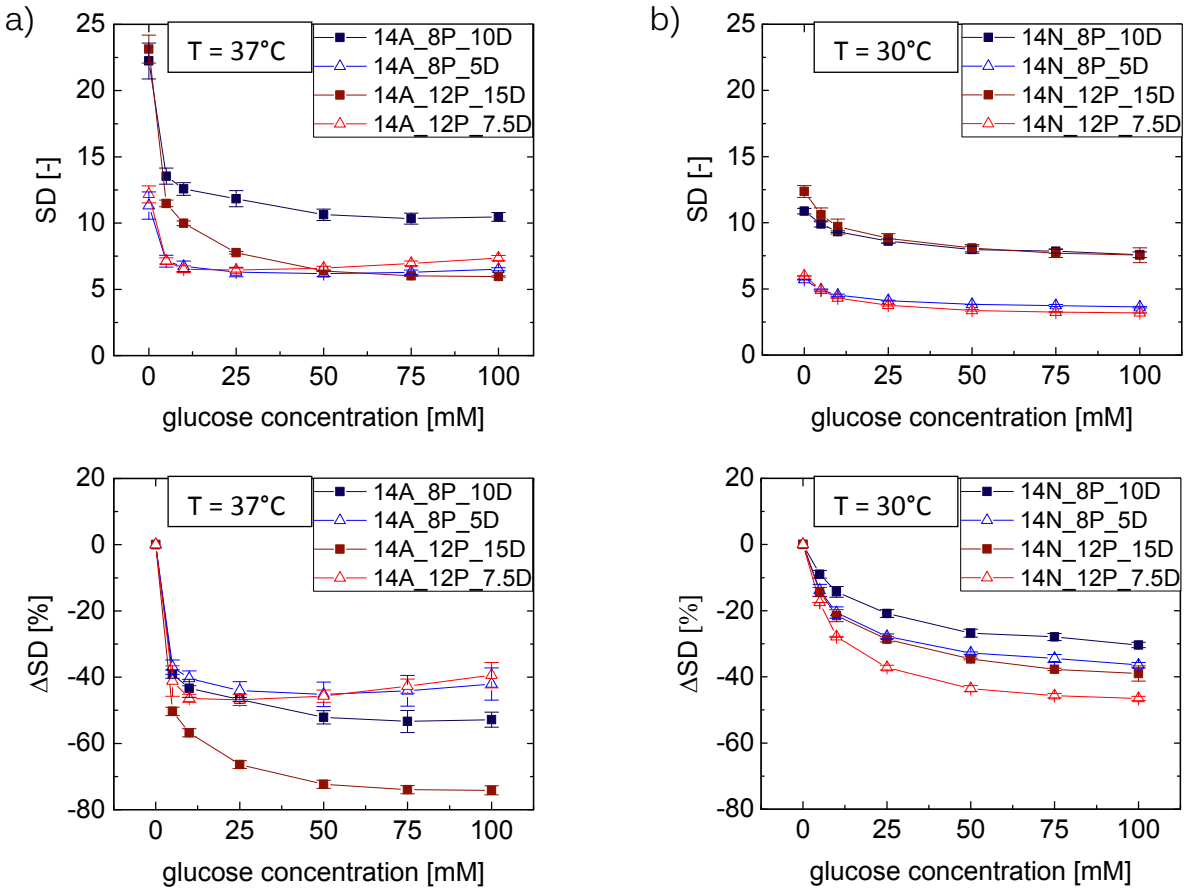

Figure 2. Swelling degree (SD) and relative change of swelling degree (ΔSD) in dependence of glucose concentration: PAAm-based hydrogels (**a**) and PNIPAAm-based hydrogels (**b**) at their optimal operating temperatures 37°C and 30°C respectively; the hydrogels were analyzed in presence of 50 mM HEPES buffer at pH 7.4.

In first experiments, the swelling degree (SD) was analyzed in a range from 0 – 100 mM to gain information about the general influence of the base material, PBA content and PBA-to-DMAPAA ratio (**Figure 2**). PAAm-based hydrogels were analyzed at the physiologically relevant temperature of 37°C. It was found that ΔSD increases with increased temperature. Since PAAm is not thermo-responsive, SD is not changing a lot in the regarded temperature range; for its dependence of SD and ΔSD on glucose concentration at 20°C see **Figure S3**. For PNIPAAm-based hydrogels, the optimal operating temperature derived from swelling pressure measurements was chosen (see **Figure S4**), to discuss the most pronounced changes. The presence of glucose caused the hydrogel to release water. The more glucose was added the larger deswelling was observed, which was found for all hydrogel compositions with either PAAm- or PNIPAAm-based materials (*cf.* **Figure 2**). It can be

concluded, that the complexation with glucose decreases the polymer network hydrophilicity. Thus, these experiments confirm earlier results.[9][10]

The SD values in absence of glucose (SD_0) (upper diagrams of **Figure 2**) clearly depend on the PBA-to-DMAPAA ratio. Hydrogels containing more PBA than DMAPAA (all open symbols, i.e. hydrogels named 8P_5D, 12P_7.5D) possess lower SD_0 values. The hydrophobic character of PBA apparently reduces the networks ability for water uptake. In contrast, higher amounts of the hydrophilic DMAPAA in the network enhance its ability for water uptake and result in higher SD values.

Additionally, the change of swelling degree in dependence of glucose concentration was measured at different pH values (*cf.* **Figure S5**). Briefly, no significant change of swelling occurs at pH 5.6, far below the pK_a of PBA. At pH 7.4 the most pronounced changes of swelling degree take place and these changes are reduced at pH 9.0 (*cf.* **Figure S5**).

Furthermore, it could be shown, that the VPTT of PNIPAAm is shifted by the introduction of PBA and DMAPAA into the hydrogel network and can be further fine-tuned by the ratio of them. Therefore, the SD was measured in dependence of the temperature in a range from 20 – 60°C; the VPTT was defined as the temperature, at which the SD displays the maximal change (according to Büning et al.[29]; see **Table S1**). The change of VPT in dependency of the comonomer ratio can also be found in swelling pressure measurements. Here, no distinct VPTT can be identified due to the continuous phase transition (see **Table S2**; *cf.* **section 3.4**).

In general, PAAm-based hydrogels show more distinct changes in SD with increasing glucose concentration and therefore larger transducer effects. These hydrogels are able to release more water in case of glucose binding compared to PNIPAAm-based hydrogels. A further influence of the PBA-to-DMAPAA ratio is especially obvious for PAAm-based hydrogels: In **Figure 2 a**, lower diagram, the solid symbols (PBA < DMAPAA) display a more pronounced deswelling compared to the open symbols (PBA > DMAPAA).

Additional swelling measurements reveal that a physiological fructose concentration would not interfere in these measurements (**Figure S6**).

3.2.2. Quantitative description of the transducer effect

Data for change of swelling degree ΔSD as function of glucose concentration were analyzed by adopting the Langmuir model according to **equation 8**. This quantitative analysis is later used for the comparison towards the binding isotherms. The calculations were conducted based on data shown in **Figure 2**. Here, the initial glucose concentration was inserted into the formula; the large initial volume of the solution guaranteed that the concentration of glucose did not change significantly upon glucose uptake. As a quantitative measure for the transducer effect the thus obtained initial slope K_{SD} and the maximal change of water content ΔSD_{max} were used (see **Figure 3**).

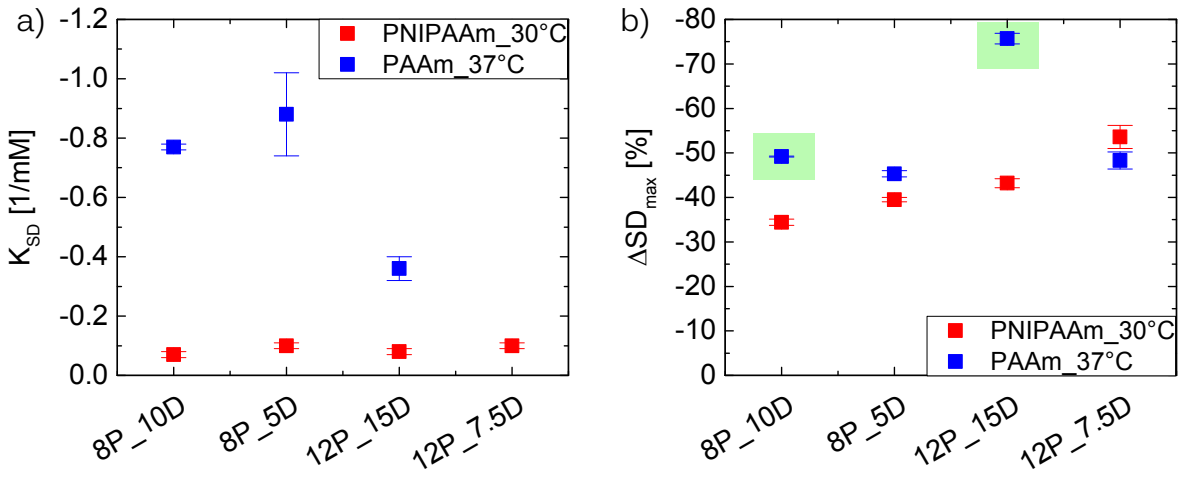

Figure 3. Comparison of initial slopes K_{SD} (**a**) and the maximal changes of water content ΔSD_{max} (**b**) of the glucose concentration dependent ΔSD curves, obtained from data analysis by adopting the Langmuir model. Comparison of PAAm- and PNIPAAm-based hydrogels at their optimal operating temperatures. The concentration ranges from 0 - 100 mM and in case of 14A_8P_10D and 14A_12P_7.5D the concentration range of 0 - 25 mM were observed. Calculations were conducted based on data shown in **Figure 2**. For 14A_12P_7.5D, no accurate value could be obtained for K_{SD}. The data for PAAm-based hydrogels are summarized in **Table S3**. For PNIPAAm-based hydrogels the data are later, in **section 3.3.3**, presented in **Table 2** (ΔSD_{max}) and **Table 3** (K_{SD}), the correlation coefficients are given in **Table S4**.

K_{SD} also shows a dependence on the hydrogel composition (**Figure 3 a**). For PAAm-based hydrogels the changes are bigger, the same is observed for materials with PBA > DMAPAA, these are the less hydrophilic ones. This is remarkable, because this behavior is opposite compared to the ΔSD_{max}, meaning that the hydrogels with the lower maximal water content

at high glucose concentrations possess the greater initial changes at low glucose concentrations and thereby represent more efficient transducers. This trend can also be found for PNIPAAm-based hydrogels, but it is not as clearly pronounced (**Figure 3 a**). The more hydrophobic compositions show the larger changes of the initial slope and hence the more pronounced transducer properties.

For PNIPAAm-based hydrogels, ΔSD_{max} (**Figure 3 b**) possesses the same trends as K_{SD}. However, the hydrophobic hydrogels (PBA > DMAPAA) show the larger transducer effect. Here, an improvement due to an increased amount of DMAPAA cannot be detected. The more PBA the hydrogels contain, the greater are the changes. In contrast, the hydrophilic PAAm-based hydrogels (PBA < DMAPAA) show the larger ΔSD_{max} (**Figure 3 b**), as it was already seen in **Figure 2**. Additionally, the higher the absolute PBA content the bigger are the ΔSD_{max}, because the presence of PBA provides more available binding sites.

3.2.3. Discussion of free swelling measurements

All hydrogels significantly release water in dependence on the glucose concentration, thus the complexation of PBA with glucose decreases the hydrophilicity of the polymer network. This behavior was analyzed by several authors, but still there is a lack of a concept, explaining this response.[4][7][9][10][14][16][28] To give more insight to the change of SD, in this study pH- and glucose-dependent measurements were conducted (*cf.* **Figure S5**). Here, it was clearly shown that at a pH ≤ 7.4 glucose binding has no effect on the swelling of hydrogels, which indicates that glucose has no relevant ability to stabilize the negative charge of PBA. At pH 7.4, the ΔSD is maximal and decreases again at pH 9.0. From pH 7.4 to pH 9.0, the SD_0 values for 8P_5D are increasing, so evidently more negative charges are introduced into the hydrogel network and the polymer chains are repelled from each other. The negative charges can still be balanced at pH 9.0 for 8P_10D due to the higher amount of positively charged DMAPAA inside these hydrogels. However, it remains unclear why glucose does not introduce a higher change of SD at pH 9.0 than at pH 7.4, although the amount of dissociated PBA is maximal. The higher the amount of charged PBA groups, the more glucose should be bound to it. This might be explained by the strong repulsion of the charges. Supposable, the charge interaction in conjunction with the increased amount of hydroxy groups is largely increasing the hydrogels hydrophilicity and hence over-compensating the

potential decrease of water content due to the introduction of hydrophobic interactions by glucose binding.

Although the amount of charges is not significantly changed by glucose binding, a general effect is occurring, which is present in both PAAm-and PNIPAAm-hydrogels. Therefore, it is assumed that glucose binding simply reduces the amount of hydrogen donor groups. These groups are necessary for water binding to the hydrogel and the more hydrogen donor groups the network contains, the more water can be taken up. Even though PBA is overall introducing hydrophobic interactions, the boronic acid group itself is hydrophilic and is able to form hydrogen bonds.[4] This effect could also be observed in case of crown ether complexation with potassium ions.[29][38]

Additionally, in these measurements the charge stabilization effect of DMAPAA is present[25] (*cf.* **Figure S5**). In case of PAAm-based hydrogels, a higher DMAPAA content evokes greater changes of water content due to its hydrophilic nature (*cf.* **Figure 3 b**).

PAAm-based hydrogels show the larger changes of the water content indicating that PAAm is better suited as base material for this application. The hydrophilic nature of this base material seems favorable for its use in glucose responsive hydrogels. This is remarkable, because temperature-responsive polymers like PNIPAAm are suggested to offer the potential for greater volume changes if the operating temperature is close to its LCST or VPTT.[38] Besides the specific effects of hydrogel composition, high initial water content in general is facilitating the change of water content in dependence of glucose concentration. In addition, these results show that also other effects do contribute to a significant volume change in dependence of glucose concentration, which cannot be clarified based on free swelling measurements only. Therefore, the substrate-receptor interactions were investigated in further experiments (see **section 3.3**). It is important for the envisioned utilization as glucose sensor that a physiological fructose concentration does not interfere with the glucose concentration dependent swelling measurements (*cf.* **Figure S6**).

In conclusion, hydrogels with PBA < DMAPAA and PAAm as base material (**Figure 3**, **highlighted in green**) are the best materials for glucose recognition in free swelling measurements. They possess the largest transducer effect and they have the highest K_{SD} indicating a high affinity towards glucose. Still, the absolute value of K_{SD} allows the accurate

determination of ΔSD in the physiological relevant concentration range. With the best material 14A_12P_15D, a maximal change of water content of 75 % could be achieved for a change of glucose concentration from 0 to 100 mM, and a change of 57 % was observed for the change of glucose concentration from 0 to 10 mM, both at 37°C.

3.3. Binding mechanism and its correlation with the transducer effect

3.3.1. Determination of binding isotherms

The newly established method for analysis of glucose bound in the hydrogel (cf. **section 2.2.5**) was used to characterize the different hydrogels. The binding isotherms were calculated according to the Langmuir model (**equation 14**), shown as continuous lines in **Figure 4**. The Langmuir and the Freundlich models, established for description of adsorption processes, are the most relevant models to describe the absorption of glucose in the hydrogels, because both models imply that a saturation of a given number of binding sites will limit the uptake. According to the Langmuir model, the binding enthalpy is not changing in dependence of the degree of complexation.[44]-[45] The Freundlich model accounts for the change of binding enthalpy with increasing degree of complexation.[44] The data were fitted according to both, Langmuir and Freundlich isotherm models. The Langmuir model yielded better fits, plausible maximal degree of complexations and affinity constants within the literature range. Additionally, also the swelling degree versus concentration curves (*cf.* **Figure 2**) could be fitted with the Langmuir model, revealing the analogous relationship between receptor and transducer effects. The Freundlich model is inferior in all of these points. Bimodal models were also considered, but these models cannot be proven consistently based on the data of **Figure 4**. The data at high concentrations, 25 and 50 mM, may point to bimodal binding site distribution, but they lack correctness for this calculation.

In **Figure 4** it is obvious that the data follow the isotherm, indicating that the Langmuir model is valid, especially at low concentrations. This is quantitatively supported by the correlation coefficients (see **Table S5**). Especially for PNIPAAm-based hydrogels good to excellent correlations are found. For PAAm-based hydrogels the lower quality of the fit can also be attributed to the lower amount of data points, in particular for hydrogels with a high

PBA content (*cf.* **Table S5**). Overall, the Langmuir model can be proven as suitable model for the description of the dependence of the degree of complexation of PBA receptor groups, Θ, on the glucose concentration.

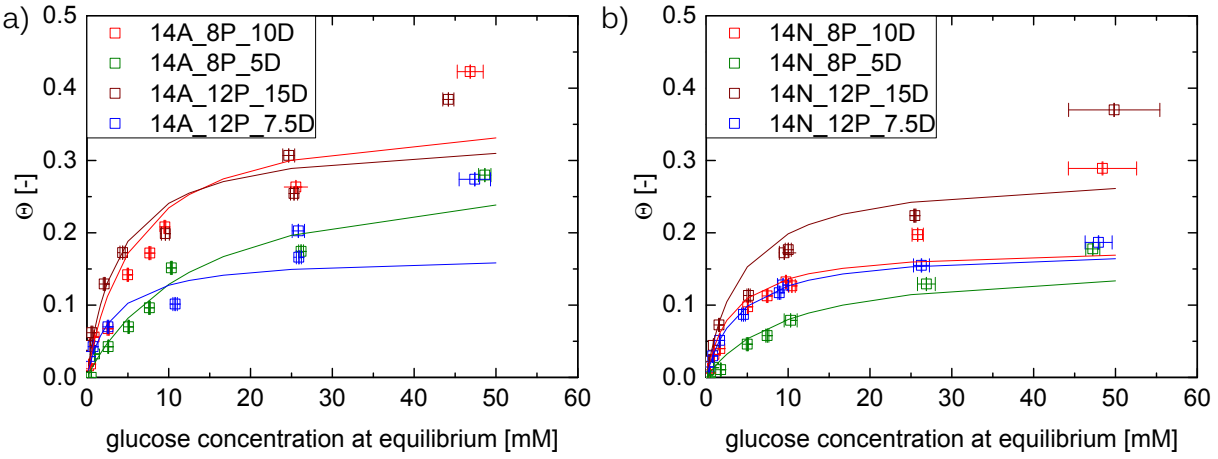

Figure 4. Degree of complexation Θ plotted against glucose concentration at equilibrium for PAAm-based hydrogels (**a**) and PNIPAAm-based hydrogels (**b**). Measurements were performed at 30°C. The hydrogels were analyzed in a background of 50 mM HEPES buffer at pH 7.4.

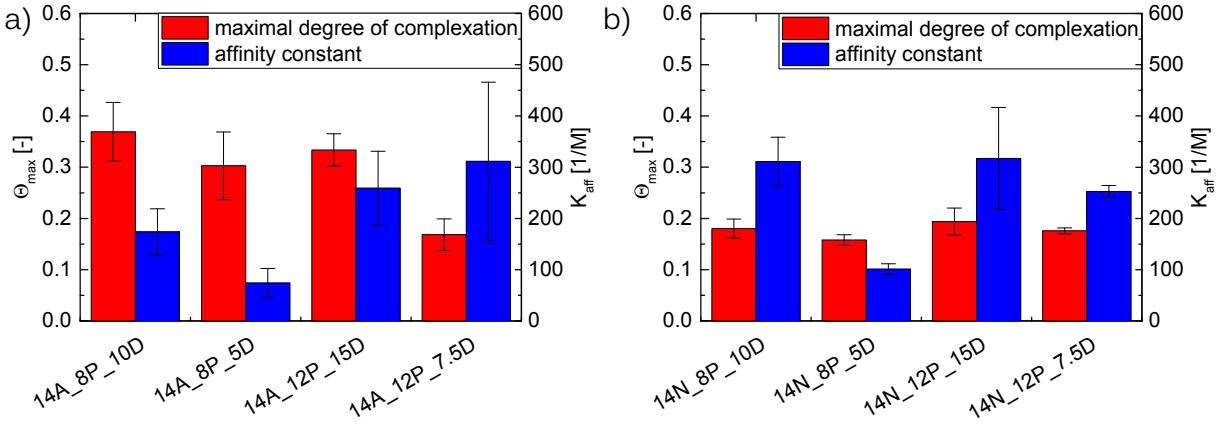

Figure 5. Maximal degree of complexation Θ_{max} and affinity constants K_{aff} for PAAm-based hydrogels (**a**) and PNIPAAm-based hydrogels (**b**). Coefficients were determined by the linearization of the Langmuir model (**equation 13**) for adsorption based on data shown in **Figure 4**.

The maximal degree of complexation Θ_{max} and the affinity constants K_{aff} (*cf.* **equation 14**) are used for quantitative comparison of the different materials and compositions (**Figure 5**). The large error for 14A_12P_7.5D is due to the small data set available for this hydrogel. Θ_{max} is rather the same for each base material and hence, independent of the composition for each base material.

3.3.2. Discussion of the binding isotherms

The results from **Figure 5** allow drawing the conclusion that for all hydrogels almost the same number of binding sites is accessible. K_{aff} increased up to three-fold compared to literature values.[4][28] Remarkably, K_{aff} values are dependent on the hydrogel composition, especially for PNIPAAm-based hydrogels. The PBA content and the PBA-to-DMAPAA ratio are the main factors influencing K_{aff}.

For the calculation of Θ, a 1:1-complexation was assumed, because so far, no method is existing that can directly provide evidence for the kind of complex formation of glucose with PBA inside hydrogels. Consequently, the values should reach 1 for 1:1 complex formation and 0.5 for 2:1 complex formation. All results show that Θ has its maximum significantly below 0.5; this strongly indicates the formation of a 2:1 complex between PBA and glucose (*cf.* **Figure 4**). Hence, these measurements are suggesting a 2:1 complex formation inside the hydrogel for the first time in literature.

Considering the maximal coverage (*cf.* **Figure 5**), 50 – 80 % of all binding sites are occupied. It reveals that for each hydrogel an equal number of PBA groups are in sufficient proximity to each other to form stable 2:1 complexes with glucose. Additionally, the density of the polymer chains is low enough to allow an efficient diffusion of glucose towards the binding sites. The accessibility of binding sites is depending on the base material as the lower maximal degree of complexation of PNIPAAm-based hydrogels shows. At the same time, also the swelling degree of these hydrogels is lower although they contain the same amount of substances as PAAm-based hydrogels with the same composition. Hence, the density of the polymer chains is higher and may therefore limit the accessibility of the binding sites. Also, PNIPAAm-based hydrogels could take up less glucose due to their hydrophobic nature. This effect could limit the amount of glucose interacting directly with the polymer network.

In general, the K_{aff} values for PBA inside the hydrogels (*cf.* **Figure 5**) are in the same order of magnitude as literature values for the complexation of boronic acid with glucose in solution.[4][28] Surprisingly, in this study only the low affinity constants (e.g. for 14A_8P_5D and 14N_8P_5D) match the literature values ($\approx 100\ M^{-1}$).[4][28] Especially 14A_12P_15D, 14A_12P_7.5D and 14N_12P_15D possess up to threefold higher affinity constants. This is presumably due to the beneficial charge stabilization by DMAPAA.[4][25] Since the PBA groups

are covalently attached to the polymeric hydrogel also multivalent binding of glucose to PBA according to the definition of Jiang et al.[46] could further enhance glucose binding affinity. This significant increase of K_{aff} allows the distinct determination of the glucose concentration in the physiological concentration range, which is 3 – 10 mM. Tailored receptors may possess affinity constants about 3000 M^{-1} or higher and may have a high selectivity towards glucose, but thereby they are shifting glucose recognition from the mM range into the µM range.[5][46]

Data in **Figure 5** also reveal that K_{aff} exhibits a significant dependence on the hydrogel composition. This is remarkable, because K_{aff} is different although the same molecular entity is used as receptor. According to Norrild and Eggert, PBA binds most likely to the furanose form of glucose, which is present only in a small fraction in solution (*cf.* **section 1**).[27] In case the furanose form is removed from the solution enough pyranose form is present to convert into the furanose form. Since the fraction of furanose form in solution is the lowest, the adjustment of the equilibria between the different forms is not limiting the complex formation between PBA and glucose. Furthermore, glucose could bind to the polymer backbone instead of binding to PBA. Kawasaki et al. studied the influence of glucose on PNIPAAm-based hydrogels.[26][43] They did observe an influence of the SD on glucose concentration but proved that glucose did not bind to the polymer (this effect will below be referred to as “sugaring out”). Similar observations were made for PAAm-based hydrogels; PAAm does not even show a change of SD in dependence of glucose concentration.[43] These results from literature could be confirmed in this study by the analysis of PNIPAAm and PAAm hydrogels without PBA or DMAPAA by the quantitative analysis of binding of glucose (*cf.* **section 2.2.5**); no binding to the polymer could be detected. Additionally, these effects would not be present in a different intensity for different PBA-containing hydrogels being comprised of the same base material, since all hydrogels possess the same amount of base material. Moreover, it was observed that at most starting concentrations the amount of bound glucose exceeded the amount of free glucose inside the hydrogel. This proves a significant amount of glucose being directly bound to the hydrogel, which then can be evoked only by the complexation of PBA. It seems reasonable to assume that simply PBA and DMAPAA can contribute to the different affinity constants. Consequently, the only possible reason is that the receptor PBA itself may change its properties since both the neutral and the dissociated forms have different affinity constants towards glucose, K_{trig} and K_{tet} (*cf.*

section 1).[4] This indicates that the amount of charged PBA is strongly depending on the surrounding conditions, because solely the charged form can efficiently contribute to glucose binding. If the amount of charged PBA groups is changing in dependence of pH and DMAPAA content, also the amount of glucose bound to PBA inside the hydrogel will change. Indeed, glucose can bind up to three times more efficiently compared to only PBA-containing hydrogel in presence of a small amount of tertiary amine groups.[4][28] Since the analysis of Θ does not allow clear distinction between the neutral and the dissociated form, K_{aff} seems to change. This is possible, because all forms are linked with each other by equilibrium reactions. Indeed, K_{aff} is the sum of K_{trig} and K_{tet} contributing to different extents depending on the surrounding and the composition of the hydrogels.

3.3.3. Comparison of the receptor interactions (degree of complexation) to the transducer effect (free swelling)

The results from free hydrogel swelling as function of glucose concentration and the glucose binding isotherms for these hydrogels could both be fitted according to the Langmuir model (**equations 9** and **14**). This should enable the comparison of the transducer effect, derived from the ΔSD data, to the receptor effect, represented by Θ, for different hydrogels.

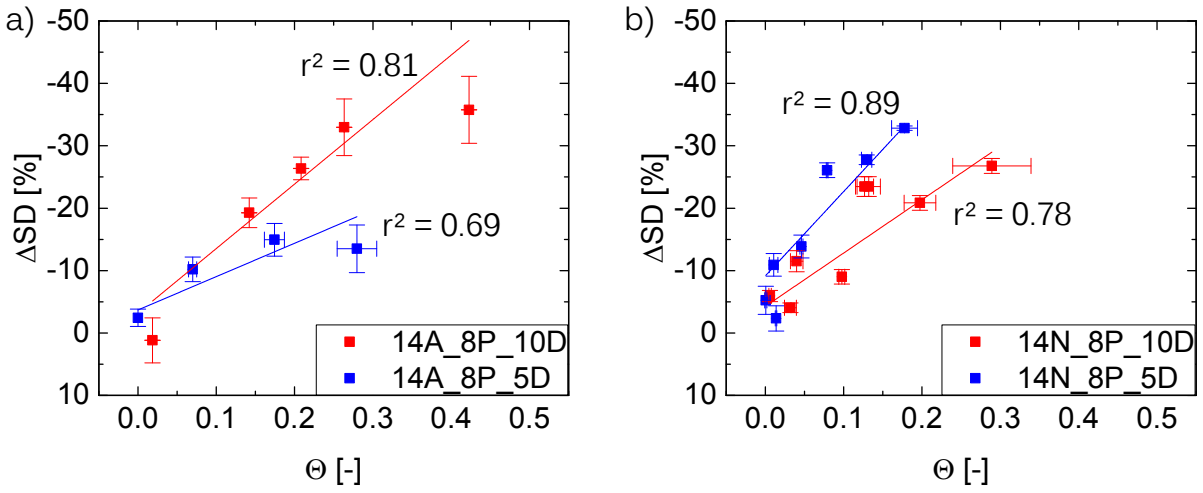

Figure 6. Dependence of relative change of swelling degree ΔSD on degree of complexation Θ with glucose for PAAm-based hydrogels (**a**) and PNIPAAm-based hydrogels (**b**); measurements were conducted at 30°C at a background of 50 mM HEPES buffer at pH 7.4.

A direct comparison of **Figure 2 a** and **Figure 4** reveals for PAAm-based hydrogels that both ΔSD and Θ show the same trends: A higher PBA content and PBA < DMAPAA enhance effects

of glucose binding and glucose recognition, although the influences are more pronounced for ΔSD. Both effects show its maximum for a glucose concentration in the range 50 – 100 mM, indicating that the change of ΔSD is a direct consequence of the increased degree of glucose binding. ΔSD is plotted against Θ (**Figure 6 a**). The linear relationship between both values demonstrates the direct dependence of the transducer effect on the degree of saturation of the receptor.

The linear relationship between ΔSD and Θ is also observed for PNIPAAm-based hydrogels (**Figure 6 b**). However, in contrast to PAAm-based hydrogels the dependence on the hydrogel composition is showing opposite trends. The hydrogel with a lower DMAPAA-content (14N_8P_5D) exhibits greater changes of ΔSD. The hydrogel with a higher DMAPAA content (14N_8P_10D) reaches also a higher degree of complexation. Evidently, the latter is possessing a smaller transducer effect in case the same amount of glucose is bound to the hydrogels.

The maximum change of free swelling (obtained from the Langmuir analysis), ΔSD_{max}, also named performance coefficient as measure for the transducer effect, is compared to Θ_{max} as measure for the receptor effect (**Figure 7 b**, **d**, **Table 2**). Also, the initial slope of ΔSD vs. glucose concentration, K_{SD} (*cf.* **equation 8**), is plotted against K_{aff} derived from the binding isotherms (**Figure 7 a**, **c**, **Table 3**). These values are crucial regarding the future application because a glucose responsive hydrogel should possess both a high sensitivity towards glucose in the physiological concentration range and a distinct transducer effect in dependence of the glucose concentration. A high sensitivity demands a distinctive change of Θ in the considered concentration range, which is defined by K_{aff}.

For PAAm-based hydrogels, the comparison of K_{SD} with K_{aff} (*cf.* **Figure 7 a**) revealed opposite influences . Here, the K_{SD} values were smaller for hydrogels with contents of PBA < DMAPAA. At the same time, these hydrogels showed higher affinity constants. (For 14A_12P_7.5D the results for K_{aff} lack accuracy and are not considered here). Indeed, the results implied that although more glucose was bound to the hydrophilic hydrogels (PBA < DMAPAA), the hydrophobic ones (PBA > DMAPAA) showed the greater changes of ΔSD. Derived from Θ at low concentrations (< 2 mM), no significant amount of glucose was bound under these conditions (see **Figure S5**). The difference to the above discussed results (*cf.* **Figure 7 a**) was that here the changes of ΔSD were larger for the more hydrophilic hydrogels. If ΔSD_{max} was

compared to Θ_{max} for PAAm-based hydrogels (*cf.* **Figure 7 b**), the results followed the familiar trends. The higher the PBA and the DMAPAA content, the higher was ΔSD_{max} and Θ_{max}. Evidently only at low concentrations the hydration of PAAm-based hydrogels was influenced by another factor than glucose binding. It is possible that glucose changes the hydration state of the hydrogel without being bound to PBA (the phenomenon called "sugaring out").

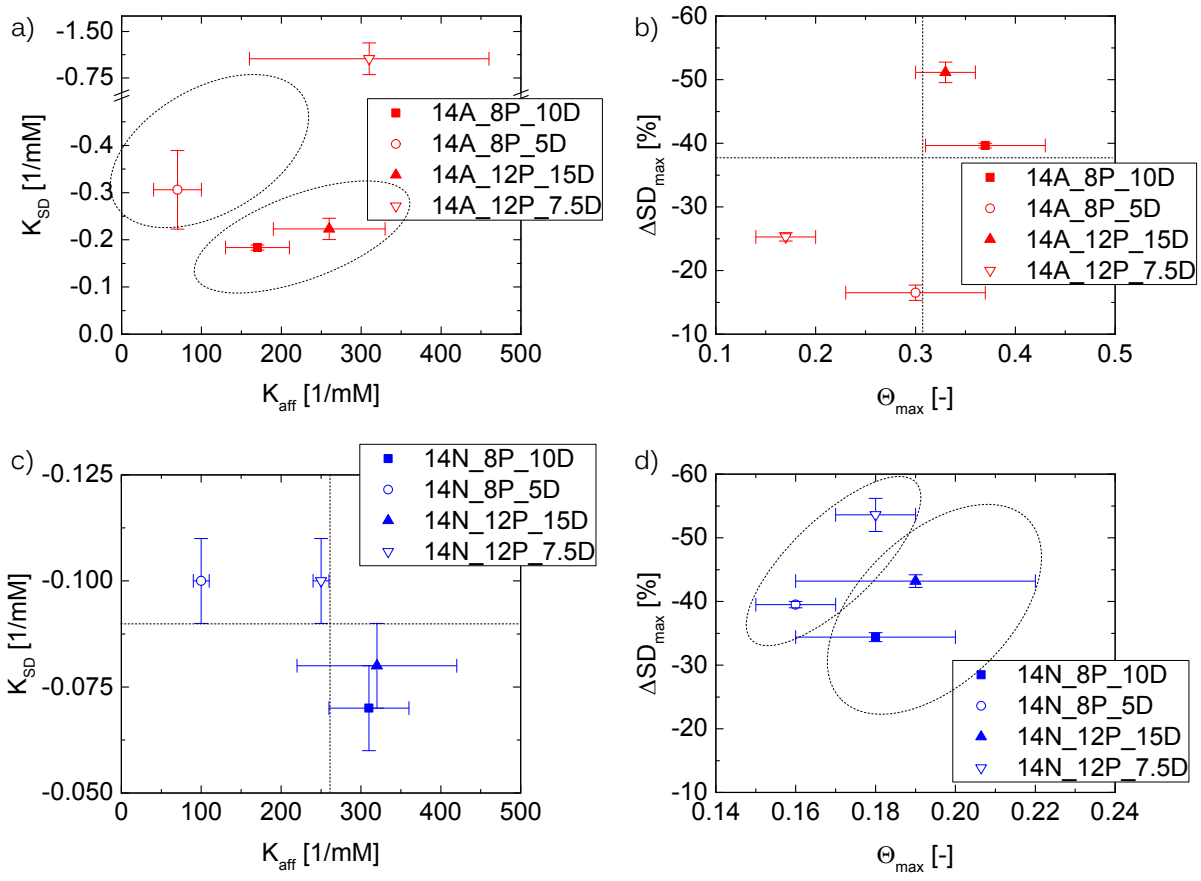

Figure 7. Relationships between initial slope of ΔSD versus glucose concentration, K_{SD}, and glucose affinity constant K_{aff} (**a**, **c**) and between ΔSD_{max} and maximal degree of complexation Θ_{max} (**b**, **d**) for PAAm-based hydrogels (**a**, **b**) and PNIPAAm-based hydrogels (**c**, **d**); the measurements were conducted at 30°C at a background of 50 mM HEPES buffer at pH 7.4.

Table 2. Comparison of ΔSD_{max} (free swelling) to Θ_{max} (from analysis of hydrogel-bound glucose). All measurements were conducted at 30°C at a background of 50 mM HEPES buffer at pH 7.4. Correlation coefficients are summarized in **Table S4** and **Table S5**.

Hydrogel	ΔSD_{max} (free swelling) [%]	Concentration range (free swelling) [mM]	Θ_{max} [-]
14A_8P_10D	-39.6 ± 0.4	0 - 100	0.37 ± 0.06
14A_8P_5D	-16.5 ± 1.2	0 - 25	0.30 ± 0.07
14A_12P_15D	-51.6 ± 1.0	0 - 100	0.33 ± 0.03
14A_12P_7.5D	-25.3 ± 0.7	0 - 50	0.17 ± 0.03
14N_8P_10D	-34.4 ± 0.7	0 - 100	0.18 ± 0.02
14N_8P_5D	-39.5 ± 0.5	0 - 100	0.16 ± 0.01
14N_12P_15D	-43.2 ± 1.0	0 - 100	0.19 ± 0.03
14N_12P_7.5D	-53.6 ± 2.6	0 - 100	0.18 ± 0.01

Table 3. K_{SD} determined according to Langmuir model applied to free swelling data; for some hydrogels the concentration range had to be reduced to gain valid results. Constants are compared with affinity constants K_{aff} determined by use of the Langmuir model based on Θ from analysis of hydrogel-bound glucose. Data are shown for the measurements at 30°C at a background of 50 mM HEPES buffer at pH 7.4. Correlation coefficients are summarized in **Table S4** and **Table S5**.

Hydrogel	K_{SD} (free swelling) [1/mM]	Concentration range (free swelling) [mM]	K_{aff} [1/M]
14A_8P_10D	-0.18 ± 0.01	0 - 100	170 ± 40
14A_8P_5D	-0.31 ± 0.08	0 - 25	70 ± 30
14A_12P_15D	-0.22 ± 0.01	0 - 100	260 ± 70
14A_12P_7.5D	-1.06 ± 0.20	0 - 50	310 ± 150
14N_8P_10D	-0.07 ± 0.01	0 - 100	310 ± 50
14N_8P_5D	-0.10 ± 0.01	0 - 100	100 ± 10
14N_12P_15D	-0.08 ± 0.01	0 - 100	320 ± 100
14N_12P_7.5D	-0.10 ± 0.01	0 - 100	250 ± 10

For PNIPAAm-based hydrogels both comparisons K_{SD} versus K_{aff} (**Figure 7 c**) and ΔSD_{max} versus Θ_{max} (**Figure 7 d**) showed opposite trends. Hydrogels with higher K_{SD} possessed smaller affinity constants and hydrogels with higher ΔSD_{max} had smaller degrees of complexation. The same trends were demonstrated in **Figure 6**. In conclusion, another effect, not only glucose binding, affects the ΔSD over the whole concentration range, especially for hydrophobic hydrogels (PBA > DMAPAA). Glucose may affect the change of water content without being bound to PBA. This is remarkable, because for the hydrophilic

PNIPAAm-based hydrogels (PBA < DMAPAA) these measurements could clearly show the benefit of DMAPAA for glucose binding due to the charge stabilization of PBA (*cf.* **Figure 4**, **Figure 7**).[10][14][25]

Putting all these results together, the presence of glucose is changing the hydration state of the polymer network itself without the need of binding to PBA. In all cases glucose is increasing the network hydrophobicity and causing the water to be repelled out of the network. This is counterintuitive, because the hydrophilic glucose is expected to interact with the hydrophilic parts of the polymer network. In conclusion, these results are an evidence for the so called "sugaring-out"-effect. This effect could be proven for pure PNIPAAm hydrogels by Kawasaki et al.,[43] explained by a decrease of the chemical potential of water in case the glucose concentration is increased. Thereby, the amount of structured water in the hydrogel is decreased and the VPTT is shifted to lower temperature. For pure PAAm hydrogels this phenomenon was not observed.[43] In contrast to the pure PAAm hydrogels of Kawasaki et. al.[43], the hydrogels of the present study contain phenyl rings of PBA, which are also surrounded by structured water due to the hydrophobic effect. This is analogous to the isopropyl groups of pure PNIPAAm hydrogels. Therefore, glucose decreases the amount of structured water also in case of PAAm-based PBA-containing hydrogels, thus causing the hydrogels to repel water upon increasing glucose concentration. Obviously, this effect occurs especially in the more hydrophobic PNIPAAm-based hydrogels with PBA > DMAPAA, because they contain the highest amounts of hydrophobic groups and, hence, structured water in absence of glucose.

The opposite effect compared to that of adding glucose is present for HEPES buffer as is pointed out in the determination of VPTT either in pure water or in HEPES buffer (*cf*. supporting information, **Table S1** and **Table S2**). In presence of HEPES, the VPTT is shifted above 60°C (14N_12P_15D) while it is around 42°C in pure water. Here, HEPES is increasing the network hydrophilicity, therefore more water can be bound in the hydrogel. This phenomenon is known as "salting in" effect. This effect is presumed to occur for electrolytes with large partial molar volume, a property valid for HEPES in comparison to the partial molar volume of water in the binary mixture.[47]-[50] However, this effect is also claimed to be caused by the ability of osmolytes, such as HEPES, to contribute to the polarization of bonds in the polymer, e.g. amide bonds.[51]-[54] As a consequence of the greater polarization, these

bonds can build more hydrogen bonds with the surrounding water.[51][52] Although the latter theory is thoroughly studied, a contribution of the large partial molar volume of HEPES cannot be excluded.

In general, PAAm-based hydrogels possess both a larger transducer effect and a higher affinity for glucose. This can be further enhanced by the appropriate ratio of comonomers, which is PBA < DMAPAA. Thus, these hydrogels are more suited as sensor materials. Another advantage of these hydrogels is the very low concentration range (< 2 mM) where the "sugaring-out" effect is influencing the transducer interactions. Since this effect is not present in the physiological concentration range (3 - 10 mM), the transducer effect is depending only on the amount of bound glucose here.

3.4. Swelling pressure measurements

3.4.1. Results of swelling pressure determination

Swelling pressure (SP) measurements were conducted in the physiologically relevant glucose concentration range (3 – 10 mM). The dependence of the SP and the ΔSP on the glucose concentration is shown in **Figure 8**. Like the SD in free swelling experiments the SP is decreasing with increased glucose concentration. However, in contrast to the SD changes with glucose concentration are almost linear. In the course of the swelling pressure measurements with variations of parameters including glucose concentration, intervals of reproducibility measurements were conducted in HEPES buffer at 20 and 37°C (**Figure S7**). The plot shows that the swelling pressure for the buffer without glucose is the same at different days during the measurement period. These data reveal that the hydrogels are mechanically long-term stable for at least 3-4 months.

Derived from data shown in **Figure S8** and **Figure S9**, the "sugaring out" effect is not as pronounced in the swelling pressure measurements but still significant.

The influence of the hydrogel composition on SP and ΔSP is obvious in **Figure 8**. As in free swelling hydrogels with PBA < DMAPAA possess the larger changes and PAAm emerges as the most suited base material. Besides the composition, the volume fraction of polymer, Φ, which is proportional to the mass of the hydrogel placed in the same swelling chamber (**equation 16**), was found to have a significant influence on the swelling behavior. At first, its

influence on the initial SP was investigated (**Figure S10 a**). The higher Φ, the higher is the measured pressure in the sensor; in these measurements each hydrogel was assigned to a certain pressure sensor housing (**Figure S10 a**, **b**). This is in accordance to the results of Wack and Ulbricht[18] In case one hydrogel is put in different pressure sensor housings, there is no relationship between Φ and the resulting SP. This is caused by the different amplifications of each individual pressure sensor and this problem could only be solved by a (re)calibration of the sensors what had not been possible in the frame of the study. These measurements also reveal that the same changes of SP are created for certain hydrogel compositions in case the same housing and the same degree of filling are used (*cf.* **Figure S10**). This proves the general reproducibility of these measurements.

$$\Phi = \frac{polymer\ mass}{volume\ of\ swelling\ chamber} \qquad (\mathbf{16})$$

Further, the influence of Φ on the maximal ΔSP in presence of 10 mM glucose was studied. As it is apparent in **Figure S10 b**, the higher Φ is, the smaller is the maximal ΔSP. Since Φ has a high influence on ΔSP, measured ΔSP data are divided by Φ to account for this influence. As shown for the ΔSP data in **Figure 8**, the base material and the PBA-to-DMAPAA ratio have the highest impact on the transducer effect. PAAm-based hydrogels show larger changes, and in case of PBA < DMAPAA the changes are higher than in case of PBA > DMAPAA. The influence of the temperature is depending on the hydrogel composition. It has a significant influence for PBA < DMAPAA. Derived from temperature-dependent measurements of ΔSP (*cf.* **Figure S4**), recorded for the glucose concentration range from 0 to 10.9 mM, PNIPAAm-based hydrogels show the largest changes at an operating temperature of 30°C. Therefore, 30°C is assumed to be the best suited operating temperature for these hydrogels. For PAAm-based no significant temperature dependence is present at temperatures between 30°C and 37°C.

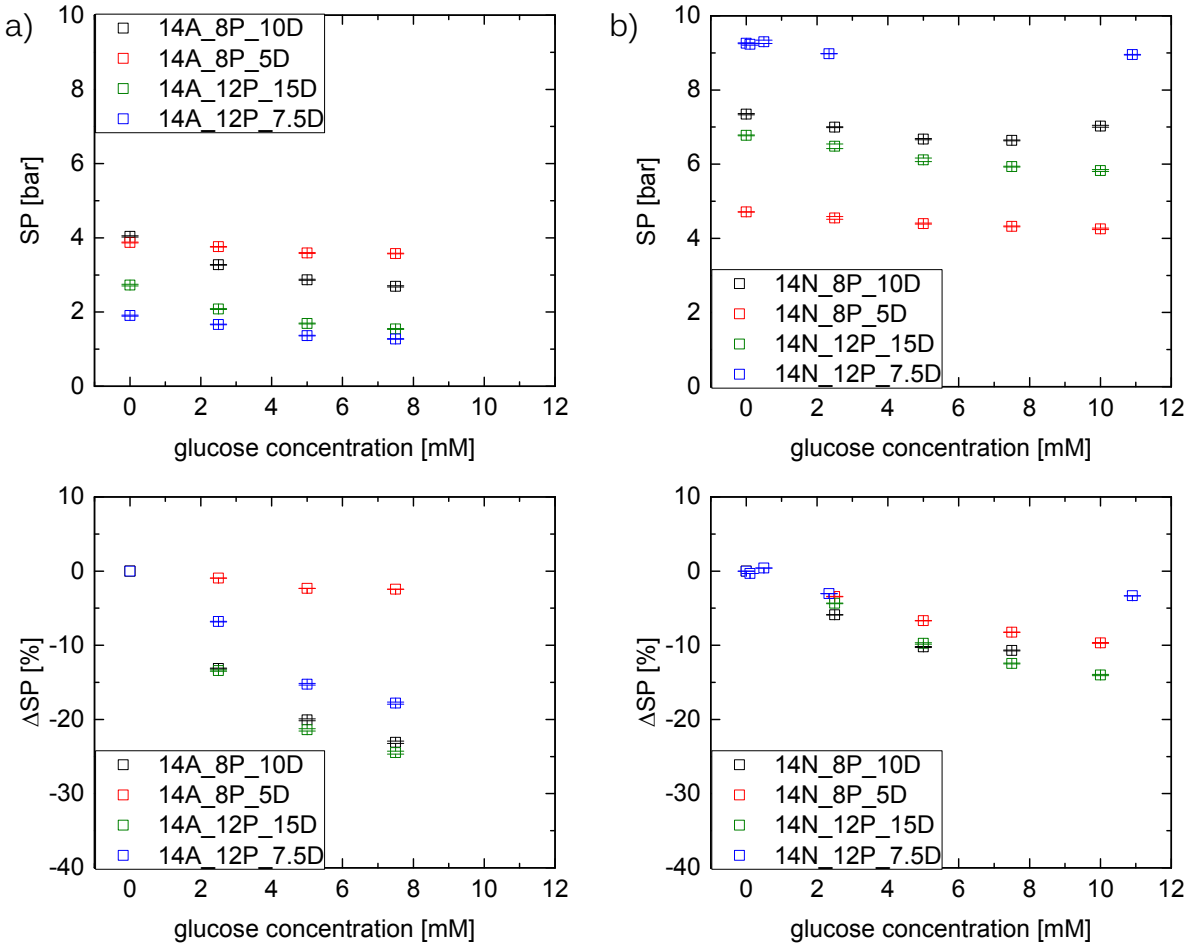

Figure 8. Swelling pressure (SP) and relative change of swelling pressure (ΔSP) in dependence of glucose concentration of PAAm-based hydrogels (**a**) and of PNIPAAm-based hydrogels (**b**); measurements were conducted at 30°C with a background of 50 mM HEPES buffer at pH 7.4.

3.4.2. Comparison of free swelling and swelling pressure measurements

The results of free swelling and swelling pressure measurements are compared, and the influences of the composition are discussed in detail based on the quantitative comparison of the maximal changes in free swelling and in swelling pressure measurements (**Figure 9**). The maximal ΔSD or ΔSP (at 10 mM) is used for the quantitative comparison; this is defined as performance coefficient. In case of the swelling pressure measurements the results are shown for the smallest Φ, which exhibits the largest changes.

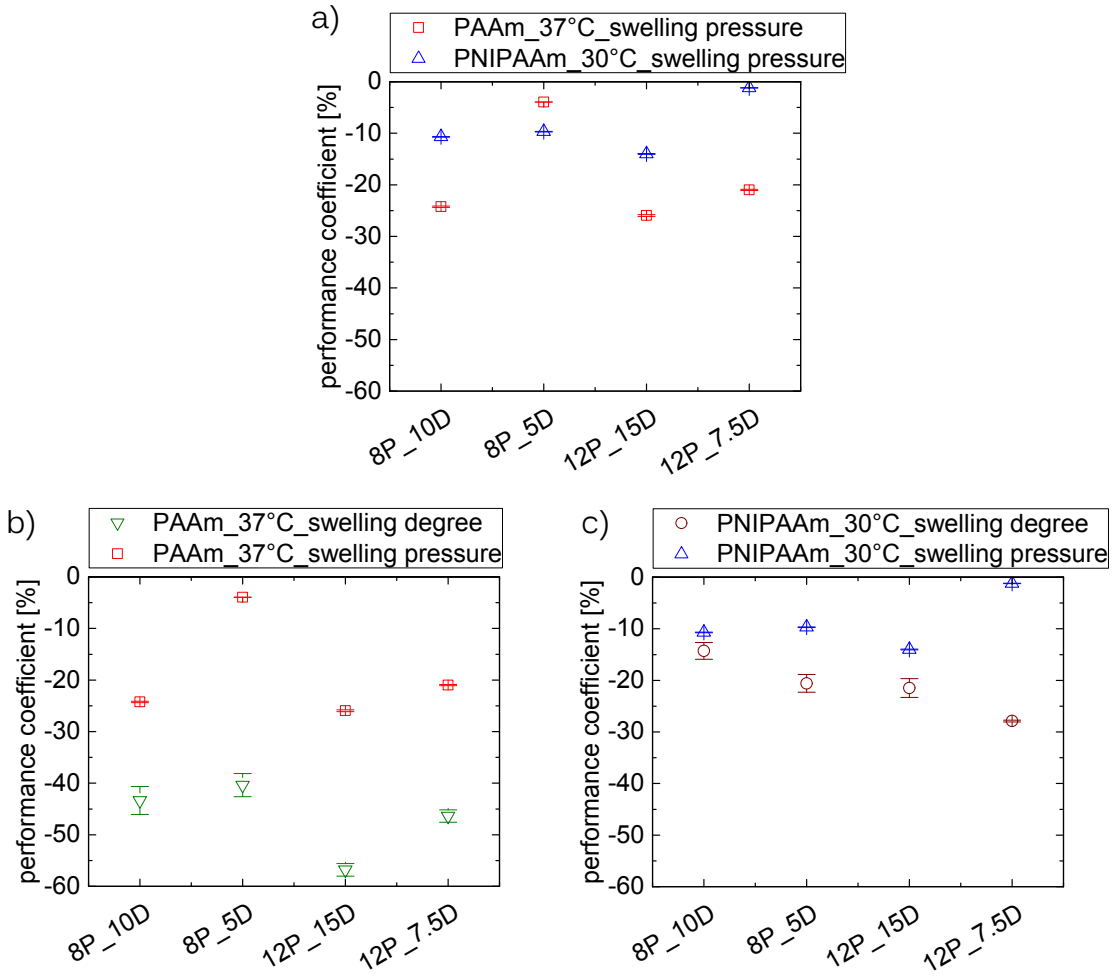

Figure 9. Comparison of performance coefficients (effect of changing glucose concentration from 0 to 10 mM) in swelling pressure setup of PAAm- and PNIPAAm-based hydrogels at 37°C and 30°C (**a**); comparison of performance coefficients in unlimited volume and in pressure sensors for PAAm-based hydrogels at 37°C (**b**) and PNIPAAm-based hydrogels at 30°C (**c**); all measurements were conducted in a background of 50 mM HEPES buffer at pH 7.4.

PAAm-based hydrogels show the larger performance coefficients (**Figure 9 a**), as it was also observed in the free swelling measurements. A clear influence of the PBA-to-DMAPAA ratio, especially for PAAm-based hydrogels, is also apparent in swelling pressure measurements (**Figure 9 a**) and these effects are more pronounced compared to measurements in unlimited volume (**Figure 9 b, c**). However, the PBA content has less influence on the change of swelling pressure.

Furthermore, the shape of the curves as function of glucose concentration is largely different. While the ΔSD curves can be fitted by use of the Langmuir model (*cf.* **Figure 2**), the ΔSP curves are rather linear (**Figure 8**). This difference is due to the change from isobaric to

isochoric conditions in the measurement setup; the different shape of curves results from the different contributions of polymer-water interactions to the change of water content (cf. **section 3.4.3**).

3.4.3. Discussion of swelling pressure data and suitability for glucose sensing

The implementation of the hydrogels in the pressure sensor setup and their resulting swelling pressure in relation with their free swelling data is crucial for establishing an application in a biomedical sensor. While free swelling occurs under isobaric conditions, the switch to isochoric conditions introduces other influencing parameters. Each pressure sensor has its own amplification, i.e. the transformation of the deformation of the pressure sensors' metal membrane into the resistance of the piezo-resistive pressure sensor, but such differences can be addressed by a calibration. However, the most important difference between both types of measurements is the water content of the hydrogels. In free swelling the amount of water inside the hydrogel is determined by the equilibrium between the thermodynamic driving force for mixing between water and polymer and the elastic forces of the polymer network.[55] Thereby, hydrogels are analyzed in swelling equilibrium. In contrast, Φ determines the amount of water inside the swelling pressure measurement system since the volume of the swelling chamber is fixed. Here, five factors are influencing the SP (**equation 17**). These effects are the mixing of polymer and solvent (Flory-Huggins model) and the elastic forces limiting the expansion of the polymers (phantom model), represented by the mixing term, p_{mixing}, and the elastic term, $p_{elastic}$.[18][55] A term regarding the Donnan potential, p_{ion}, is included since the polymer network contains ionic groups.[18] The model is completed by a term for binding including crosslink formation between PBA and glucose, $p_{complexation}$, and a term for the change in hydration state of the polymer backbone due to the presence of glucose, $p_{hydration}$ (considering the "sugaring out" effect). For swelling pressure measurements, the contributions of the mixing term and the elastic forces are present but significantly reduced in comparison to free swelling measurements, thus the expansion of the polymer, and hence the water uptake, are limited. Consequently, the action of the intrinsic elastic forces of the polymer network is (partly) replaced by the confinement within the hydrogel chamber. Unoccupied binding sites for hydrogen bonds are prerequisite for the generation of swelling pressure, so these hydrogels are analyzed below swelling equilibrium. Therefore, Φ influences the absolute values of SP and ΔSP. And the

effects of glucose concentration on SP and ΔSP can be described by the terms $p_{complexation}$ and $p_{hydration}$ in **equation 17**.

$$SP = p_{mixing} + p_{elastic} + p_{ion} + p_{complexation} + p_{hydration} \qquad (17)$$

The impact of the different initial water content becomes obvious when the results for the maximal ΔSD and the maximal ΔSP are compared (*cf.* **Figure 9 b** and **c**). In all cases, the maximal changes are larger in free swelling. First, these hydrogels contain more water (at 0 mM glucose), which can be released due to glucose binding. Second, under isobaric conditions a high contraction of the hydrogels, i.e. the release of water in conjunction with a reduction of the hydrogel volume, can be achieved. At sufficiently large Φ, a contraction of hydrogels is not possible in pressure sensor measurements because of the fixed volume of the polymer inside the swelling chamber (in order to utilize the hydrogel as transducer, it is actually mandatory to avoid that the volume of the swollen hydrogel is smaller than the volume of the chamber because under such condition no change in swelling pressure can be recorded). Instead, the hydration state of the polymer is changed in presence of glucose (*cf.* **equation 17**).

Using an analogy, in SP measurements the polymer behaves like a compressed spring. The more polymer is filled into the swelling chamber, the bigger is the force on the surfaces of this chamber in case the dry polymer is hydrated. This explains why a higher Φ causes higher SP values. Relatively it can be taken up more water if Φ is smaller, meaning that the hydration is more effective in this case. If glucose is added, it binds to PBA and therefore occupies possible binding sites for hydrogen bonds. This is the reason why a smaller Φ is causing larger changes of ΔSP.[18][19] The lower Φ of the hydrogel inside the chamber, the more water it contains at the beginning of the measurement and the more bound water can be released due to glucose binding. Although it is generating a smaller initial pressure,[18] the impact on the relative change is larger. More important, the reduced amount of binding sites for hydrogen bonds reduces the driving force for water uptake. Therefore, the force of the polymer network imposed onto the chamber surface is reduced; the more bound water is replaced by glucose the greater is this reduction. While the water content inside the swelling chamber is approximately constant, the bound water is converted into free water as consequence of glucose binding. For amphiphilic polymeric networks, the release of bound water may also trigger the additional release of structured water (associated with

hydrophobic groups). Additionally, glucose may evoke the "sugaring-out" effect under certain circumstances. This also reduces the amount of hydrogen bonds towards the polymer and decreases the amount of structured water around hydrophobic domains.[56][57] In contrast to SD measurements, the amount of free water is limited during SP measurements (under isochoric conditions; keeping the "spring" in a partially compressed state). However, the reduction of fractions of both bound and structured water causes the release of a large amount of free water from the hydrogel under isobaric conditions, i.e. free swelling (the relaxation of the "spring" is only limited by the elastic forces of the network). This may also be the reason for another observation. The shape of the SP versus glucose concentration graphs is linear while the SD versus glucose concentration graphs can be described with the Langmuir model (*cf.* **Figure 8** and **Figure 2**). The amount of the released bound water is proportional to the glucose concentration. Apparently, the cooperative effect, meaning the release of structured water resulting from the release of bound water, is not as pronounced in swelling pressure measurements as in free swelling. Hence, under isobaric conditions (in free swelling) the release of bound water initializes the release of both structured and free water due to the cooperative effect, causing the large decrease of ΔSD. Since under isochoric conditions (swelling pressure measurements) this cooperative effect is weakened due to the change from free swelling towards an enclosed hydrogel, a linear dependency of SP on the glucose concentration is observed.

PNIPAAm-based hydrogels are known to undergo a 1st order VPT under isobaric conditions,[29] which, for boronic acid-containing derivatives, is still maintained in presence of glucose.[37] Nevertheless, hydrogels in this study show a linear dependence of ΔSP on the temperature. This is due to the measurement conditions. Thus, the VPT is 1st order in free swelling,[29] and it is clearly 2nd order in swelling pressure measurements (**Figure S11**). As for the addition of glucose, the cooperative effect is causing the release of a large amount of structured water and, resulting from that, free water in free swelling. In case the hydrogels are fixed in the swelling chamber, the cooperative effect is reduced. As a result, the change of swelling pressure is not as pronounced and shows a linear dependence on temperature.

Because PNIPAAm-based hydrogels are prone to the "sugaring out" effect in the whole analyzed concentration range (0 – 100 mM, *cf.* **section 3.3.3**), this base material is less suited in a biomedical pressure sensor application. The reason is, that a simple mathematical

description of the dependence of ΔSP on glucose concentration would certainly be beneficial for the pressure sensor calibration. However, in case of PNIPAAm-based hydrogels, the transducer effects due to glucose binding to PBA and the "sugaring out" effect follow two separate mathematical models. In contrast, PAAm-based hydrogels exhibit the "sugaring out" effect predominantly below physiological concentrations (*cf.* **section 3.3.3** and **Figure S8**), therefore a simple mathematical model applies for the description of the dependence of ΔSP on glucose concentration.

Overall, for the best suited hydrogel, 14A_12P_15D, a maximal change of ΔSP of 26 % can be achieved in the pressure sensor setup upon an increase of glucose concentration from 0 to 10 mM glucose analyzed at 37°C. These changes are still sufficient for a distinct measurement of the glucose concentration in the swelling pressure application, which is further facilitated through the linear dependence of the swelling pressure on the glucose concentration.

4. Conclusions

In this work, three aspects which are important for the application of glucose responsive hydrogels as biosensors were highlighted. The hydrogels were analyzed in free swelling to test if temperature-responsive PNIPAAm is better suited as base material in comparison to PAAm. Additionally, these measurements gave insights into influences of comonomer compositions on the transducer effects. These results were complemented with data for degree of complexation of phenylboronic acid units with glucose. Thereby, it was analyzed if the transducer effects were simply caused by glucose binding to PBA. Finally, swelling pressure measurements were conducted to evaluate if the hydrogel's behavior inside an isochoric biosensor is the same as in free swelling. Hereby, the long-term use of hydrogels in this pressure sensor setup was also established. Hydrogels could be kept inside the sensor housings for three months without showing a reduction of the pressure signal sensitivity to glucose addition.

First, regarding the role of the base polymer, in contrast to expectations, PAAm is better suited for the use in glucose responsive hydrogels as was demonstrated by the results of free

swelling measurements. Although the VPTT of PNIPAAm-based hydrogels could be significantly increased by the incorporation of DMAPAA, the expected enhancement of the transducer effect compared to PAAm was not observed. A temperature dependent analysis of PNIPAAm-based glucose recognition in swelling pressure measurements revealed that there was a decrease of the swelling pressure change in the physiologically relevant temperature range.

Second, the complexation of PBA units with glucose was, for the first time, analyzed inside polymeric hydrogels; binding isotherms and the respective parameters could be determined. It was shown that glucose binding follows the Langmuir model and predominately 2:1 complexes are formed between PBA and glucose in a glucose concentration range up to 50 mM. Both swelling and binding results underline that at physiological pH glucose preferably binds to PBA groups in dissociated charged state, and that the fraction of these groups can be increased by the presence of DMAPAA. By an appropriate ratio between PBA and DMAPAA, the affinity constant K_{aff} was shown to be three-fold higher compared to literature values for PBA with glucose in free solution.[4][28] This allows an occupation of 50 - 80 % of all binding sites at saturated conditions, obtained at a glucose concentration of about 50 mM. The results from free swelling and the determination of the degree of complexation of PBA were then compared to find out if the transducer effect is solely attributed to glucose binding. It was found that the hydration of the PAAm-based polymer network is significantly influenced by the presence of glucose at low concentrations (< 2 mM glucose), although glucose is not bound to PBA. This phenomenon is called “sugaring out” effect. Advantageously, this effect occurs only at glucose concentrations below the physiologically relevant values, which simplifies the mathematical description of the sensor response, because the swelling degree in the relevant range is solely changed by glucose binding to PBA-containing receptor groups. In contrast, PNIPAAm-based hydrogels show this “sugaring out” effect over the whole glucose concentration range (0 – 100 mM). This is even more pronounced for hydrogels containing more PBA than DMAPAA (more hydrophobic hydrogels), which bind less glucose in the specific receptor units.

Third, it was assumed that the hydrogels show the same effects in response to glucose addition in free swelling and in the pressure sensor setup. However, the comparison of these two measurements reveals that hydrogels exhibit the larger transducer effect in free

swelling. It was found that the water content of a hydrogel is crucial for detecting the glucose recognition. The higher the initial water content, the higher is the transducer effect in case of PAAm-based hydrogels. Since hydrogels cannot be analyzed in swelling equilibrium in the swelling pressure setup, they contain less water and therefore show a less pronounced transducer effect. However, the responsivity towards glucose, which was adjusted by the hydrogel composition, can be preserved in swelling pressure measurements. This can be explained by the direct influence of specific glucose binding to the total swelling pressure. An additional parameter significantly influences the change of swelling pressure, i.e. the polymer volume fraction ϕ. The lower ϕ the higher is ΔSP because the hydrogel can be hydrated more effectively. The more water the hydrogel contains the greater is the change of the elasticity of the hydrogel in dependence of glucose concentration. For the most suited PAAm-based composition 14A_12P_15D, maximal changes of 57 % in free swelling and 26 % in the swelling pressure setup could be achieved when changing the glucose concentration from 0 to 10 mM, at 37°C and pH 7.4. Moreover, the change of swelling pressure in dependence of glucose concentration was linear, which will allow an accurate determination of the glucose concentration in the physiologically relevant concentration range.

In conclusion, PAAm is the better suited base material for a pressure sensor application. By free swelling as well as direct measurements of glucose binding, it could be shown that more glucose is bound to PAAm-based hydrogels with PBA and DMAPAA receptor units and they are highly sensitive towards glucose in the physiological concentration range. These hydrogels exhibit the larger transducer effect, because they possess a higher water content, which can be released due to glucose binding. Moreover, these hydrogels are not as sensitive towards the reduction of the amount of structured water due to an increased glucose concentration in the physiologically relevant concentration range. Furthermore, PAAm-based hydrogels are not thermo-responsive, their operating temperature can be easily adjusted and the ΔSD values for these hydrogels increase with a temperature raise to 37°C. These advantages were also found in the pressure sensor setup, with a pronounced linear change of the swelling pressure in dependence of the glucose concentration even under physiological temperature and pH value.

Abbreviations

ΔSD	relative change of swelling degree
ΔSD_{max}	maximal relative change of swelling degree
ΔSP	relative change of swelling pressure
ΔSP per Φ	normalized relative change of swelling pressure with respect to the polymer volume fraction
Θ	degree of complexation
Θ_{max}	maximal degree of complexation
Φ	polymer volume fraction
AAm	acrylamide
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
DMAPAA	(*N*-(3-dimethylaminopropyl))-acrylamide
DMSO	dimethyl sulfoxide
HEPES	4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid
IC	inorganic carbon
K_{aff}	affinity constant
K_{SD}	initial slope of ΔSD vs. glucose concentration curve (according to Langmuir model)
MBAAm	*N,N'*-methylenebis(acrylamide)
NIPAAm	*N*-isopropylacrylamide
PAAm	polyacrylamide
PBA	3-acrylamidophenylboronic acid
PBS	phosphate buffer saline
PNIPAAm	poly-*N*-isopropylacrylamide
rt	room temperature
SD	swelling degree
SD_0	swelling degree at a glucose concentration of 0 mM glucose
SP	swelling pressure
TC	total carbon
TOC	total organic carbon
TRIS	tris(hydroxymethyl)aminomethane
VPT	volume phase transition
VPTT	volume phase transition temperature

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Supporting Information

- Depiction of procedure for determination of bound glucose
- Detailed description of swelling pressure measurement setup including accompanying schemes
- Diagrams showing SD and ΔSD in dependence of glucose concentration at 20°C (only PAAm-based hydrogels)
- Diagrams showing ΔSP for glucose concentration of 10.9 mM at temperatures varied between 25 and 45°C
- pH-dependent swelling degree determination
- VPTT values for PNIPAAm-based hydrogels in water and HEPES buffer
- VPT transition with related parameters (temperature at start and end, slope, r^2) in swelling pressure measurement setup
- Results for physiological glucose and fructose concentration in 150 mM NaCl at 37°C
- ΔSD_{max} and K_{SD} derived from Langmuir fits (swelling degree) for PAAm-based hydrogels at 37°C
- Correlation coefficients for Langmuir fits (swelling degree) at 30°C (all hydrogels)
- Intercept, slope and correlation coefficients for Langmuir fits (binding isotherms) at 30°C (all hydrogels)
- Long-term stability measurements: Plot of SP against time
- Comparison of ΔSD, ΔSP and Θ at low concentrations (0 – 10.9 mM)
- Comparison of slope of ΔSP versus glucose concentration curve normalized by polymer volume fraction in dependence of concentration range

- Dependence of initial pressure SP (0 mM glucose) plotted on polymer volume fraction ϕ
- Dependence of SD and SP of PNIPAAm-based hydrogels on temperature

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Graphical Abstract