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Swelling Behavior of a Crosslinked pHEMA Hydrogel in pH and Salt Environments

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ABSTRACT

The swelling behavior of non-ionic crosslinked poly hydroxyethyl methacrylate (pHEMA) is investigated in alkaline, acidic, and electrolyte solutions. The swelling behavior is affected by the crosslinker concentration, pH, and swelling time. An intermediate crosslinker concentration requires 24 h to reach equilibrium swelling in which the final swelling extent decreases with increasing pH. For swelling times of 90 min with lower and intermediate crosslinker concentrations, the swelling first increases followed by a decrease with increasing pH. In electrolyte solutions of monovalent cations, swelling follows the direct Hoffmeister series, whereas in electrolyte solutions of divalent cations, the swelling follows the indirect Hoffmeister series. The precise swelling mechanisms are dictated by specific Ion-pHEMA-water interactions. This work shows that the swelling behavior of pHEMA is dictated by the precise swelling conditions and environment. Therefore, careful analysis of the relevant processes is essential to accurately characterize the swelling behavior of non-ionic hydrogels, which determines their functionality and applicability.

1 | Introduction

Hydrogels are hydrophilic polymer networks that can absorb from 10%–20% up to thousands of times their dry weight in water [1]. Hydrogels are called “reversible” or “physical” gels when the network is held together by molecular entanglements and/or secondary forces (including ionic, H-bonding, or hydrophobic forces). This differs from chemically crosslinked hydrogels where the network structure is permanent.

Hydrophilic gels for biomedical applications were already introduced as early as 1960 by Otto Wichterle and Drahoslav Lim [2]. One hydrophilic gel proven to be promising was poly hydroxyethyl methacrylate (pHEMA). This polymer was successfully used to fabricate hydrogel contact lenses [3]. From that time on, pHEMA has found many biological applications such as bone tissue generation, wound healing, cancer therapy [4], and fabrication of artificial skin [5]. Apart from biomedical

applications, a macroscopic super porous monolithic structure was fabricated by Kovačič et al. [6]. Such porous structures could be used for the deposition of inorganic materials such as salt hydrates [7], act as scaffolds for tissue engineering [8], or be used in chromatography [9].

As in such applications, the polymer (hydrogel) can be exposed for relatively short time to various liquid environments containing electrolytes. The swelling behavior of such highly porous pHEMA structure is of particular interest as it can affect the material behavior.

Swelling of polymers in an aqueous solution is dependent on many different factors. The first important factor is the nature of the polymer. Hydrophilic polymers display a high solubility in/affinity to water as a consequence of their nature/functional groups present along the polymeric chain. In pH-responsive hydrogels functional groups responding to a

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change in pH are attached to the polymer main chain [10]. The water-affine functionalities may be categorized according to their nature into polycationic, polyanionic, non-ionic, and polyampholytes (containing cationic and anionic) functionalities. Cationic gels which contain protonatable groups (like amines) show enhanced swelling in acidic media due to the protonation of a proton accepting group. This creates an electrostatic repulsion between the positively charged groups. When maximum protonation and repulsion is achieved, addition of more ions results in screening of the positive charges by the excess of counterions and swelling decreases. Anionic gels contain groups which can be deprotonated (like carboxylic acids) and will result in negatively charged groups attached to the polymer chain in alkaline environments. Therefore, swelling of anionic gels is observed in alkaline environments. When dealing with polyampholyte polymers the swelling curves can be thought of as a superposition of a cationic and anionic hydrogel as the polymer contains both proton accepting groups and groups which can be deprotonated [11]. Addition of salts to ionic polymer can influence swelling by ion exchange [12], ionic crosslinking [13] and charge screening [11].

When dealing with non-ionic polymers such as pHEMA, protonation and deprotonation events do not happen. Swelling is now dependent on the specific interactions between water, dissolved ions, and polymer. A general way to describe swelling of polymers in electrolyte solutions is by means of the Hoffmeister series. The Hoffmeister series indicates the power of an ion to salt out or salt in proteins. Salting in proteins refers to the potential of the ion to weaken the protein-protein interaction and make the protein-solvent interaction more favorable. Salting out refers to the opposite and indicates the potential to weaken the protein-solvent interaction while strengthening the protein-protein interactions.

In general, anions with high charge densities tend to salt out proteins whereas low charge density ions tend to salt in proteins. For cations this trend is reversed and ions with high charge densities tend to salt in proteins whereas low charge density ions salt out proteins. These two series are named the direct and indirect Hoffmeister series [14]. However, using this series to explain the swelling behavior of a neutral hydrogel is not always straightforward since the specific ion interactions depend largely on the environment of the ions.

Careful analysis of the relevant processes is essential to accurately characterize the swelling behavior of non-ionic hydrogels, which determines their functionality and applicability. Therefore, this work will elucidate the specific ion-polymer-water interactions observed in neutral pHEMA, which is based on the work of Kovačič et al. [6]. The deeper understanding of these interactions contributes to a more reliable design and tuning of the behavior of neutral hydrogels.

2 | Materials and Methods

2.1 | Hydrogel Synthesis

The porous hydrogel was synthesized according to a modified method [7] based on work by Kovačič et al. [6]. About 2.2 g of hydroxyethyl methacrylate (HEMA, Sigma Aldrich) were added

to 5.2 mL of deionized water. The desired amount of methyl bisacrylamide (MBAA, Sigma Aldrich), 0.07 g of ammonium persulfate (APS, Sigma Aldrich) and 1.5 g of Kolliphor P188 (Sigma Aldrich) were dissolved in the HEMA solution in an ultrasonic bath. 26 mL of cyclohexane (Sigma Aldrich) were added dropwise to the monomer solution under constant stirring (500 RPM). The solution was stirred for 30 min at 500 RPM to produce the emulsion. After 30 min stirring was slowed down to 250 RPM while Tetramethylethylenediamine (TEMED, Sigma Aldrich) was added. After additional stirring for 3 min the emulsion was transferred into the molds. The material was cured at lab temperature (20°C–21°C) for 1 h and washed with demi water 5 times. Afterwards it was washed one time with 2-propanol, cut into smaller pieces, and immersed in 2-propanol for 24 h. Afterwards the polymer samples were dried under vacuum for 6 h (6 pressurizing/depressurizing cycles, 1 per hour). Afterwards the dry polymer is stored in an airtight vial.

2.2 | Impregnation Swelling

Vacuum impregnation swelling experiments were performed by submerging the dried polymer into an already prepared (salt/pH) solution for a given time. After this time five vacuum/pressurizing cycles were applied until no air bubbles were visible leaving the polymer. This was done to enforce complete wetting of the polymer. The wet samples were taken from the solution, and the volumes were measured using a Mitutoyo digital caliper. Care was taken not to compress the filled polymer during volume measurements. The wet volume change (swelling) Q [%] was determined using

$$Q = \left(\frac{V_f}{V_i} - 1 \right) * 100\% . \quad (1)$$

here V_i [m³] and V_f [m³] represent the initial and final volumes respectively. For each datapoint 3 samples are used from which the average wet volume change and error are determined.

2.3 | pH Measurements

Measurements of the solution pH was done using a Metrohm 913 pH meter equipped with a Unitrode Pt 1000 combined temperature and pH electrode. This way the pH was determined with an accuracy of 0.01 in the temperature range of 20°C–21°C. A three-point calibration was performed using Certipur buffer solutions purchased from Merck.

3 | Results and Discussion

3.1 | Swelling in Water at Various pH

Swelling experiments were performed using deionized water acidified with hydrochloric acid (HCl) or basified using sodium hydroxide (NaOH). HCl and NaOH were used since solutions of these molecules have a negligible ionic strength. This way, the sole influence of the solution pH could be investigated. Different wetting times and crosslinker densities were investigated. The polymers with higher or lower crosslink densities were made by increasing

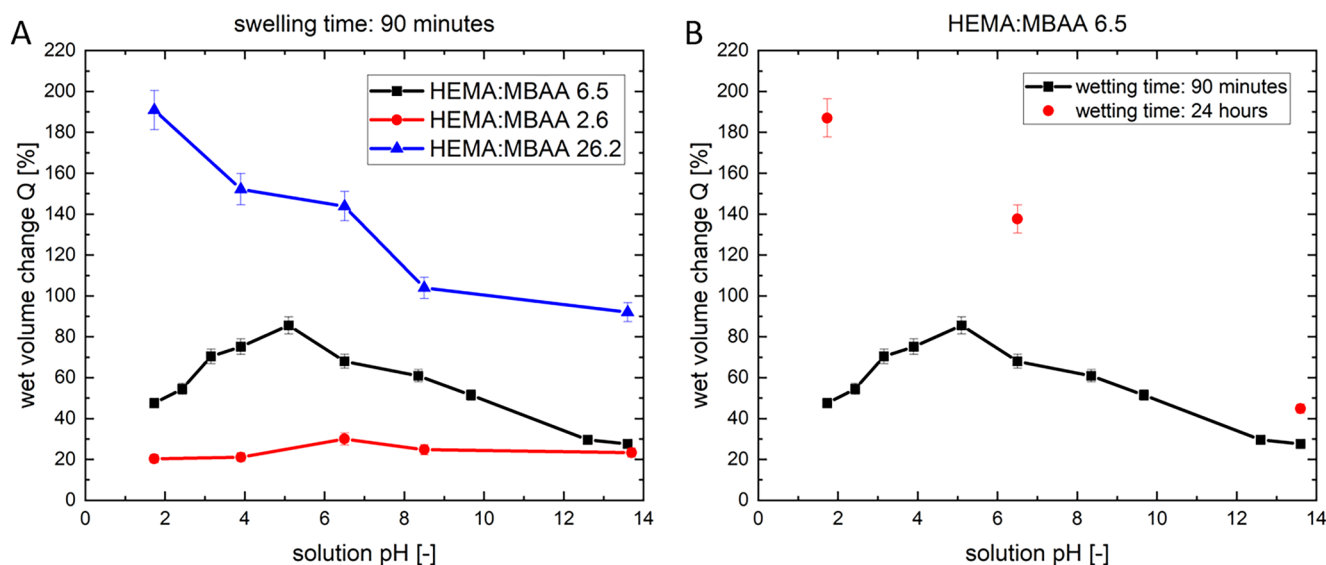


FIGURE 1 | The wet volume change as a function of the external solution pH for a fixed swelling time of 90 min (A) and a fixed ratio of HEMA:MBAA equal to 6.5 (B). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

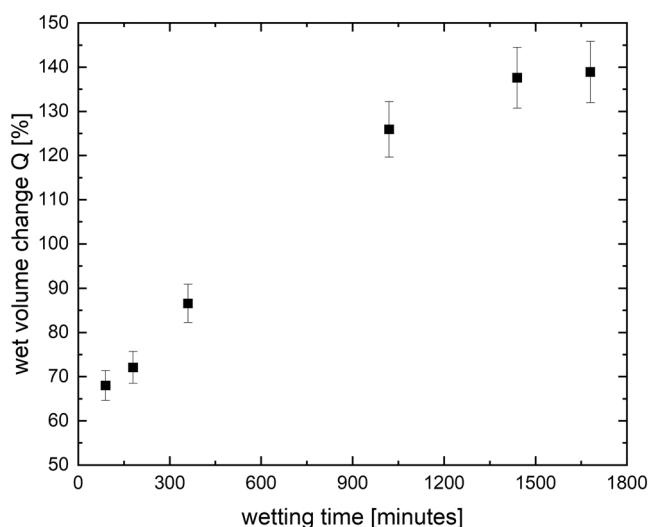


FIGURE 2 | The wet volume changes as the function of time for a fixed solution pH of 6.5 and a ratio of HEMA:MBAA of 6.5.

or decreasing the MBAA content while keeping all other parameters constant. The resulting wet volume changes as a function of pH are displayed in Figure 1. The ratio of HEMA:MBAA represents the molar ratio of monomers to crosslinker.

First swelling of a hydrogel with a HEMA:MBAA ratio of 6.5 after 90 min of immersion is discussed. It is observed that upon acidifying the solution (starting from the most basic measurement point) the wet volumetric swelling increases inversely proportionally with the solution pH until a pH of 5, after which the swelling decreases with decreasing pH. Increasing the crosslinker fraction decreases the overall swelling, whereas decreasing it increases the overall swelling, especially in acidic conditions. Comparing Figure 1A with Figure 1B shows that increasing the swelling time of a hydrogel with an intermediate content of crosslinker from 1.5 to 24 h results in a similar swelling extent as determined for the hydrogel with a smaller content of crosslinker for shorter swelling time.

Different literature studies have shown that swelling of hydrogels can take up to several hours to reach the equilibrium swelling degree [15–18]. Therefore, in Figure 2, the extent of swelling versus time is given for a hydrogel with a monomer ratio HEMA:MBAA of 6.5 (intermediate crosslinker content), immersed into a solution of pH 6.5. After ~24 h the swelling plateaus. From this observation, two conclusions can be drawn. First, short swelling times (90 min) for high and intermediate crosslinker concentration will not reach a swelling equilibrium (black and red datapoints in Figure 1A). Second, swelling behavior for the intermediate crosslinked sample in Figure 1B after 24 h (red circles) is at equilibrium swelling.

As a result, the swelling of the intermediate crosslinked sample in Figure 1B after 24 h (red circles) can be described according to the equilibrium interactions of the polymer and solvent. Hamri and Bouchaour observed a similar inversely proportional swelling behavior as a function of pH for a pHEMA/pHBMA interpenetrating network [17]. The authors attribute the decreased swelling at basic conditions to the strong (short) hydrogen bonding of hydroxide (HO^-) ions with water molecules. Consequently, a strong hydrogen-bonded water network is formed, limiting the adsorption of water molecules onto the polymer. The increased swelling in acidic medium is attributed to penetration of hydronium ions (H_3O^+) inside the polymer. Here the hydronium ion is strongly hydrogen-bonding to the OH functionality of the HEMA building block, creating an effective positively charged group. These effective positively charged groups create repulsion between the polymer chains, increasing the space to insert water and consequently increase the swelling ability [17].

As the samples with intermediate and high crosslink density at a shorter swelling time of 90 min (red and black lines in Figure 1A) are out of equilibrium, the observed behavior should be described according to the swelling kinetics. Wu et al. predict that for a crosslinked polyethylene glycol diacrylate (PEGDA), the diffusion coefficients of water and ions decrease with increasing crosslink density [19]. In their study, the diffusion coefficient decreases faster for ions compared to that

of water molecules with increasing crosslink density due to different hydrodynamic radii of the solute. The hydrodynamic radius is larger than pure water when ions are present in the solution. Additionally, water molecules will not enter the polymer without accompanying ions since this would otherwise create an ion concentration gradient, and with that an osmotic pressure difference. This explains the non-equilibrium swelling observed when using a swelling time of 90 min with high and intermediate crosslink density.

For the lowest crosslinked sample (blue line in Figure 1A) it is hypothesized that the swelling behavior is close to equilibrium as the swelling trend resembles the swelling of the intermediate crosslinked sample after 24 h in Figure 1B.

It is concluded that swelling of pHEMA depends on the degree of crosslinking, wetting time, and pH. When the system is not at equilibrium, the swelling is determined by diffusion of ions into the polymer, which is directly affected by the crosslink density. However, when the system comes close to equilibrium, the pH (equilibrium properties) determines the final swelling degree.

3.2 | Swelling in Salt Solutions

The swelling of pHEMA (HEMA:MBAA 6.5, intermediate crosslinker content) in salt solutions for 90 min was investigated to elucidate the effect of different ions on the swelling dynamics. At this crosslink density and swelling time, the system is not expected to reach equilibrium. The effect of different cations and anions was investigated to investigate the specific ion effects on the volumetric swelling (Figure 3).

When considering solutions of both monovalent cations and anions (LiCl and NaCl), a continuous increase in swelling with increasing concentration is observed. For monovalent cations combined with divalent anions (Na_2SO_4 , Na_2CO_3 and K_2CO_3)

the opposite is observed. This clear division in behavior can be explained using the Hoffmeister series in which salting out salts tend to decrease the swelling (promote polymer-polymer interaction) and salting in salts increase swelling (promote polymer-solvent interaction) [14].

Livney and coworkers have shown by titration that for neutral nonionic polyacrylamide salting out salts leads to a negative excess of salt near the polymer surface compared to the bulk, whereas salting in salts leads to a positive excess [20]. A positive excess of salt on the polymer surface reduces the chemical potential of the water (increases osmotic pressure). When zones of different polymer chains containing an excess of salt overlap, there exists a driving force for water diffusion into these areas, pushing the two chains apart (swelling). On the other hand, a negative excess results in a decrease in osmotic pressure, expelling water from the region between two polymer chains, pushing the chains towards each other [20]. For a detailed graphical representation, the reader is referred to reference [20].

First focusing on anions and monovalent cations (Figure 3A), in the Hoffmeister series one finds the carbonate (CO_3^{2-}) and sulphate (SO_4^{2-}) to be strongly salting out anions, whereas the chloride (Cl^-) ion is only weakly salting out [21]. This is due to the strong interaction of carbonate and sulphate ions with water molecules. These ions coordinate water molecules radially, with one hydrogen pointing inward and the other outward. This strong bonding prevents the anion from approaching the first hydration layer of the polymer, making the solvent a poorer solvent. Additionally, the outward facing hydrogens of the coordinated water molecules lose some of their electron pair acceptance ability towards the polymer hydroxyl groups. These effects push the hydrated anion away from the polymer, creating a negative excess (Figure 4A,B). This matches with the observations for the salts consisting of monovalent cations where the ones with strong salting out anions show a continuous decrease in swelling with increasing salt concentration [20].

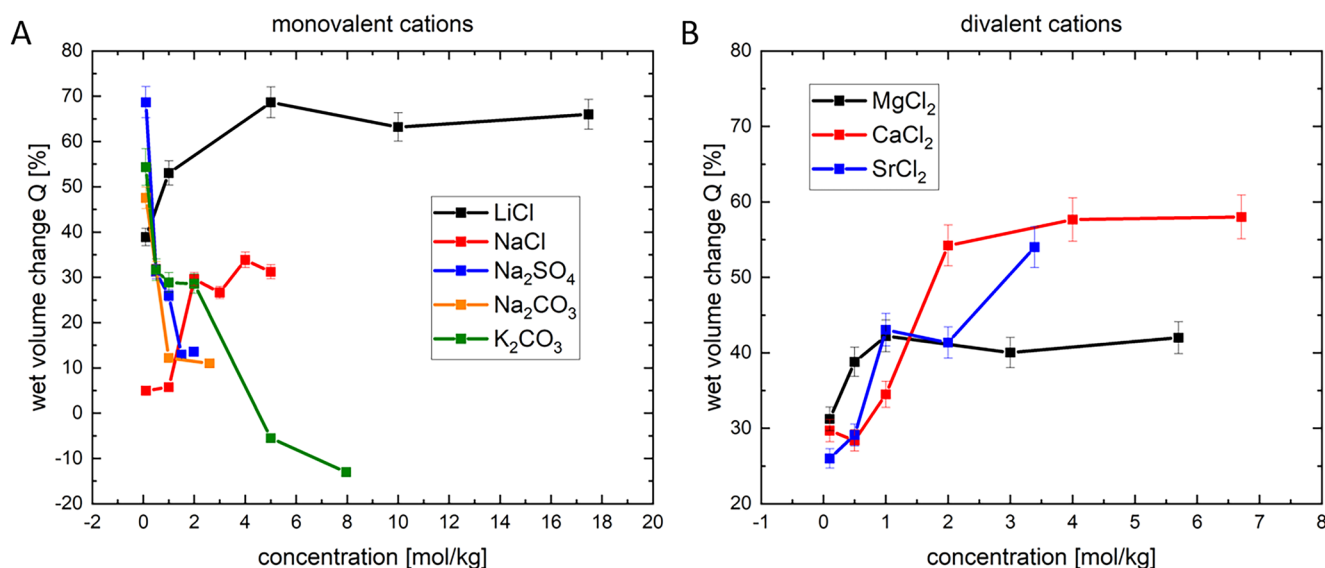


FIGURE 3 | Wet volume change versus concentration for monovalent cations (A) and divalent cations (B) by submerging into the salt solution for 90 min. Note that the lowest concentration corresponds to $0.1 \text{ mol} \cdot \text{kg}^{-1}$. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

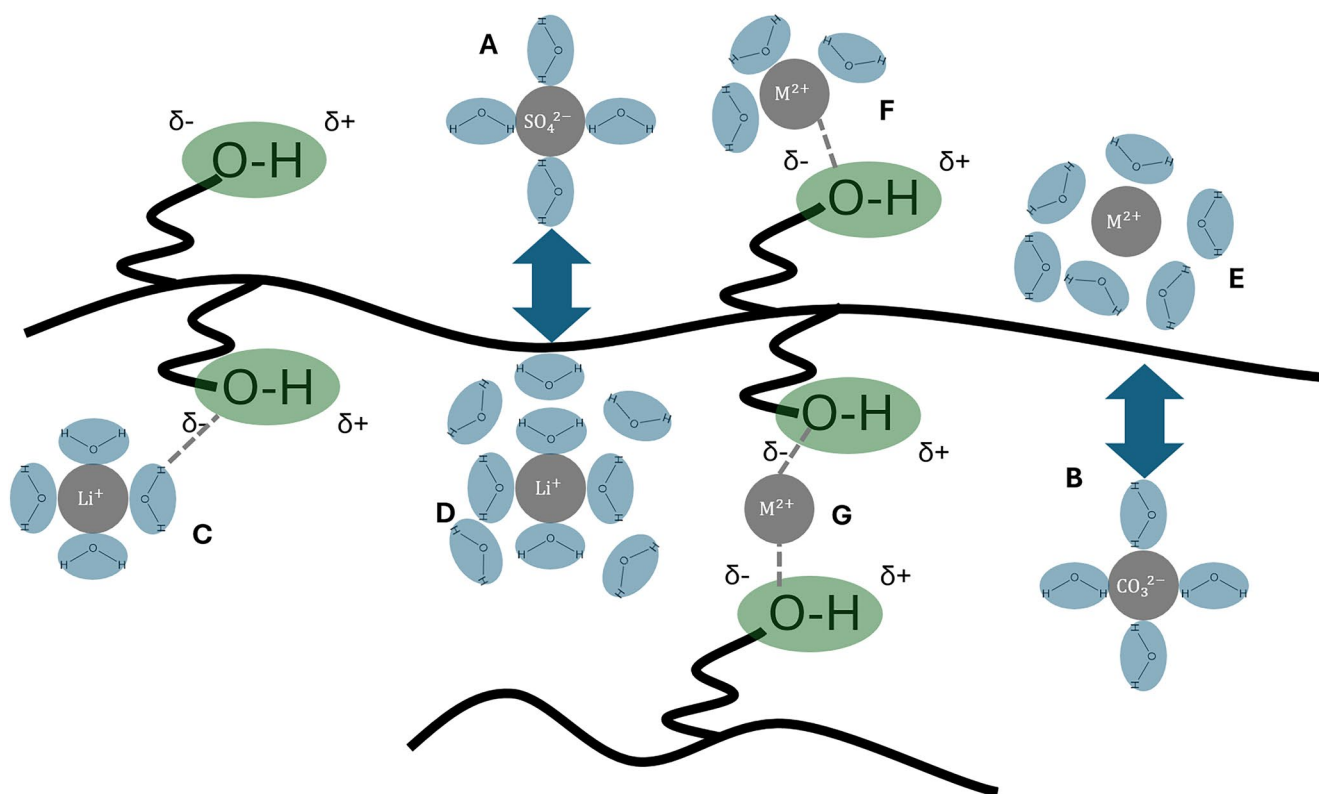


FIGURE 4 | Schematic representation of the interactions of pHEMA with monovalent and divalent electrolyte solutions. Repulsion of high charge density divalent anions (A and B). Interaction of the lithium ion with the hydroxyl dipole (C) and hydrophobic surface (D). Interaction of a divalent cation with the hydrophobic surface (E) and hydroxyl dipole (F). Crosslinker formation due to the interaction of a divalent cation with hydroxyl dipoles (G). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/app.71196)]

LiCl and NaCl are considered salting in salts. Therefore, both salts show a continuous swelling increase with increasing concentration. Comparing LiCl and NaCl it is found that LiCl results in an overall larger degree of swelling compared to NaCl. Even though lithium ions (Li^+) have a higher charge density compared to sodium ions (Na^+). This is due to two reasons. First, the lithium ion has a strong attraction to polar groups due to the higher charge density, which causes water molecules to orient with oxygen towards the metal ion [22]. This makes the water hydrogen atoms good electron pair acceptors, and the coordinated water molecules have more positive hydrogen atoms than in bulk water [20]. This ability to accept electron pairs enhances their coordination with dipoles of the hydroxyl groups (Figure 4C) [20, 23]. Second, due to the effective larger radius of the hydrated lithium ions, the hydrated ions also tend to associate at hydrophobic surfaces such as the carbon backbone (Figure 4D) [22]. The plateau observed in LiCl is due to complete coverage of the polymer [24].

In the case of multivalent cations, a continuous increase is observed since all salts are salting in salts. Mg^{2+} , Ca^{2+} and Sr^{2+} are all three salting in ions. The overall level for swelling follows the trend of enhanced swelling with decreased charge density, where Mg^{2+} has the highest and Sr^{2+} the lowest charge density. This is considered an indirect Hoffmeister series for cations [23, 25]. The indirect series is observed as Mg^{2+} coordinates water molecules the strongest due to its high charge density, whereas Ca^{2+} and Sr^{2+} coordinate water more loosely due to a lower charge density. This makes it the most difficult for Mg^{2+} to

let the hydrophobic surface enter its solvation shell (Figure 4E) or coordinate with hydroxyl moieties (Figure 4F), whereas for Ca^{2+} and Sr^{2+} this is easier [20, 22, 26]. It is hypothesized that the interaction with the hydroxyl groups is the predominant swelling factor.

There is also the possibility for multivalent cations to create effective crosslinks between hydroxyl moieties, decreasing the swelling (Figure 4G) [27]. However, this effect is assumed to be less than the interactions with the hydrophobic surface and hydroxyl groups, as the swelling extent increases with the concentration of salt.

For various salting in salts, a plateau in swelling is observed at higher concentrations (LiCl , MgCl_2 and CaCl_2). This plateau is most probably indicating maximum coverage of the polymer by the ions [24]. Unfortunately, the curve of SrCl_2 is limited by the salt solubility. The swelling behavior in water containing salting in or salting out ions is summarized in Figure 4.

4 | Conclusion

The swelling behavior of pHEMA submerged in alkaline, acidic, and electrolyte solutions was investigated. The swelling behavior is affected by the crosslinker density and swelling time. For swelling times of 90 min with lower and intermediate crosslinker concentrations, the swelling first increases followed by a

decrease with increasing pH. For the lowest crosslinker concentration, a continuous decrease in swelling behavior is observed with increasing solution pH. pHEMA with intermediate crosslinker concentrations achieved equilibrium swelling in 24 h and showed a continuous decrease in swelling behavior with increasing solution pH.

The swelling behavior in electrolyte solutions of monovalent cations follows the direct Hoffmeister series dictated by the anions whereas in divalent cations the swelling follows the indirect Hoffmeister series for divalent cations. This is due to specific ion-pHEMA-water interactions.

This work shows that the swelling behavior of pHEMA is dictated by the precise swelling conditions and environment and deepens the understanding of crosslinked non-ionic hydrogel swelling in electrolyte and pH solutions. It must be noted that this work examines the first swelling cycle of pHEMA. Therefore, investigation into the effect of multiple swelling cycles on the final properties is of future interest.

Author Contributions

Olaf Adan: conceptualization, funding acquisition, writing – review and editing, supervision. **Joey Aarts:** conceptualization, investigation, methodology, writing – original draft, visualization. **Henk Huinink:** conceptualization, funding acquisition, writing – review and editing, supervision. **Hartmut Fischer:** conceptualization, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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